TET2 is differentially expressed in coronavirus infections.

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The novel coronavirus SARS-CoV-2 has infected nearly 20,000,000 in less than one year^{1,2}. We mined published and public data³⁻⁸ to discover the most significant transcriptional changes induced in host cells and tissues following coronavirus infections. We identified the ten eleven (TET) methylcytosine dioxygenase TET2 as a differentially expressed gene following infection of human cells with the Middle East Respiratory Syndrome (MERS) coronavirus, Human Coronavirus 229E, and in the lungs of mice infected with the Severe Acute Respiratory Syndrome (SARS) coronavirus SARS-CoV-1. TET2 may be a relevant target of study for understanding coronavirus pathogenesis.

Keywords: Coronavirus, SARS-CoV-1, SARS-CoV-2, MERS-CoV, HCoV-229E, TET2, epigenetics of coronavirus infection.

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 data³⁻⁸ to understand in an unbiased fashion
3 transcriptional behavior of host cells and tissues following coronavirus infection and identified
4 the ten eleven (TET) methylcytosine dioxygenase TET2 as among the genes most differentially
5 expressed following infection with a series of human coronaviruses, most significantly following
6 infection with MERS-CoV. TET2 may be important for establishment of coronavirus infection.
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9 **Methods**

11 We used datasets GSE56677³, GSE100509⁴, GSE59185⁵, GSE68820⁶, GSE22581⁷, and
12 GSE89167⁸ for this analysis of gene expression changes following coronavirus infection.
13 GSE56677 was generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray
14 039381 technology with $n=3$ each for CALU3 2B4 cells infected with MERS-CoV London
15 (Eng1): at 0 hours, 3 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection. GSE100509
16 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology
17 with $n=5$ each for primary human microvascular endothelial cells infected with MERS-CoV at 0
18 hours, 12 hours, 24 hours, 36 hours and 48 hours. GSE59185 was generated using
19 Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray technology with $n=3$ lung tissue
20 from mock-infected BALB/c mice and $n=3$ lung tissue from SARS-CoV-1-infected BALB/c
21 mice, both at 2 days post-infection. GSE68820 was generated using Agilent-014868 Whole
22 Mouse Genome Microarray 4x44K G4122F technology with $n=5$ for lung tissue from mock-
23 infected C57BL6/NJ mice and $n=5$ for lung tissue from SARS-CoV-1-infected C57BL6/NJ mice,
24 both at 2 days post-infection. GSE22581 was generated using Affymetrix Canine Genome 2.0
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Array technology with $n=3$ each for ferret lung on day 0, day 1 and day 2 post-infection through the intranasal route with SARS-CoV-1, and for ferret blood, $n=3$ uninfected ferret blood on day 0 and $n=4$ for ferret blood at 2 days post-infection with SARS-CoV-1. GSE89167 was generated using 039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with $n=2$ HuH-7 cells and $n=2$ HCoV-229E infected HuH-7 cells.

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. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined six independent published and public microarray datasets³⁻⁸ to determine in an unbiased and systematic fashion genes most transcriptionally perturbed following infection of host cells and tissues with coronaviruses capable of infecting humans: two genetically distinct isolates of MERS-CoV, SARS-CoV-1, and human coronavirus 229E (HCoV-229E).

TET2 is differentially expressed in primary human microvascular epithelial cells following infection with MERS-CoV.

We identified the ten eleven (TET) methylcytosine dioxygenase TET2 as differentially expressed in primary human microvascular epithelial cells following infection with MERS-CoV.

When sorting all of the genes expressed in primary microvascular epithelial cells based on change in expression when comparing uninfected and infected cells, TET2 ranked 285 out of 34127 total transcripts (Table 1), equating to differential expression more significant than 99.2% of the measured transcriptome. Differential expression of TET2 was statistically significant (Table 1; $p=3.32E-24$).

TET2 is differentially expressed in CALU3 2B4 cells following infection with MERS-CoV London (Eng1).

TET2 was also differentially expressed in CALU3 cells following infection with MERS-CoV London (Eng1), a genetically distinct MERS isolate. When sorting all of the genes expressed in CALU3 cells based on change in expression when comparing uninfected and infected cells, TET2 ranked 60 out of 28653 total transcripts (Table 2), equating to differential expression more significant than 99.8% of the measured transcriptome. Differential expression of TET2 in MERS-CoV London-infected CALU3 cells was statistically significant (Table 2; $p=2.13E-13$).

TET2 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1.

