Pan-cancer analysis of RNA expression of ANGIOTENSIN-CONVERTING ENZYME II (ACE2) and cellular proteases reveals high variability and possible impact on COVID-19 clinical outcomes

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Article
Abstract

Patients with cancer demonstrate particularly poor outcomes from COVID-19. To provide information essential for understanding the biologic underpinnings of this association, we analyzed whole-transcriptome RNA expression data obtained from a large cohort of cancer patients to characterize expression of ACE2, TMPRSS2, and other proteases that are involved in viral attachment to and entry into target cells. We find substantial variability of expression of these factors across tumor types and identify subpopulations expressing ACE2 at very high levels. In some tumor types, especially in gastrointestinal cancers, expression of ACE2 and TMPRSS2 is highly correlated. Furthermore, we found infiltration with T-cell and natural killer (NK) cell infiltration to be particularly pronounced in ACE2-high tumors. These findings suggest that subsets of cancer patients exist with heightened susceptibility to SARS-CoV-2 infection in whom malignant tumors function as viral reservoir and possibly promote the frequently detrimental hyper-immune response in patients infected with this virus.

Introduction

The new severe acute respiratory syndrome coronavirus, SARS-CoV-2, has led to a devastating pandemic affecting large segments of the global population with 11,159,301 confirmed cases and at least 527,827 deaths as of July 4, 2020\(^1\). The disease resulting from SARS-CoV-2 infection, COVID-19, shows particularly severe courses in men and the elderly, a relationship that is further aggravated by comorbidities\(^2\). Malignant diseases are comorbidities of interest in this context, as cancer patients, whose frequent treatment with immune-suppressive drugs might add to their vulnerability, have demonstrated particularly severe courses of the disease\(^3,4\). Despite the similarities of SARS-CoV-2 to other coronaviruses\(^5–7\), the biologic underpinnings of increased COVID-19 morbidity remain unclear.

SARS-CoV-2 utilizes the angiotensin-converting enzyme-II (ACE2) cell surface receptor for host cell entry\(^8\). Interaction with ACE2 is mediated by the viral spike protein, which undergoes proteolytic pre-activation, or priming, by cellular proteases TMPRSS2 and cathepsins (CTSB/CTSL)\(^5\). The SARS-CoV-2 spike protein also contains a novel cleavage site for the proprotein convertase FURIN that is absent in SARS-CoV\(^6\). FURIN cleavage of the SARS-CoV-2 can preactivate the spike protein for cell entry, and TMPRSS2 and CTSB/CTSL have cumulative effects with FURIN on SARS-CoV-2 entry\(^9\). Interestingly, increased expression of ACE2 and FURIN have also been observed in oral epithelial cells\(^10\).

Non-small cell lung cancer (NSCLC) patients infected by SARS-CoV-2 have a higher risk of severe symptoms and higher mortality rate than patients without\(^11,12\). As ACE2 expression is related to the severe acute respiratory syndromes induced by SARS-CoV via mediating the production of cytokines and the resultant adaptive immune response\(^13,14\), we examined transcriptomic markers of immune and stromal cell populations in the tumor microenvironment (TIME) of NSCLC specimens\(^15\). Understanding how the tumor microenvironment changes in NSCLC as ACE2 expression increases may provide additional insight into the associated immune system deregulation that may underlie the severe
comorbidities observed in NSCLC patients and may provide additional insight into an effective treatment for those suffering from COVID-19.

Initial data suggest that some cancers express high levels of ACE2\textsuperscript{16, 17}. In such cases, malignant masses could function as viral reservoirs, sustaining and amplifying SARS-CoV-2 infection in patients with cancer. Additionally, increased co-expression of cellular proteases with ACE2 may further identify subsets of cancer patients with increased vulnerability to infection\textsuperscript{18, 19}. Here, we endeavored to systematically characterize ACE2 and key cellular protease gene expression in normal and malignant tissues, taking advantage of a large database of tumors profiled by whole-transcriptome sequencing (WTS). We hypothesized that ACE2 and protease gene expression would vary across tumor types, with high expression presumed to associate with increased risk of infection and severe course of disease. We found that ACE2 expression varies and correlates with expression of proteases in a tumor type-specific manner, which may underlie the severe disease progression observed in patients with cancer.

