Differential expression of CARD6 in coronavirus infections.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

The novel coronavirus SARS-CoV-2 has infected nearly 20,000,000 in less than one year^{1,2}. We mined published and public data³⁻⁷ to discover the most significant transcriptional changes induced in host cells and tissues following coronavirus infections. We identified the caspase activation and recruitment domain protein CARD6 as a differentially expressed gene following infection with the Middle East Respiratory Syndrome (MERS) coronavirus in human cells and, to a lesser extent, in the lungs of mice infected with the Severe Acute Respiratory Syndrome (SARS) coronavirus SARS-CoV-1. CARD6 may be a relevant target of study for understanding coronavirus pathogenesis.

Keywords: Coronavirus, SARS-CoV-1, MERS-CoV, HCoV-229E, CARD6.

1 The novel coronavirus SARS-CoV-2 has infected nearly 20,000,000 in less than one
2 year^{1,2}. We mined published and public microarray data³⁻⁷ to understand in an unbiased fashion
3 transcriptional behavior of host cells and tissues following coronavirus infection and identified
4 CARD6 as differentially expressed, most significantly following infection with MERS-CoV.
5 CARD6 may be important for establishment of coronavirus infection.
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8 **Methods**

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10 We used datasets GSE56677³, GSE100509⁴, GSE59185⁵, GSE68820⁶ and GSE89167⁷ for
11 this analysis of gene expression changes following coronavirus infection. GSE56677 was
12 generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381
13 technology with $n=3$ each for CALU3 2B4 cells infected with MERS-CoV London (Eng1): at 0
14 hours, 3 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection. GSE100509 was
15 generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology with
16 $n=5$ each for primary human microvascular endothelial cells infected with MERS-CoV at 0
17 hours, 12 hours, 24 hours, 36 hours and 48 hours. GSE59185 was generated using
18 Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray technology with $n=2$ lung tissue
19 from mock-infected C57BL6/NJ mice and $n=2$ lung tissue from SARS-CoV-1-infected C57BL6/
20 NJ mice, both at 2 days post-infection. GSE68820 was generated using Agilent-014868 Whole
21 Mouse Genome Microarray 4x44K G4122F technology with $n=5$ for lung tissue from mock-
22 infected BALB/c mice and $n=5$ for lung tissue from SARS-CoV-1-infected BALB/c mice, both
23 at 2 days post-infection. GSE89167 was generated using 039494 SurePrint G3 Human GE v2
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1 8x60K Microarray 039381 technology with $n=2$ HuH-7 cells and $n=2$ HCoV-229E infected
2 HuH-7 cells.

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4 The Benjamini and Hochberg method of p -value adjustment was used for ranking of
5 differential expression but raw p -values were used for assessment of statistical significance of
6 global differential expression. Log-transformation of data was auto-detected, and the NCBI
7 generated category of platform annotation was used. Only p -values less than 0.05 were
8 considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)
9 (455).
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12 **Results**

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14 We mined five independent published and public microarray datasets³⁻⁷ to determine in
15 an unbiased and systematic fashion genes most transcriptionally perturbed following infection of
16 host cells and tissues with coronaviruses capable of infecting humans: two genetically distinct
17 isolates of MERS-CoV, SARS-CoV-1, and HCoV-229E.
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20 CARD6 is differentially expressed in primary human microvascular epithelial cells following 21 infection with MERS-CoV.

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23 We identified CARD6 as differentially expressed in primary human microvascular
24 epithelial cells following infection with MERS-CoV. When sorting all of the genes expressed in
25 primary microvascular epithelial cells based on change in expression when comparing uninfected
26 and infected cells, CARD6 ranked 6980 out of 34127 total transcripts (Table 1). Differential
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1 expression of CARD6 was statistically significant (Table 1; $p=1.62E-14$).

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4 CARD6 is differentially expressed in CALU3 2B4 cells following infection with MERS-CoV
5 London (Eng1).