We mined other published and public microarray datasets to determine if TET2 differential expression occurred after infection with other coronaviruses capable of infecting humans. TET2 was differentially expressed in the lungs of C57BL6/NJ mice at 2 days post-

infection with SARS-CoV-1, albeit less markedly so than following *in vitro* MERS-CoV infection. Differential expression of TET2 in the lungs of SARS-CoV-1-infected mice was statistically significant (Table 3; $p=1.9\text{E-}02$). Though TET2 differential expression in the lungs of SARS-CoV-1-infected C57BL6/NJ mice was statistically significant, when sorting all transcripts expressed in the mouse lung based on change in expression between mock-infected and infected mice, TET2 ranked 18028 out of 29649 total transcripts (Table 3), equating to differential expression more significant than only 39.2% of the measured transcriptome.

TET2 was not differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1 to a statistically significant extent (Table 4; $p=1.74\text{E-}01$), with 38.3% of total transcripts measured undergoing more significant gene expression changes than TET2.

We queried a third *in vivo* infection model of SARS-CoV-1 in the ferret. TET2 was differentially expressed in the lungs of ferrets infected intranasally with SARS-CoV-1 (Table 5; $p=1.68\text{E-}05$). When sorting each transcript based on significance of change in expression when comparing mock-infected lung to SARS-CoV-1-infected lung, TET2 ranked 1087 out of 43045 total transcripts, equating to differential expression more significant than 97.5% of the measured transcriptome. TET2 differential expression in ferret blood after intranasal infection with SARS-CoV-1 was not statistically significant (Table 6; $p=6.77\text{E-}02$); when sorting each transcript based on significance of change in expression when comparing blood from non-infected ferrets (day 0) to blood from SARS-CoV-1-infected ferrets at 2 days post-infection, TET2 ranked 4091 out of 43045 total transcripts, equating to differential expression more significant than 90.5% of the measured transcriptome.

1 In addition to MERS-CoV and SARS-CoV-1, we analyzed microarray data from human
2 coronavirus 229E infection in the Huh-7 human cell line. TET2 was also differentially expressed
3 following infection with HCoV-229E *in vitro* (Table 5; $p=0.037879$). When sorting all
4 transcripts measured based on significance of change in gene expression when comparing
5 HCoV-229E infected and uninfected cells, TET2 ranked 3180 out of 62796 total transcripts
6 (Table 5), equating to differential expression more significant than 95.0% of the measured
7 transcriptome.
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10 Finally, we assessed kinetics of TET2 expression changes in CALU3 cells following
11 infection with MERS-CoV London. At 3 hours post-infection, the fold change in TET2 gene
12 expression was 0.9906 ± 0.0070 , and at 7 hours 1.0173 ± 0.0165 ; by 12 hours post-infection,
13 statistically significant transcriptional induction of TET2 could be observed with a mean fold
14 induction of 1.0668 ± 0.0027 (Figure 1; $p<0.0001$); at 18 hours post-infection, TET2 mRNA
15 levels increased to 1.1687 ± 0.0079 (Figure 1; $p<0.0001$) and continued to increase with mean
16 fold induction of 1.2523 ± 0.0046 at 24 hours (Figure 1; $p<0.0001$).
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19 Thus, we found differential expression of the gene encoding TET2 in three human cell
20 types following infection with MERS-CoV and HCoV-229E, and to a lesser extent, in the lungs
21 of C57BL/6/NJ mice infected with SARS-CoV-1. TET2 was rapidly transcribed following
22 infection of human cells with MERS-CoV London.
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Discussion

The ten eleven translocation (TET) enzyme TET2 functions in epigenetic modification of the DNA and is related to TET1 which functions in the conversion of 5-methylcytosine moieties to 5-hydroxymethylcytosine for DNA demethylation⁹. TET2 and TET3 but not TET1 associate with the O-linked beta-N-acetylglucosamine (O-GlcNAc) transferase (OGT), which functions in the addition of O-GlcNAc to serine and threonine residues and this interaction can be found at transcription start sites¹⁰. TET2 can be found at active enhancers and functions in the recruitment of the estrogen receptor alpha (ER α). Depletion of TET2 results in global accumulation of methyl marks on DNA, supporting a role for TET2 in DNA demethylation¹¹.

TET2 has important roles in embryonic stem cells¹² and in blood cancers affecting the myeloid lineage^{13,14}. TET2 gene expression is regulated by the embryonic stem cell transcription factor Oct4, and expression of TET2 is induced in parallel with accumulation of 5-hydroxymethylcytosine (5hmC) during induction of pluripotency in fibroblasts¹². Depletion of TET2 in murine hematopoietic precursors results in lineage commitment defects skewed towards the monocyte and macrophage lineage¹³. Mutation of TET2 is found in acute myeloid leukemia and myeloproliferative neoplasms, and 5hmC on DNA is reduced in bone marrow from patients with TET2 mutations¹⁴. Tet2 deficiency in mice results in expansion of the hematopoietic stem cell compartment, and competitive transplant revealed enhancement of stem cell renewal *in vivo* in the absence of Tet2¹⁴. Tet2 $-/-$ mice develop a cancer of the myeloid lineage that resembles human chronic myelomonocytic leukemia¹⁴.

