Differential expression of UVSSA in the blood in cases of viral co-infection.

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The human coronavirus SARS-CoV-2¹ has resulted in the death of over 200,000 Americans in less than one year². Infection of a person already suffering from a viral infection, a phenomena known as co-infection can potentially pose a problem during the upcoming influenza season. We mined published microarray data³ to identify genes most differentially expressed in the whole blood of patients suffering from viral co-infections, including co-infections involving a human coronavirus. We found that the UVSSA gene, encoding the UV stimulated scaffold protein A, was among the genes whose expression changed most significantly transcriptome-wide when in the blood of four patients suffering from two different types of co-infections: human coronavirus HKU1 and human rhinovirus, as well as in human coronavirus OC43 and influenza A co-infection. UVSSA may be of relevance to biology underlying some viral co-infections.

Keywords: coronavirus, HCoV HKU1, HCoV OC43, SARS-CoV-2, viral co-infection, UVSSA, systems biology of viral infection.

Co-infection refers to a process by which more than one pathogen have infected a cell or host concurrently. Infections from the severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, has resulted in the death of over 200,000 Americans in less than one year². Our understanding of the transcriptional changes that accompany coronavirus co-infection is limited, but a recent study of patients in Northern California found that of 116 patients testing positive for a SARS-CoV-2, 24 (20.7%) possessed a co-infection⁴. We mined published microarray data³ to study gene expression in the blood of humans with different viral co-infections to determine in an unbiased fashion genes most differentially expressed in patient blood upon coronavirus co-infection. We found significant differential expression of UVSSA specifically in the blood of four patients with two different types of viral co-infections involving human coronaviruses.

Methods

We used dataset GSE68310³ for this differential gene expression analysis of the blood of patients with viral co-infections. GSE68310 was generated using Illumina HumanHT-12 V4.0 expression beadchip technology; analysis was performed with platform GPL10558. In the study from which this microarray data was obtained, investigators collected peripheral blood from patients at baseline, day 0 of infection, correlating with a visit to the clinic upon infection with a respiratory illness; day 2, day 4, day 6, and day 21 of infection following the clinic visit, as well as in the season following infection (“Spring”). We compared the blood of $n=1$ patient with HCoV HKU1 infection (ID: JR0336) to the blood of $n=3$ patients with HCoV HKU1 and human rhinovirus co-infection (ID: JR207, JR611 and JR1702). We compared the blood of $n=1$ patient with Influenza A infection (ID: JR0021) to the blood of $n=1$ patient with Influenza A and HCoV

OC43 co-infection (ID: JR1829). We compared the blood of $n=2$ patients with HCoV HKU1 and human rhinovirus co-infection (IDs: JR0207 and JR1702) at baseline, day 0, day 2, day 4, day 6, and day 21. The Benjamini 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 used to evaluate the significance of difference of UVSSA gene expression between the blood of a patient or patients with a single infection and the blood of a patient or patient(s) with co-infection using either a two-tailed, unpaired t-test with Welch's correction or a one-way ANOVA with Dunnett's multiple comparisons test. All statistical tests were performed with PRISM 8.4.0 (455).

Results

We mined a published microarray dataset³ to determine in an unbiased fashion genes most differentially expressed in the blood of patients with coronavirus co-infection.

UVSSA is differentially expressed in the blood of patients with co-infection of Human Rhinovirus and Human Coronavirus HKU1.

We found that the gene encoding UVSSA was among those most differentially expressed when comparing the blood of one patient with human coronavirus (HCoV) HKU1 infection to the blood of three patients with co-infection of human rhinovirus and HCoV HKU1. When comparing all transcripts measured by microarray based on change in expression between HCoV HKU1 single infection patient blood and the blood of patients with HCoV HKU1 and rhinovirus

co-infection, UVSSA ranked 193 out of 47254 total transcripts, equating to 99.6% differential expression. Differential expression of UVSSA in the blood of patients with HCoV HKU1 / rhinovirus co-infection was statistically significant (Table 1; $p=4.1E-04$).