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7 CARD6 was also differentially expressed in CALU3 cells following infection with
8 MERS-CoV London (Eng1), a genetically distinct MERS isolate. When sorting all of the genes
9 expressed in CALU3 cells based on change in expression when comparing uninfected and
10 infected cells, CARD6 ranked 345 out of 28653 total transcripts (Table 2). Differential
11 expression of CARD6 in MERS-CoV London-infected CALU3 cells was statistically significant
12 (Table 2; $p=6.3E-11$).
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16 CARD6 is differentially expressed in the lungs of C57BL6/NJ mice following infection with
17 SARS-CoV-1.
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19 We mined other published microarray datasets to determine if CARD6 differential
20 expression could be found after infection with other coronaviruses capable of infecting humans.
21 CARD6 was differentially expressed in the lungs of C57BL6/NJ mice at 2 days post-infection
22 with SARS-CoV-1. When sorting all of the genes expressed in the mouse lung based on change
23 in expression when comparing mock-infected and infected mice, CARD6 ranked 12707 out of
24 29649 total transcripts (Table 3). Differential expression of CARD6 in the lungs of SARS-
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27 CoV-1-infected mice was statistically significant (Table 3; $p=9.85E-04$).
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CARD6 was not differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1 (Table 4; $p=6.72E-01$), not was it differentially expressed in Huh-7 cells following infection with human coronavirus 229E (Table 5; $p=0.0837348$). However, while CARD6 differential expression after infection of Huh-7 cells with HCoV-229E was not statistically significant, changes in CARD6 expression post-infection with HCoV-229E was more significant than 88.7% of the measured transcriptome.

Thus, we discovered that CARD6 was among the genes most differentially expressed in two types of human cells following infection with MERS-CoV, and to a lesser extent, in the lungs of mice infected with SARS-CoV-1.

Discussion

We observed significant differential expression of the caspase activation and recruitment domain protein CARD6 following infection a human cell line with MERS-CoV London (Eng1) and following infection of primary human cells with MERS-CoV wild-type. Less marked yet still statistically significant differential expression of CARD6 in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1 suggests this may be a shared feature among specific types of coronaviruses, but not all, as BALB/c mice failed to differentially express CARD6 following infection with SARS-CoV-1. CARD6 is a modulator of NF- κ B activation downstream of NOD1 and RIP2⁸. Future studies can determine whether host genetic factors confer unique requirements for changes in CARD6 gene expression following coronavirus infections.

References

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Rank	ID	p-value	F	Gene	Gene name
6980	A_23_P41854	1.62E-14	102.9544	CARD6	caspase recruitment domain family, member 6

Table 1: CARD6 is differentially expressed in primary human microvascular epithelial cells following infection with MERS-CoV.

Rank of differential expression, probe ID, p-value with respect to differential expression, F-statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
345	A_33_P3340847	6.3E-11	97.3527	CARD6	caspase recruitment domain family, member 6

Table 2: CARD6 is differentially expressed in CALU3 2B4 cells following infection with MERS-CoV London (Eng1).

Rank of differential expression, probe ID, p-value with respect to differential expression, F-statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
12707	A_52_P303904	9.85E-04	-4.53508	-1.937876	Card6	caspase recruitment domain family, member 6

Table 3: CARD6 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1.

Rank of differential expression, probe ID, p-value with respect to differential expression, t-statistic, B, the log-odds of differential expression when comparing mock-infected and infected lung tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
48915	15190	6.72E-01	-4.41E-01	-7.36956	Card6	caspase recruitment domain family, member 6

Table 4: CARD6 is not differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1.

Rank of differential expression, probe ID, p-value with respect to differential expression, t-statistic, B, the log-odds of differential expression when comparing mock-infected and infected lung tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
7130	43915	0.0837348	2.0471561	-3.986	CARD6	caspase recruitment domain family, member 6

Table 5: CARD6 is not differentially expressed in Huh-7 cells following infection with HCoV-229E.

Rank of differential expression, probe ID, p-value with respect to differential expression, t-statistic, B, the log-odds of differential expression when comparing uninfected and infected cells, gene and gene name are listed in this chart.