1 TET2 has been described as important for the inflammatory response and production of
2 cytokines in immune cells¹⁵⁻¹⁷. A specific target of TET2-mediated gene expression is the
3 cytokine interleukin IL-6 in dendritic cells and macrophages; deficiency of TET2 results in
4 induction of multiple inflammatory mediators in response to lipopolysaccharide. Indications that
5 TET2 is important *in vivo* for control of immune responses include increased susceptibility of
6 dextran-sulfate sodium colitis and endotoxin shock in Tet2-deficient mice¹⁵. 5-hmC is enriched
7 at lineage-specific genes in T-lymphocytes and supports cytokine production in Th1 and Th17-
8 lineage cells; deficiency of Tet2 is associated with impairment of p300 recruitment concomitant
9 to impaired cytokine production¹⁶. In plasmacytoid dendritic cells, TET2 is recruited by CXXC5
10 and could be found at the Irf7 promoter and the CpG island (CGI) of Irf7 was marked by higher
11 than normal levels of DNA methylation¹⁷. Tet2-deficient plasmacytoid dendritic cells produced
12 less Irf7 transcript than wild-type pDCs, and levels of the H3K36me3 and H3K4me3 marks were
13 significantly decreased at the Irf7 CGI¹⁷. Moreover, 5hmC at the CGI of two separate
14 inflammatory mediators, Cxcl2 and Tnfa was significantly decreased; reduction of CGI 5-hmC at
15 these genes was accompanied by reduction of RNA pol II promoter binding¹⁷. TET2 is
16 important for effector function in immune cells.

23 Infection with the Epstein-Barr Virus (EBV) results in repression of TET2 gene
24 expression involving EBV transcripts BARF0 and LMP2A¹⁸. When TET2 was depleted using
25 small hairpin RNA (shRNA), de novo DNA methylation after EBV infection was significantly
26 increased, suggesting TET2 functions to restrict induction of DNA methylation during viral
27 infection with EBV¹⁸.

1 We present here the first report of differential induction of the TET2 DNA demethylase in
2 coronavirus infection. It is of interest to determine whether interfering with TET2 function alters
3 outcomes in coronavirus infection, including in the novel SARS-CoV-2 (“COVID-19”).
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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. Coronavirus disease (COVID-19) pandemic. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>.
3. Selinger, C., Tisoncik-Go, J., Menachery, V.D., Agnihothram, S., Law, G.L., Chang, J., Kelly, S.M., Sova, P., Baric, R.S. and Katze, M.G., 2014. Cytokine systems approach demonstrates differences in innate and pro-inflammatory host responses between genetically distinct MERS-CoV isolates. *BMC genomics*, 15(1), p.1161.
4. GSE100509. Baric R, Sims A, Heller N, Waters KM, Einfeld AJ, Kawaoka Y. PNNL. 902 Battelle Blvd. Richland, USA.
5. Regla-Nava, J.A., Nieto-Torres, J.L., Jimenez-Guardeño, J.M., Fernandez-Delgado, R., Fett, C., Castaño-Rodríguez, C., Perlman, S., Enjuanes, L. and DeDiego, M.L., 2015. Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates. *Journal of virology*, 89(7), pp.3870-3887.
6. Tatura, A.L., Whitmore, A., Agnihothram, S., Schäfer, A., Katze, M.G., Heise, M.T. and Baric, R.S., 2015. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *MBio*, 6(3), pp.e00638-15.
7. Danesh, A., Cameron, C.M., León, A.J., Ran, L., Xu, L., Fang, Y., Kelvin, A.A., Rowe, T., Chen, H., Guan, Y. and Jonsson, C.B., 2011. Early gene expression events in ferrets in response to SARS coronavirus infection versus direct interferon-alpha2b stimulation. *Virology*, 409(1), pp.102-112.
8. Poppe, M., Wittig, S., Jurida, L., Bartkuhn, M., Wilhelm, J., Müller, H., Beuerlein, K., Karl, N., Bhujju, S., Ziebuhr, J. and Schmitz, M.L., 2017. The NF-κB-dependent and-independent transcriptome and chromatin landscapes of human coronavirus 229E-infected cells. *PLoS pathogens*, 13(3), p.e1006286.
9. Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L. and Rao, A., 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*, 324(5929), pp. 930-935.