**Methods**

As part of routine comprehensive molecular profiling of cancer samples at the Caris Life Sciences laboratory, a NextGen RNA sequencing panel was utilized that uses a hybrid bait-capture method to pull down RNA transcripts and sequence them. FFPE biopsies are obtained by the lab, sliced to 0.7 mm by microtome, and mounted on slides. Tumor tissue is identified by pathologists and microdissected. Tumor percentage is estimated. RNA preparation and cDNA reverse transcription follows manufacturer's guidelines (Qiagen). Library prep is standard for Illumina NextSeq two-color sequencing. RNA preparation follows manufacturer's guidelines. RNA transcripts are pulled down by the Agilent V7 Whole Exome bait panel. The bait panel is the Agilent SureSelect Whole Human Exome hg19 V7. For their whole-exome panel they avoided pseudogenes and highly redundant genes, resulting in 22,192 annotated transcripts. The RNA-Seq assay sequences the captured exonic mRNAs and assembles them onto the hg19 genome scaffold by STAR Aligner\textsuperscript{20}.

The Illumina NovaSeq 6500 was used to sequence the whole transcriptome from patients to an average of 30M paired end reads. Raw data was demultiplexed by Illumina Dragen BioIT accelerator, trimmed, counted, PCR-duplicates removed and aligned to human reference genome hg19 by STAR aligner\textsuperscript{20}. NGS RNA sequencing captures 22,192 exonic regions. For transcription counting, transcripts per million molecules (TPM) was used (Salmon expression pipeline\textsuperscript{21}). TPM expression data were pulled from a relational database containing retrospective clinical sample information. In addition to the large cohort of tumor samples tested, a small panel of patients where non-tumor tissue was extracted and tested was examined for ACE2 expression. Pathway analysis was performed using KEGG pathway colored mapping on Z-scores of fold change between groups\textsuperscript{22}. All analyses were performed on deidentified, retrospective cases. As such, this research was covered under IRB Exemption, reviewed and determined by the Western Institutional Review Board (WIRB). Expression data were used to build data distributions, correlate cohorts, and examine gene expression outliers, and perform statistical tests. Statistical analyses were
performed using R version 3.6.1. Pearson’s product moment correlations were calculated and associated p-values generated via the cor.test function in the stats package.

Results

Patient and tumor characteristics and correlations with ACE2 expression

A total of 38,628 tumors specimens, representing 36 tumors types, that underwent comprehensive molecular profiling at Caris Life Sciences between 2019 and 2020 were retrospectively reviewed. Molecular profiles of adjacent normal (non-cancerous) tissue from 127 tumor specimens, representing 24 tumor types, were reviewed as a control. Key demographic characteristics of these cohorts are shown in Table. Average ACE2 expression was significantly increased in patients under 65 years compared to patients 65 years and older (5.8 vs 4.8 TPM, p<0.0001), and male patients exhibited significantly higher average ACE2 expression compared to female patients (6.1 vs 4.8 TPM, p<0.0001). Pathway analysis shows that ACE2 is the most enriched gene in the renin-angiotension pathway in male gender and underexpressed in patients older than 55 years (Supplementary Figure 1, 2)