UVSSA is differentially expressed in the blood of a patient with co-infection of influenza A and human coronavirus OC43.

We then asked whether UVSSA differential expression was specific HCoV HKU1 co-infection or could be observed in human coronavirus co-infections of other types. We found significant differential expression of UVSSA in the blood of one patient with influenza A to the blood of one patient with co-infection of influenza A and HCoV OC43. When comparing all transcripts measured by microarray based on change in expression between the blood of a patient with influenza A single infection and the blood of a patient with co-infection of HCoV OC43 and influenza A, UVSSA ranked 240 out of 47254 total transcripts, equating to 99.5% differential expression. Differential expression of UVSSA in influenza A / HCoV OC43 co-infected patient blood was statistically significant (Table 2; $p=8.2E-04$).

While we found significant differential expression of UVSSA in the blood of patients with two different types of human coronavirus co-infections, interpretation of these data is not straightforward as there can be inherent differences in mRNA expression levels from patient to patient. We then asked whether UVSSA was significantly differentially expressed in the blood of patients with a human coronavirus co-infection, not from patient to patient but comparing the same patients across the course of infection, from baseline to day 21 following infection.

UVSSA is not significantly differentially expressed in the blood of patients with co-infection of human coronavirus HKU1 and human rhinovirus over the course of infection.

We queried microarray data from the blood of two patients with co-infection of HCoV HKU1 and human rhinovirus, comparing total gene expression at baseline, day 0, day 2, day 4, day 6 and day 21, to determine if UVSSA was differentially expressed in the blood of patients with a viral co-infection across time, and not based on comparison between a patient with co-infection versus a patient with infection with a single virus. We found that UVSSA was not significantly differentially expressed in the blood during co-infection with HCoV HKU1 and human rhinovirus; when sorting each of the genes expression in the blood during HCoV HKU1 and human rhinovirus co-infection based on significance of change in expression across infection time points as compared to baseline, UVSSA ranked 19672 out of 47254 total transcripts (Table 3), equating to 58.4% differential expression; this was not statistically significant (Table 3; $p=0.4505434$).

UVSSA expression is significantly higher in HCoV HKU1 and Rhinovirus co-infection and in HCoV NL63 and rhinovirus co-infection.

We then obtained exact mRNA expression levels of UVSSA in the blood of each patient with human coronavirus co-infection to directly compared UVSSA expression levels between patients with and without human coronavirus co-infection. In the blood of three patients with HCoV HKU1 and rhinovirus co-infection, UVSSA was expressed at lower levels when compared to the blood of a patient with infection of HCoV HKU1 infection only; lower expression of UVSSA in HCoV HKU1 and rhinovirus co-infection was statistically significant

(Figure 1; patient 1: $p=0.0290$; patient 2: $p=0.0145$; patient 3: $p=0.0019$). We calculated a mean fold change in UVSSA expression of 0.9903 ± 0.0055 , 0.9889 ± 0.0039 , and 0.9857 ± 0.0037 in the blood of three patients with HCoV HKU1 co-infection to that of rhinovirus co-infection (Table 1).

In the blood of a patient with influenza A and HCoV OC43 co-infection, we also observed significantly higher expression of UVSSA when compared to the blood of a patient with influenza A infection only (Figure 2; $p=0.0003$). We calculated a mean fold change in UVSSA expression of 1.0146 ± 0.0056 in HCoV OC43 and influenza A co-infected patient blood (Table 2).

Thus, we identified UVSSA as among the most differentially expressed genes in the blood of four patients with viral co-infections involving human coronavirus HKU1 and OC43. UVSSA was differentially expressed in the blood in HCoV HKU1 viral co-infection when comparing patients with co-infection to a patient with infection of HCoV HKU1 only, but when evaluating differential expression across the course of infection amongst the same patients.

