The La ribonucleoprotein domain family member 6, LARP6 is differentially expressed and transcriptionally induced in 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 two independent datasets (3, 4), public (3) and published (4) containing transcriptome data from infection models of the Middle East respiratory syndrome (MERS) coronavirus and human coronavirus (HCoV) to discover genes that are differentially expressed in coronaviruses and identify potential therapeutic targets and host cell vulnerabilities. We identified the La ribonucleoprotein domain family member 6 (LARP6) as a conserved differentially expressed gene following coronavirus infection. LARP6 may be involved in the cellular response to COVID19 infection.

Keywords: LARP6, 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 (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 (10-12), 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 multiple types of coronavirus, including MERS-CoV and HCoV 229E using two independent datasets (3, 4). From these datasets were mined transcriptome data following coronavirus infection in human cell culture and in primary human endothelial cells. Across both of these datasets, we identified LARP6 as among the genes most differentially expressed following coronavirus infection. LARP6 represents a transcriptional target of the host cell gene expression program following infection with coronaviruses in human cells.

Methods

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

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

6 A statistical test was performed to evaluate the significance of difference between mRNA
7 expression levels of LARP6 in HuH-7 cells with and without HCoV 229E infection using a two-
8 tailed, unpaired t-test. A statistical test was performed to evaluate the significance of difference
9 between mRNA expression levels of LARP6 in human primary microvascular endothelial cells
10 after infection with MERS-CoV using a one-way ANOVA with multiple comparisons, all
11 compared to baseline infection at 0 hours. Only p-values less than 0.05 were considered
12 statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

15 Results

16 We mined two independent microarray datasets, public (3) and published (4) containing
17 transcriptome data from models of coronavirus infection in primary human cells and cell
18 culture. Across experimental models, we identified LARP6 as differentially expressed following
19 coronavirus infection.

21 LARP6 is differentially expressed in primary human microvascular endothelial cells when
22 comparing cells infected with wild-type Middle East respiratory syndrome coronavirus (MERS-
23 CoV), icMERS-CoV EMC2012 and uninfected cells.

24 We identified LARP6 as differentially expressed following infection of primary human
25 microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus
26 (MERS-CoV), icMERS-CoV EMC2012 when compared to non-infected cells (Table 1) (3). When
27 sorting all of the transcripts expressed in human microvascular endothelial cells measured by
28 microarray based on change in expression with and without infection, LARP6 ranked 214 out of
34127 transcripts. Differential expression of LARP6 in primary human microvascular

endothelial cells following infection with MERS-CoV was statistically significant (Table 1; $p=8.97E-25$).

LARP6 is differentially expressed in the human cell line HuH-7 when comparing cells with human coronavirus 229E and uninfected cells.

We also identified LARP6 as differentially expressed in the human cell line HuH-7 when comparing cells with human coronavirus 229E with uninfected cells (Table 2) (4). When sorting all of the transcripts expressed in human HuH-7 cells measured by microarray based on change in expression with and without infection with human coronavirus 229E, LARP6 ranked 145 out of 62976 transcripts. Differential expression of LARP6 in HuH-7 following infection with HCoV 229E was statistically significant (Table 2; $p=0.00047777$).

LARP6 is transcriptionally repressed following infection of primary human microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV EMC2012.

We extracted exact mRNA expression values for LARP6 from primary human microvascular endothelial cells infection with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV, and from uninfected primary human microvascular endothelial cells in order to compare expression levels of LARP6 between these two groups rather than relative to the rest of the transcriptome as assessed in differential gene expression analysis. This dataset contained transcriptome information from infection of primary human microvascular endothelial cells at 12 hours, 24 hours, 36 hours and 48 hours post-infection at compared to baseline (0 hours). We also performed a statistical test to evaluate whether the difference in expression of LARP6 in primary human microvascular endothelial cells infection with and without MERS-CoV infection was statistically significant. LARP6 was expressed at significantly higher levels at 12 hours, 24 hour, 36 hours, and 48 hours post-infection with MERS-CoV as compared to cells infected at baseline (0 hours) (Figure 1; $p<0.0001$). LARP6 expression peaked at 24 hours, then decreased from 24 hours to 36 hours and from 36 hours to 48 hours (Figure 1; $p<0.0001$ between 24 and 36 hours, and between 36 and 48 hours).