ACE2 expression across tumor types

Among the 36 tumor types investigated, median ACE2 expression was increased in 9 tumor types compared to normal tissue (0.99 TPM), with the highest ACE2 expression observed in colorectal (7.08 TPM) and kidney (5.35 TPM) cancers (Figure 1). In addition to colorectal cancer, ACE2 expression in other gastrointestinal (GI) cancers, including small intestinal (2.62 TPM), gastric (2.04 TPM), cholangiocarcinoma (1.92 TPM), esophageal/esophagogastric junction (1.29 TPM), and pancreatic (1.11 TPM) cancers, was increased relative to normal tissue. While non-small cell lung cancer (NSCLC) exhibited slightly decreased median ACE2 expression (0.95 TPM) compared to normal tissue, a subset of NSCLC tumors (N=64, 0.85%) demonstrated severely increased expression (>50 TPM). The lowest ACE2 expression was observed in melanoma (0.19 TPM), mesothelioma (0.18 TPM), and meningioma (0.16 TPM).

Protease gene expression across tumor types

Entry of SARS-CoV-2 into human cells is facilitated by priming of the viral spike protein by cellular proteases (Hoffman et al., 2020). While inhibition of the serine protease TMPRSS2 blocks SARS-CoV-2 infection of lung cells (Hoffman et al., 2020), other proteases, including cathepsin B and L (CTSB/CTSL) and FURIN, mediate spike protein priming and have cumulative effects with TMPRSS2 on viral entry (Shang et al. 2020). Median TMPRSS2 expression was increased in 16 tumor types, including NSCLC (10.35 TPM), compared to normal tissue (3.82 TPM), most notably in prostate cancer (265.43 TPM) (Figure 2). Similar to ACE2 expression, increased TMPRSS2 expression was observed in many GI cancers (colorectal: 44.02 TPM, gastric: 40.12 TPM, small intestinal: 35.69 TPM, esophageal/esophagogastric junction: 31.16 TPM, pancreatic: 17.96 TPM, cholangiocarcinoma: 14.75 TPM, liver: 9.55 TPM, and anal: 6.78 TPM). CTSB, CTSL, and FURIN expression was increased in most tumor types relative to normal
tissue. CTSB expression was highest in thyroid cancer (1287.97 TPM; normal: 166.61 TPM), CTSL expression was highest in gastrointestinal stromal tumors (376.26 TPM; normal: 30.36 TPM), and FURIN expression was highest in liver cancer (55.37 TPM; normal: 16.89 TPM).

**Co-expression of ACE2 and key protease genes across tumor types**

We examined the co-expression of ACE2 with key protease genes to evaluate the correlation between ACE2 and protease gene expression (Figure 3). In normal tissue, the correlation with ACE2 expression was strongest for TMPRSS2 (Pearson’s correlation \( r=0.44 \), slope \( m=0.95 \)) compared to CSTB \( (r=0.09, m=0.09) \), CSTL \( (r=-0.10, m=0.05) \), and FURIN \( (r=-0.04, m=0.14) \). Interestingly, most tumor types exhibited weaker ACE2/TMPRSS2 and ACE2/CTSB correlations compared to normal tissue, whereas the ACE2/CTSL and ACE2/FURIN correlations in normal tissue are weaker than that of most cancer types ([include Supplementary table of correlation coefficients]). Tumor type-specific deviations from the primary ACE2/TMPRSS2 cluster were observed for prostate \( (r=-0.05, m=0.06) \) and GI cancers \( (r=0.27, m=0.35) \), while expression in NSCLC tumors \( (r=0.07, m=0.46) \) largely overlapped with the primary cluster. Tumor type-specific deviations in ACE2/protease co-expression were less prominent for CTSB, CTSL, and FURIN compared to TMPRSS. Notably, ACE2/protease co-expression in GI cancers deviated from the primary cluster due to increased ACE2 expression (CTSB: \( r=-0.02, m=-0.0001 \); CTSL: \( r=-0.04, m=-0.02 \); FURIN: \( r=0.03, m=0.02 \)). Together, this suggests the co-expression of ACE2 and protease genes may be more predictive of susceptibility to viral infection by SARS-CoV-2 than ACE2 expression alone, with GI cancers exhibiting the highest potential susceptibility.