Discussion

UVSSA was isolated as part of a protein complex ubiquitinated upon exposure to ultraviolet (UV) light. UVSSA is important for transcription-coupled nucleotide-excision repair (TC-NER) as depletion of UVSSA results in defects in TC-NER. UVSSA functions in stabilization of ERCC6/CSB by associating with the deubiquitinase Usp7 in TC-NER complexes at UV-induced lesions; UVSSA also interacts with RNA polymerase II at these sites^{5,6}. The A

1 group of UV sensitive syndrome (UV^{SS}) (UV^{SS}-A) is caused by mutations in UVSSA⁶. UVSSA
2 can also function to promote ubiquitination of stalled RNA Pol IIo at sites of DNA damage⁷. The
3 transcription-coupled repair complex includes CSA, CSB and UVSSA; CSB can interact with
4 RNA Pol IIo; subsequent recruitment of CSA by CSB permits UVSSA association with TC-NER
5 complex⁸. In conjunction with CSA and CSB, UVSSA is then able to function in recruitment of
6 transcription factor IIH (TFIIH) to TC-NER sites for DNA damage repair⁸. However, a separate
7 study argued that UVSSA is recruited to DNA damage sites independent of CSA and CSB, and
8 that while interactions between UVSSA and CSA required the Vps/Hrs/STAM domain (VHS),
9 recruitment of UVSSA to transcription-blocking DNA lesions (TBLs) required the domain of
10 unknown function (DUF2043)⁹. A subunit of the FACT chaperone for H2A/H2B, Sp16, has also
11 been described to be important for UVSSA recruitment to sites of TC-NER⁹. While CSB is
12 stabilized through deubiquitination by Usp7, ubiquitination-mediated UV-induced degradation of
13 CSB is performed by an E3 ligase complex containing DDB1, Cul4A, and ROC/Rbx1 in
14 association with the COP9 signalosome¹⁰, a regulator of cullin E3 ligase function.

20 By mining published microarray data³ from the blood of patients with viral infections, we
21 found significant differential expression of the UV stimulated scaffold protein A, UVSSA, in the
22 blood of four patients with viral co-infections with two types of human coronavirus co-
23 infections, indicating modulation of UVSSA expression occurs in some viral co-infections and
24 suggesting UVSSA function might be important in some types of human coronavirus co-
25 infections.

References

1. Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C. and Garry, R.F., 2020. The proximal origin of SARS-CoV-2. *Nature medicine*, 26(4), pp.450-452.
2. World Health Organization. United States of America Situation. <https://covid19.who.int/region/amro/country/us>.
3. Zhai, Y., Franco, L.M., Atmar, R.L., Quarles, J.M., Arden, N., Bucasas, K.L., Wells, J.M., Nino, D., Wang, X., Zapata, G.E. and Shaw, C.A., 2015. Host transcriptional response to influenza and other acute respiratory viral infections—a prospective cohort study. *PLoS Pathog*, 11(6), p.e1004869.
4. Kim, D., Quinn, J., Pinsky, B., Shah, N.H. and Brown, I., 2020. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *Jama*.
5. Schwertman, P., Lagarou, A., Dekkers, D.H., Raams, A., van der Hoek, A.C., Laffeber, C., Hoeijmakers, J.H., Demmers, J.A., Foustari, M., Vermeulen, W. and Marteijn, J.A., 2012. UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair. *Nature genetics*, 44(5), pp.598-602.
6. Zhang, X., Horibata, K., Saijo, M., Ishigami, C., Ukai, A., Kanno, S.I., Tahara, H., Neilan, E.G., Honma, M., Nohmi, T. and Yasui, A., 2012. Mutations in UVSSA cause UV-sensitive syndrome and destabilize ERCC6 in transcription-coupled DNA repair. *Nature genetics*, 44(5), pp.593-597.
7. Nakazawa, Y., Sasaki, K., Mitsutake, N., Matsuse, M., Shimada, M., Nardo, T., Takahashi, Y., Ohyama, K., Ito, K., Mishima, H. and Nomura, M., 2012. Mutations in UVSSA cause UV-sensitive syndrome and impair RNA polymerase II processing in transcription-coupled nucleotide-excision repair. *Nature genetics*, 44(5), p.586.
8. van der Weegen, Y., Golan-Berman, H., Mevissen, T.E., Apelt, K., González-Prieto, R., Goedhart, J., Heilbrun, E.E., Vertegaal, A.C., van den Heuvel, D., Walter, J.C. and Adar, S., 2020. The cooperative action of CSB, CSA, and UVSSA target TFIIH to DNA damage-stalled RNA polymerase II. *Nature Communications*, 11(1), pp.1-16.
9. Wienholz, F., Zhou, D., Turkeyilmaz, Y., Schwertman, P., Tresini, M., Pines, A., van Toorn, M., Bezstarosti, K., Demmers, J.A. and Marteijn, J.A., 2019. FACT subunit Spt16 controls UVSSA recruitment to lesion-stalled RNA Pol II and stimulates TC-NER. *Nucleic acids research*, 47(8), pp.4011-4025.

