IFI44 is differentially expressed in models of coronavirus infection *in vivo*.

Shahan Mamoor, MS¹

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 two independent published datasets (3-4), containing transcriptome data from infection models of the severe acute respiratory distress syndrome SARS coronavirus in mice and in ferrets to discover genes that are differentially expressed in coronaviruses *in vivo* and identify potential therapeutic targets and host cell vulnerabilities. We identified IFI44 as a conserved differentially expressed gene following coronavirus infection in the blood of infected ferrets and in the lungs of infected mice. IFI44 may be involved in the cellular response to COVID19 infection or a host cell vulnerability, exploited by coronaviruses.

Keywords: IFI44, coronavirus, *in vivo* models of coronavirus infection, SARS coronavirus, systems biology of viral infection, COVID19.

Viruses are classified according to a system known as the “Baltimore” classification of viruses (5) 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 (6). Their genome is largest of all RNA viruses, ranging from 27 to 33 kb in size (7). They obtain their name from the crown-like appearance of the viral particle imparted by the structure of the large-surface glycoprotein (7). The coronaviridae family includes seven viruses capable of infecting humans, including the severe acute respiratory distress syndrome, or SARS coronavirus (8), the Middle East respiratory syndrome coronavirus, or MERS coronavirus (9), the human coronaviruses (HCoV) 229E, OC43, HKU and NL63 (10-14), and the novel human coronavirus now known as COVID19 (13, 14). As of March 19, 2020, the World Health Organization reported 209,839 cases of COVID19 and 8778 deaths from COVID19 infection world-wide (15). 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 vivo* using two independent datasets (3, 4). From these datasets we mined transcriptome data following SARS coronavirus infection in the lungs of infected mice and in the blood of infected ferrets. Across both of these datasets, we identified the interferon induced protein IFI44 among the genes most differentially expressed following coronavirus infection. IFI44 represents a transcriptional target of the host cell gene expression program following infection with coronaviruses *in vivo*.

Methods

We used datasets GSE68820 (3) and GSE22581 (4) for this systems-level differential gene expression analysis of coronavirus infections in conjunction with GEO2R. GSE68820 was

generated using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F technology. GSE22581 was generated using Affymetrix Canine Genome 2.0 Array technology.

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 IFI44 in the lungs of C57BL6/NJ 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 IFI44 in uninfected ferret blood at day 0, and infected ferret blood at day 2 using a two-tailed, unpaired t-test with Welch's correction. 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 two independent microarray datasets containing transcriptome data from models of coronavirus infection *in vivo*, in mice and in ferrets (3, 4). Across experimental models, we identified IFI44 as differentially expressed following coronavirus infection.

IFI44 is differentially expressed the lungs of mice infected with SARS coronavirus as compared to the lungs of mock-infected mice.

We identified IFI44 as differentially expressed in the lungs following infection of mice with SARS coronavirus when compared to the lungs of mock-infected mice (Table 1) (3). When sorting all of the transcripts expressed in the mouse lung measured by microarray based on change in expression with and without infection, IFI44 ranked 91 out of 29649 transcripts. Differential expression of IFI44 in the mouse lung following infection with SARS-CoV was statistically significant (Table 1; $p=1.64E-12$).

IFI44 is differentially expressed in the blood of ferrets infected with SARS coronavirus when compared to blood from uninfected ferrets.

We also identified IFI44 as differentially expressed in the ferret blood in a ferret model of coronavirus infection *in vivo*. When comparing the blood of ferrets infected with SARS coronavirus and the blood of uninfected ferrets, IFI44 was among the genes whose expression changed most significantly (Table 2) (5). When sorting all of the transcripts expressed in the ferret blood measured by microarray based on change in expression with and without infection with SARS coronavirus, IFI44 ranked 75 out of 43035 transcripts. Differential expression of IFI44 in ferret blood following infection with SARS coronavirus was statistically significant (Table 2; $p=8.44E-04$).

IFI44 is transcriptionally induced in the lungs of mice infected with SARS coronavirus.

We also extracted exact mRNA expression values for IFI44 from the lungs of mice following infection with SARS coronavirus and from the lungs of mock infected mice in order to compare expression levels of IFI44 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 IFI44 in the lungs of mice following infection with SARS coronavirus as compared to the lungs of mock-infected mice was statistically significant. IFI44 was expressed at significantly higher levels in the lungs of infected mice following infection with SARS coronavirus (Figure 1; $p<0.0001$).

IFI44 is transcriptionally induced in the ferret blood following infection of ferrets with SARS coronavirus.