**ACE2 expression and association with cell population abundance in the TME in NSCLC**

In addition to its function as a receptor for SARS-CoV-2, ACE2 is suggested to be involved in various aspects of post-infection processes, and the expression of ACE2 in lung has been shown to associate with innate and acquired immune responses\(^{23}\). We used the Microenvironment Cell Populations (MCP)-counter\(^{24}\) to estimate cell population abundance in the TME, inferred by RNA expression tested by WTS in NSCLC tumors of adenocarcinoma histology. Consistent with the previous observation of ACE2 expression level correlating with neutrophils, NK cells and various Th cells in SARS-CoV infected cells\(^{23}\), we observed in NSCLC tumor tissues significant positive correlations of ACE2 expression with T cells, NK cells, B cells, monocytic lineage, dendritic cells, neutrophils, as well as endothelial cells \( (p<0.0001) \), Figure 4.

**Discussion**

The study presented here provides, to our knowledge, the first systematic assessment of RNA expression of key molecules involved in the infectious process leading to COVID-19 in advanced cancer using data from a large cohort of patients who have undergone routine molecular profiling of their malignancies. We found extensive variability of expression of the main receptor for SARS-CoV-2, ACE2, in particular in NSCLC and gastrointestinal cancers. Our findings are of immediate relevance for an improved
understanding of the dynamics of the current COVID-19 pandemic since recent studies highlight the particular vulnerability of patients with cancer in terms of increased mortality but also worse severity of the disease\textsuperscript{3,4}. While there are manifold potential pathophysiologic underpinnings for this concerning finding, including immune-suppression and smoking history, differences in expression of the primary viral receptor in normal and malignant tissues might contribute. Knowledge of such systematic differences could lead to improved risk prediction for patients with cancer.

Our analysis of normal tissue samples demonstrates significantly higher ACE2 levels in males and in patients of age less than 55 compared to females and older patients, respectively. This finding mirrors the clinical observation of increased mortality and morbidity in male patients in Asian and Western populations while not in agreement with the worse outcomes from the infection in older patients\textsuperscript{2,25,26}. Thus, it is conceivable that differences in expression levels of ACE2 in normal tissues contribute to differences in risk of infection and disease severity in men but also highlight the possible impact of co-morbidities in the elderly. Interestingly, multiple components of the renin-angiotensin system appear to be co-regulated with ACE2 in normal tissues, indicating that there is a potential complex interplay between the infection and pre-existing conditions, which might contribute to the worse outcome for patient with hypertension and other cardiovascular diseases\textsuperscript{25}.

Examination of our extensive database of molecular profiles obtained from patients with advanced malignancies revealed wide-ranging variability in expression of ACE2 in samples from advanced cancers. Highest ACE2 RNA expression levels were observed in colorectal, renal, and NSCLC cancers as well as in cholangio- and gastric carcinomas. These findings are in agreement with previous observations in the TCGA dataset, which consists mostly of data from resected, non-metastatic tumors\textsuperscript{17} while our dataset is overwhelmingly derived from patients with advanced cancers. Our analysis of expression levels of several proteases known to function as coreceptors demonstrates variable correlation with ACE2. Correlation of ACE2 expression with protease expression was particularly pronounced in GI cancers suggesting these patients may have heightened susceptibility for SARS-CoV-2 infection and a more severe course of COVID-19. From a clinical perspective, of particular interest is our finding that subgroups of patients with intensely increased ACE2 expression levels exist in several cancer types, including NSCLC and breast cancers, as well as in glioblastoma and melanoma. While, in general, little is known about the interaction of human viruses with malignant tumors, data from studies of oncolytic viruses demonstrate that viruses can replicate in malignant masses and sustain ongoing viremia if viral receptors are present on tumor cells\textsuperscript{27}. Thus, it is conceivable that malignant tumors with particularly high ACE2 and protease expression function as viral reservoirs leading to sustained viremia and increased disease severity. This hypothesis needs to be tested clinically since it would suggest intensified isolation measures or even prophylactic antiviral treatment for patients with tumors that express ACE2 at very high level. In addition, our finding of increased presence of various immune-cell lineages, including T cells and NK cells, in tumors with high ACE2 expression highlights the possibility that such tumors are not only susceptible to SARS-CoV-2 infection but could also promote the severe inflammatory response observed in many patients with COVID-19. On the other hand, intratumoral injection of flu vaccine can promote conversion
“cold” tumors to hot, generates systemic CD8+ T cell-mediated antitumor immunity, and sensitizes resistant tumors to checkpoint blockade. Thus, one could speculate that in some cases SARS-CoV-2 infection might promote tumor regression in the context of immune checkpoint inhibitors.