10. Chen, Q., Chen, Y., Bian, C., Fujiki, R. and Yu, X., 2013. TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature*, 493(7433), pp.561-564.
11. Wang, L., Ozark, P.A., Smith, E.R., Zhao, Z., Marshall, S.A., Rendleman, E.J., Piunti, A., Ryan, C., Whelan, A.L., Helmin, K.A. and Morgan, M.A., 2018. TET2 coactivates gene expression through demethylation of enhancers. *Science advances*, 4(11), p.eaau6986.
12. Koh, K.P., Yabuuchi, A., Rao, S., Huang, Y., Cunniff, K., Nardone, J., Laiho, A., Tahiliani, M., Sommer, C.A., Mostoslavsky, G. and Lahesmaa, R., 2011. Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. *Cell stem cell*, 8(2), pp.200-213.
13. Ko, M., Huang, Y., Jankowska, A.M., Pape, U.J., Tahiliani, M., Bandukwala, H.S., An, J., Lamperti, E.D., Koh, K.P., Ganetzky, R. and Liu, X.S., 2010. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*, 468(7325), pp.839-843.
14. Moran-Crusio, K., Reavie, L., Shih, A., Abdel-Wahab, O., Ndiaye-Lobry, D., Lobry, C., Figueroa, M.E., Vasanthakumar, A., Patel, J., Zhao, X. and Perna, F., 2011. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer cell*, 20(1), pp.11-24.
15. Zhang, Q., Zhao, K., Shen, Q., Han, Y., Gu, Y., Li, X., Zhao, D., Liu, Y., Wang, C., Zhang, X. and Su, X., 2015. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature*, 525(7569), pp.389-393.
16. Ichiyama, K., Chen, T., Wang, X., Yan, X., Kim, B.S., Tanaka, S., Ndiaye-Lobry, D., Deng, Y., Zou, Y., Zheng, P. and Tian, Q., 2015. The methylcytosine dioxygenase Tet2 promotes DNA demethylation and activation of cytokine gene expression in T cells. *Immunity*, 42(4), pp.613-626.
17. Ma, S., Wan, X., Deng, Z., Shi, L., Hao, C., Zhou, Z., Zhou, C., Fang, Y., Liu, J., Yang, J. and Chen, X., 2017. Epigenetic regulator CXXC5 recruits DNA demethylase Tet2 to regulate TLR7/9-elicited IFN response in pDCs. *Journal of Experimental Medicine*, 214(5), pp. 1471-1491.
18. Namba-Fukuyo, H., Funata, S., Matsusaka, K., Fukuyo, M., Rahmutulla, B., Mano, Y., Fukayama, M., Aburatani, H. and Kaneda, A., 2016. TET2 functions as a resistance factor against DNA methylation acquisition during Epstein-Barr virus infection. *Oncotarget*, 7(49), p.81512.

Rank	ID	p-value	F	Gene	Gene name
285	A_33_P3238685	3.32E-24	726.1898	TET2	tet methylcytosine dioxygenase 2

Table 1: TET2 is among the genes most 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	FC	Gene	Gene name
60	A_23_P350187	2.13E-13	211.1306	<u>3hr</u> : 0.9906 ± 0.0070 <u>7hr</u> : 1.0173 ± 0.0165 <u>12hr</u> : 1.0668 ± 0.0027 <u>18hr</u> : 1.1687 ± 0.0079 <u>24hr</u> : 1.2523 ± 0.0046	TET2	tet methylcytosine dioxygenase 2

Table 2: TET2 is among the genes most 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
18028	A_51_P340083	1.9E-02	-2.77373	-4.992566	Tet2	tet methylcytosine dioxygenase 2

Table 3: TET2 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
24089	38798	1.74E-01	1.50	-6.3649	Tet2	tet oncogene family member 2

Table 4: TET2 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	F	Gene	Gene name
1087	CfaAffx.16978.1.S1_s_at	1.68E-05	6.73E+01	TET2	tet methylcytosine dioxygenase 2

Table 5: TET2 is differentially expressed in the lungs of ferrets 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 lung tissue from non-infected ferrets and ferrets at 2 days post-infection, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
4091	CfaAffx.16981.1.S1_at	6.77E-02	2.183217	-3.9233	TET2	tet methylcytosine dioxygenase 2

Table 6: TET2 is not differentially expressed in the blood of ferrets 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 blood from non-infected ferrets (day 0) and ferrets at 2 days post-infection, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
3180	62031	0.037879	2.6102675	-3.459	TET2	tet methylcytosine dioxygenase 2

Table 7: TET2 is 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.

Figure 1: Transcriptional induction of TET2 following MERS-CoV London infection in CALU3 2B4 cells.

TET2 mRNA expression level is graphically represented at 0 hours (baseline), 3 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection, with mean mRNA expression level marked and *p*-value denoting statistical significance.