LARP6 is transcriptionally induced in the human cell line HuH-7 following infection with human coronavirus 229E.

We also extracted exact mRNA expression values for LARP6 from HuH-7 cells following infection with human coronavirus 229E and from uninfected HuH-7 cells in order to compare expression levels of LARP6 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 LARP6 in HuH-7 cells following infection with human coronavirus 229E as compared to uninfected HuH-7 cells was statistically significant. Consistent with the induction of LARP6 after infection of primary human microvascular endothelial cells with MERS-CoV, LARP6 was expressed at significantly higher levels in HuH-7 cells following infection with HCoV 229E (Figure 2; $p=0.0169$).

Thus, we found that LARP6 was among the genes whose expression changed most significantly after infection of human cells with MERS-CoV and HCoV 229E, and that LARP6 was transcriptionally induced following infection of primary human microvascular endothelial cells with MERS-Cov and following infection of HuH-7 cells with HCoV 229E coronavirus.

Discussion

COVID19 is a newly discovered coronavirus that infects humans and whose spread has lead to a global pandemic (15) 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 two 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 (3, 4). Across experimental models, we identified LARP6 as one of the genes whose expression changed most significantly following infection with a coronavirus. In both experimental models analyzed, LARP6 expression significantly increased after infection with coronavirus.

LARP6, or La ribonucleoprotein domain family member 6, binds to a stem loop structure contained at the 5' untranslated region (UTR) of type I collagen messenger RNAs (16). The region of LARP6 that binds diverse RNAs is composed of two domains, the La motif (LaM) and RRM1 which together are known as the La module. In the canonical La protein, the La module binds to the 3' UUU_{OH} sequences of nascent RNAs generated by RNA polymerase III, functioning as an RNA chaperone (17). RNA binding by LARP6 involving its LaM is structurally distinct from RNA binding by the LaM of La, and requires synergy by the LaM and RRM1 (17). LARP6 knockdown reduced translation of collagen mRNAs by reducing loading of collagen mRNAs onto polysomes, suggesting that LARP6 is positive regulator of translation for collagen mRNAs (16). In the smooth muscle cells of the aorta, the insulin-like growth factor IGF-1 increases expression of LARP6 as well as the rate of collagen synthesis (18). In lung fibroblasts, LARP6 is phosphorylated at 8 serine residues, 6 of which are located in its carboxy-terminal domain (19). The protein kinase Akt is at least in part responsible for this phosphorylation, which follows a hierarchical order, first at serine residue 451. Expression of a LARP6 S451A, a mutant that cannot be phosphorylated, leads to a decrease in collagen synthesis and secretion, demonstrating that phosphorylation of LARP6 at serine residue 451 is important for collagen translation (19). LARP6 is also phosphorylated by mTORC1 at serine residues at positions 348 and 409 (20). Lack of LARP6 phosphorylation by mTORC1 at S348 and S409 results in LARP6 sequestration at the ER membrane (20). The relationship between LARP6 and collagen synthesis in fibrotic disease processes is evident in the importance of LARP6 for the development of both atherosclerotic plaques as well as kidney fibrosis in end stage renal disease (18, 21, 22). With respect to a role for LARP6 in viral infections, LARP6 is differentially expressed and up-regulated after infection of Huh 7.5 cells with hepatitis C virus (23).

We found that LARP6 was differentially expressed in a human cell line following infection with human coronavirus 229 E as well as in primary human endothelial cells after infection with Middle East respiratory syndrome coronavirus. LARP6 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 induction of LARP6 as seen after infection with MERS-CoV and HCoV 229E. The relationship between LARP6, collagen synthesis and viral infection is unclear but modulation of LARP6 expression could potentially 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
214	A_23_P117782/ NM_018357	8.97E-25	812.731	LARP6	La ribonucleoprotein domain family, member 6

Table 1: LARP6 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
145	38945/ NM_018357	0.00047777	7.03	0.451707	LARP6	La ribonucleoprotein domain family, member 6

Table 2: LARP6 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.

Figure 1: LARP6 is transcriptionally induced following infection of primary human microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV.

Expression of LARP6 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: LARP6 is transcriptionally induced in the human cell line HuH-7 following infection with human coronavirus 229E.

Expression of LARP6 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 LARP6 with and without HCoV infection.