In summary, our investigation demonstrates significant differences in ACE2 and protease expression in normal and malignant tissues with a subgroup of patients expressing very high levels of ACE2 RNA. These findings, together with the increased presence of inflammatory cells in tumors displaying high ACE2 levels might contribute to the developing complex pathophysiologic picture of COVID-19 and help guide prophylactic measures in patients with solid malignancies.

References

1. 10.1016/S1473-3099(20)30120-1
   Dong E, Du H, Gardner L: An interactive web-based dashboard to track COVID-19 in real time [Internet]. The Lancet Infectious Diseases 20:533–534, 2020Available from: http://dx.doi.org/10.1016/S1473-3099(20)30120-1
2. 10.1371/journal.ppat.1008520
   Meng Y, Wu P, Lu W, et al: Sex-specific clinical characteristics and prognosis of coronavirus disease-19 infection in Wuhan, China: A retrospective study of 168 severe patients [Internet]1–13, 2020Available from: http://dx.doi.org/10.1371/journal.ppat.1008520
3. Liang W, Guan W, Chen R, et al: Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. The Lancet Oncology 21:335–337, 2020
4. Dai M-Y, Liu D, Liu M, et al: Patients with Cancer Appear More Vulnerable to SARS-CoV-2: A Multi-Center Study During the COVID-19 Outbreak. SSRN Electronic Journal, 2020
5. Hoffmann M, Kleine-Weber H, Schroeder S, et al: SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 181:271–280.e8, 2020
6. Alexandra C. Walls, Young-Jun Park, M. Alejandra Tortorici, Abigail Wall, Andrew T. McGuire DV: Structure, Function, and Antigenicity of the SARS-. Cell, 2020
7. Wang Q, Zhang Y, Wu L, et al: Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell 181:894–904.e9, 2020
8. 10.1038/s41586-020-0212-7
   Zhou P, Yang X lou, Wang XG, et al: A pneumonia outbreak associated with a new coronavirus of probable bat origin [Internet]. Nature 579:270–273, 2020Available from: http://dx.doi.org/10.1038/s41586-020-0212-7
9. Shang J, Wan Y, Luo C, et al: Cell entry mechanisms of SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America 117, 2020
10. 10.1038/s41368-020-0074-x
    Xu H, Zhong L, Deng J, et al: High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa [Internet]. International Journal of Oral Science 12:1–5, 2020Available from:
11. Kong Q, Xiang Z, Wu Y, et al: Analysis the Susceptibility of Lung Cancer Patients to SARS-CoV-2 Infection. SSRN Electronic Journal 1–5, 2020

12. Rogado J, Pangua C, Serrano-Montero G, et al: Covid-19 and lung cancer: A greater fatality rate? Lung Cancer 146:19–22, 2020

13. He L, Ding Y, Zhang Q, et al: Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2 + cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. [Internet]. The Journal of pathology 210:288–97, 2006Available from: http://www.ncbi.nlm.nih.gov/pubmed/17031779