1 10. Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J.I., Saijo, M., Drapkin, R., Kisselev,
2 A.F., Tanaka, K. and Nakatani, Y., 2003. The ubiquitin ligase activity in the DDB2 and CSA
3 complexes is differentially regulated by the COP9 signalosome in response to DNA
4 damage. *Cell*, 113(3), pp.357-367.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
193	ILMN_1810225	4.1E-04	-4.0824185	-0.18837	P1: 0.9903 ± 0.0055 P2: 0.9889 ± 0.0039 P3: 0.9857 ± 0.0037	UVSSA	UV stimulated scaffold protein A

Table 1: Differential expression of UVSSA in the blood of patients with co-infection of HCoV HKU1 and human rhinovirus.

Rank of global differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change of UVSSA mRNA expression when comparing co-infected patient blood to the blood of a patient with infection of HCoV HKU1 only, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
240	ILMN_1810225	8.2E-04	4.28685	-0.5176	1.0146 ± 0.0056	UVSSA	UV stimulated scaffold protein A

Table 2: Differential expression of UVSSA in the blood of a patient with co-infection of HCoV OC43 and influenza A.

Rank of global differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change of UVSSA mRNA expression when comparing co-infected patient blood to the blood of a patient with infection of influenza A only, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
19672	ILMN_1810225	0.4505434	1.05702	UVSSA	UV stimulated scaffold protein A

Table 3: UVSSA is not differentially expressed in the blood of patients with co-infection of HCoV HKU1 and human rhinovirus when comparing patient blood amongst the same patients at time points across the course of infection.

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

Figure 1: UVSSA is expressed at significantly higher levels in the blood of patients with co-infection of HCoV HKU1 and human rhinovirus when compared to a patient with human rhinovirus infection only.

The mRNA expression level of UVSSA in the blood of each patient is graphically represented with mean expression level marked and the result of a statistical test evaluating significance of difference in UVSSA expression between the blood of patients with HCoV HKU1 / rhinovirus co-infection and the blood of a patient with HCoV HKU1 infection only, a *p*-value, listed above each comparison. *n*=1 patient for HCoV HKU1 infection and *n*=3 patients for HCoV HKU1 / Rhinovirus co-infection. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient with HCoV HKU1 infection only is lacking day 6 sample; 1 patient with HCoV HKU1 and rhinovirus co-infection is lacking baseline sample.

Figure 2: UVSSA is expressed at significantly higher levels in the blood of a patient with co-infection of HCoV OC43 and influenza A when compared to a patient with influenza A infection only.

The mRNA expression level of UVSSA in the blood of each patient is graphically represented with mean expression level marked and the result of a statistical test evaluating significance of difference in UVSSA expression between the blood of $n=1$ patient for influenza A infection and $n=1$ patient for HCoV OC43 / influenza A co-infection. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.