We also extracted exact mRNA expression values for IFI44 in the ferret blood following infection of ferrets with SARS coronavirus, and from the blood of uninfected ferrets in order to compare expression levels of IFI44 between these two groups rather than relative to the rest of the transcriptome as assessed in differential gene expression analysis. We compared expression of IFI44 at baseline (0 hours) in the blood of uninfected ferrets, and in the blood of

1 ferrets on day 2 after infection. We also performed a statistical test to evaluate whether the
2 difference in expression of IFI44 in the ferret blood with and without SARS infection was
3 statistically significant. IFI44 was expressed at significantly higher levels in the blood of ferrets
4 on day 2 post-infection with SARS-CoV as compared to the uninfected ferret blood at baseline
5 (0 hours). IFI44 expression increased dramatically and significantly from 0 to 48 hours (Figure
6 2; $p=0.0244$).
7

8 **Discussion**

10 COVID19 is a newly discovered coronavirus that infects humans and whose spread has
11 lead to a global pandemic (19) with zero available therapeutic strategies. To facilitate target
12 discovery and to further understanding of the basic transcriptional program of cells and tissues
13 following coronavirus infection, we mined two independent microarray datasets containing
14 transcriptome data from *in vivo* models of coronavirus infection, from the lungs of mice
15 infected with SARS coronavirus, and in the blood of ferrets after infection with SARS
16 coronavirus. Across both experimental models, we identified IFI44 as one of the genes whose
17 expression changed most significantly following infection with a coronavirus. In both mice and
18 ferrets, SARS-CoV infection lead to a significant induction of IFI44 transcript *in vivo*.
19

20 IFI44 is a member of the IFI family of interferon induced proteins and possesses a GTP-
21 binding domain (16). Enforced expression of IFI44 results in cell cycle arrest and a general
22 anti-proliferative state in cell lines (16). While IFI44 is transcriptionally induced during human
23 papillomavirus (HPV) infections and human rhinovirus (HRV) infections, it is transcriptionally
24 repressed during human immunodeficiency virus (HIV) infections (17-19). *In vitro*, IFI44
25 suppresses HIV replication and blocks HIV-1 long-terminal region (LTR) activity, resulting in an
26 inhibitory effect on viral transcription (20). One study concluded that despite the fact that
27 interferon-stimulated genes (ISGs) are thought to restrict viral replication that IFI44 was a
28 positive regulator of viral infectivity by negative regulation of anti-viral effectors IFIT2, IFI27 and

1 IFN λ 1, but this was in response to plpC stimulation (21). Knockdown of IFI44 did however lead
2 to decreases in influenza A virus (IAV) and lymphocytic choriomeningitis virus (LCMV)
3 replication *in vitro* (21). In mice infected with *Plasmodium berghei*, a murine model of malaria
4 infection, liver-stage infection resulted in a significant induction of IFI44 expression (22).
5 Deletion of IFI44 resulted in a significantly higher severe respiratory syncytial virus (RSV)
6 infectivity, demonstrating that IFI44 controls RSV infection (23). Together, these data point to
7 mixed roles for IFI44 in viral infectivity but clearly show that IFI44 is associated with viral
8 infections, is induced during viral infections, and has the ability to control multiple types of
9 virus infection despite the fact that some viruses may contrarily be dependent on IFI44 to
10 complete their life cycle.

12 We found that IFI44 was differentially expressed in the lungs of mice after infection with
13 SARS coronavirus and in ferret blood after infection with SARS coronavirus. IFI44 expression
14 should be evaluated in the cells and tissues of patients infected with the novel coronavirus
15 COVID19 to assess whether COVID19 infection similarly leads to induction of IFI44 mRNA
16 levels as seen after infection with SARS coronavirus. Modulation of IFI44 expression may
17 represent a therapeutic strategy in coronavirus infection broadly and specifically in COVID19
18 infection.

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Plasmodium activate innate immunity against liver-stage infection. *Nature medicine*, 20(1),
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Rank	ID	p-value	t	B	Gene	Gene name
91	A_52_P550858	1.64E-12	38.2295	19.424003	Ifi44	interferon-induced protein 44

Table 1: IFI44 is differentially expressed in the lungs of mice infected with SARS coronavirus as compared to the lungs of mock-infected mice.

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	t	B	Gene	Gene name
75	CfaAffx. 31148.1.S1_at	8.44E-04	5.7793	-0.5424	IFI44	interferon-induced protein 44

Table 2: IFI44 is differentially expressed in the blood of ferrets infected with SARS coronavirus when compared to the blood of ferrets infected with SARS and the blood 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: IFI44 is transcriptionally induced in the lungs of mice following infection with SARS coronavirus at 2 days post-infection.

Expression of IFI44 at the mRNA level is graphically represented with the mean value marked and the p-value listed for each comparison relative to mock infection.

Figure 2: The expression of IFI44 in the blood of ferrets significantly increases within 48 hours of infection with SARS coronavirus.

Expression of IFI44 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 IFI44 in ferret lung with and without SARS infection.