14. Rockx B, Baas T, Zornetzer GA, et al: Early Upregulation of Acute Respiratory Distress Syndrome-Associated Cytokines Promotes Lethal Disease in an Aged-Mouse Model of Severe Acute Respiratory Syndrome Coronavirus Infection. Journal of Virology 83:7062–7074, 2009

15. Bernstein KE, Khan Z, Giani JF, et al: Angiotensin-converting enzyme in innate and adaptive immunity [Internet]. Nature Reviews Nephrology 14:325–336, 2018Available from: http://dx.doi.org/10.1038/nrneph.2018.15

16. Li Y, Xu Q, Ma L, et al: Systematic profiling of ACE2 expression in diverse physiological and pathological conditions for COVID-19/SARS-CoV-2. [Internet]. Journal of cellular and molecular medicine 1–5, 2020Available from: http://www.ncbi.nlm.nih.gov/pubmed/32639084

17. Chai P, Yu J, Ge S, et al: Genetic alteration, RNA expression, and DNA methylation profiling of coronavirus disease 2019 (COVID-19) receptor ACE2 in malignancies: a pan-cancer analysis. [Internet]. Journal of hematology & oncology 13:43, 2020Available from: http://www.ncbi.nlm.nih.gov/pubmed/32366279

18. Katopodis P, Anikin V, Randeva H, et al: Pancancer analysis of transmembrane protease serine 2 and cathepsin L that mediate cellular SARSCoV2 infection leading to COVID-19. International Journal of Oncology 2:533–539, 2020

19. Drak Alsibai K: Expression of angiotensin-converting enzyme 2 and proteases in COVID-19 patients: A potential role of cellular FURIN in the pathogenesis of SARS-CoV-2. Medical Hypotheses 143, 2020

20. Dobin A, Davis CA, Schlesinger F, et al: STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 29:15–21, 2013

21. Patro R, Duggal G, Love MI, et al: Salmon14:417–419, 2017

22. Kanehisa M, Goto S: KEGG: kyoto encyclopedia of genes and genomes. [Internet]. Nucleic acids research 28:27–30, 2000Available from: http://www.ncbi.nlm.nih.gov/pubmed/10592173

23. Li G, He X, Zhang L, et al: Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19 [Internet]. Journal of Autoimmunity 102463, 2020Available from: https://doi.org/10.1016/j.jaut.2020.102463

24. 10.1186/s13059-016-1070-5
Becht E, Giraldo NA, Lacroix L, et al: Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression [Internet]. Genome Biology 17:1–20, 2016Available from: http://dx.doi.org/10.1186/s13059-016-1070-5

25. Garg S, Kim L, Whitaker M, et al: Hospitalization Rates and Characteristics of Patients Hospitalized with Morbidity and Mortality Weekly Report 69:458–464, 2020

26. 10.1101/2020.03.25.20037721v2
Hu L, Chen S, Fu Y, et al: Risk Factors Associated with Clinical Outcomes in 323 COVID-19 Patients in Wuhan, China [Internet]. medRxiv 2020.03.25.20037721, 2020Available from: https://www.medrxiv.org/content/10.1101/2020.03.25.20037721v2

27. 10.1016/j.jmthe.2017.03.026
Russell SJ, Peng KW: Oncolytic Virotherapy: A Contest between Apples and Oranges [Internet]. Molecular Therapy 25:1107–1116, 2017Available from: http://dx.doi.org/10.1016/j.jmthe.2017.03.026

28. Newman JH, Chesson CB, Herzog NL, et al: Intratumoral injection of the seasonal flu shot converts immunologically cold tumors to hot and serves as an immunotherapy for cancer. Proceedings of the National Academy of Sciences of the United States of America 117:1119–1128, 2020

Table

| Characteristic         | Cancer |                        | Normal |                        | p-value |
|------------------------|--------|-------------------------|--------|-------------------------|---------|
|                        | N samples | Mean | Median | Range | p-value | N samples | Mean | Median | Range | p-value |
| **Gender**             |         |      |       |       |         |         |      |       |       |         |
| Female                 | 22493   | 4.797 | 0.74491 | 0.006422-3385.94 | <0.0001 | 75   | 3.527 | 0.737993 | 0-102.946 | 0.02 |
| Male                   | 16135   | 5.999 | 0.88101 | 0.007438-833.672 |         | 52   | 21.98 | 1.587625 | 0-409.937 |       |
| **Age Group (years)**  |         |      |       |       |         |         |      |       |       |         |
| <65                    | 19944   | 5.802 | 0.804511 | 0.006422-3385.94 | <0.0001 | 70   | 8.624 | 1.231935 | 0-202.582 | 0.24 |
| ≥65                    | 18684   | 4.762 | 0.790774 | 0.007306-1495.36 |         | 57   | 14.1 | 0.94495 | 0-409.937 |       |
| **Smoking Status**     |         |      |       |       |         |         |      |       |       |         |
| Current Smoker         | 1039    | 3.486 | 0.784241 | 0.008451-204.511 |         | 3    | 6.496 | 0.567184 | 0.125167-18.794 | 0.42 |
| Lifelong Non-smoker    | 322     | 3.379 | 0.9901145 | 0.008251-105.225 | 0.029    | 0    | NA   | NA   | NA   |         |
| Light Smoker (<15 packs/yr) | 2479 | 4.13 | 0.900375 | 0.008565-549.111 | 0.042    | 6   | 69.06 | 1.005194 | 0.091297-409.937 |       |
| Not Reported           | 34788   | 5.454 | 0.7865375 | 0.006422-3385.94 |         | 118  | 8.252 | 1.02695 | 0-208.806 |       |

Table 1: ACE2 expression associated with patient demographics of normal control and cancer cohorts. P-values were calculated using the Kruskal-Wallis rank sum test for numeric variables and Pearson's Chi-squared test for categorical variables.

Supplementary Table

A supplementary table was not included with this version.

Figures
Figure 1

ACE2 gene expression in normal and cancerous tissues. Box plots indicate the median and upper/lower quartiles, with individual data points representing outlier values greater than 1.5x the interquartile range above or below the upper or lower quartile, respectively.
Figure 2

Protease gene expression in normal and cancerous tissues. Box plots indicate the median and upper/lower quartiles, with individual data points representing outlier values greater than 1.5x the interquartile range above or below the upper or lower quartile, respectively.
Figure 3

Co-expression of ACE2 and protease genes in normal and cancerous tissues. Data points are color-coded for normal (red), gastrointestinal cancers (purple), NSCLC (cyan), prostate cancer (green), and all other cancer types (black). Regression lines represent log-transformed fits.
### Figure 4

| Cell populations               | ACE2 Expression Quartile Groups | p value (Q4 vs. REST) |
|-------------------------------|---------------------------------|-----------------------|
|                               | Q1 (N=750)                      | Q2 (N=750)            | Q3 (N=749) | Q4 (N=750) |<0.0001 | 0.0013 | 0.6944 | <0.0001 | 0.0001 | <0.0001 | <0.0001 | 0.8858 |
| T cells                       | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| CD8 T cells                   | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| Cytotoxic lymphocytes         | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| NK cells                      | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| B lineage                     | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| Monocytic lineage             | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| Myeloid dendritic cells       | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| Neutrophils                   | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| Endothelial cells             | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |
| Fibroblasts                   | Blue                            | Blue                  | Blue       | Blue       | Red     | Red     | Red      | Red     | Red     | Red     | Red     | Red     | White |

### Figure 4

Association between ACE2 expression and estimated immune cell infiltration in NSCLC tumors. MCP-counter results for immune and stromal cell population abundance in subgroups based on ACE2 expression. Heatmap indicates relative cell population abundance, with values color-coded by maximum (red), minimum (blue), and median (white).

### Supplementary Files

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