

Expression and clinical significance of SARS-CoV-2 human targets in lung tissues: normal, primary tumor and metastasis

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Abstract

Background: The recent COVID-19 outbreak in China led to a worldwide pandemic associated with a severe acute respiratory illness. A higher incidence of COVID-19 infection was demonstrated in cancer patients, including patients with lung cancer. This study was conducted to get insights into the reasons for this enhanced frequency of COVID-19 infection.

Methods: Using different bioinformatic tools, the expression and methylation pattern of ACE2 and TMPRSS2 gene were analyzed in healthy and malignant tissues with a focus on lung adenocarcinoma (LUAD) and correlated to clinical parameters and smoking history.

Results: ACE2 and TMPRSS2 were heterogeneously expressed across 36 healthy tissues with the highest expression in digestive, urinary and reproductive organs, while their expression was significantly lower in 36 cancer tissues. In LUAD, ACE2, but not TMPRSS2 was overexpressed, which inversely correlated to the promoter methylation. An age-dependent upregulation of ACE2 expression was found in LUAD compared to normal lung tissues. In a healthy lung, TMPRSS2 expression was dependent on sex and smoking history and downregulated in LUAD of smokers. Cancer progression was associated with decreased TMPRSS2, but unaltered ACE2 expression, while ACE2 expression in lung metastases of different cancers was higher than in metastasis of other sites. TMPRSS2, but not ACE2 expression, was associated with LUAD patients' survival.

Conclusions: Comprehensive molecular analyses revealed a heterogeneous, distinct expression and methylation profile of ACE2 and TMPRSS2 in healthy lung vs LUAD tissues across sex, age and smoking history, which is associated with clinical parameters and might have implications for COVID-19 disease.

Key words: COVID-19, metastasis, lung adenocarcinoma, ACE2, TMPRSS2, clinical relevance

Background

Human coronaviruses (CoV) were described for the first time in 1960 and infections were associated with respiratory diseases [1-3], such as severe acute respiratory syndrome (SARS)-CoV [4] and the Middle East respiratory syndrome (MERS)-CoV [5]. Recently, a novel CoV belonging to the Betacoronavirus subfamily [6, 7], named SARS-CoV2/COVID-19, was identified in December 2019 in Wuhan, China [8-10]. Due to its high human-to-human transmission rate, it quickly spread across the world and has developed as a worldwide respiratory viral pandemic [11-13].

Infection with SARS-CoV, MERS-CoV and COVID-19 caused a severe acute respiratory illness with a mild to severe patients' outcome. In patients with severe disease manifestation, diffuse alveolar damage with severe capillary congestion was detected, suggesting a vascular dysfunction and inflammation [14, 15].

COVID-19 enters the cell via the angiotensin-converting enzyme 2 (ACE2), while the host transmembrane protease serine 2 (TMPRSS2) is used for COVID-19 priming [16], but other proteases also facilitate the COVID-19 entry [17, 18].

There exist several reports on an increased susceptibility and incidence of cancer patients to COVID-19 infection compared to the overall population [19-24], in particular in patients older than 60 years with lung carcinoma as the most common [25]. Upon infection, cancer patients often display more severe disease courses with multiple organ dysfunctions [22], higher mortality and intensive care unit admission [24]. Furthermore, smokers have a higher incidence and severity of COVID-19 infection than non-smokers [26]. This might be associated with structural alterations, e.g., fibrosis leading to changes in lung architecture and intra- and peritumoral microenvironment and inflammation due to tobacco-related lung damage and lung cancer [26].

Profiling of ACE2 and TMPRSS2 expression and their distribution across different cell types in lung tissues and in cells derived from subsegmental bronchial branches demonstrated high expression levels of TMPRSS2 in all the tissues, whereas ACE2 was heterogeneously expressed in the transient secretory cell type of the subsegmental bronchial branches suggesting that bronchial branches might be more vulnerable for COVID-19 infection [27]. ACE2 expression significantly differed between lung cancer subtypes. A decreased ACE2 expression was found in all non-small cell lung cancer (NSCLC) tissues when compared to healthy control, which negatively correlated with the intensity of neoangiogenesis and sensitivity to cytostatics of tumor cells [28, 29]. Concerning NSCLC subentities, ACE2 expression was significantly upregulated in lung adenocarcinoma (LUAD) and remained unchanged in lung squamous cell carcinoma [30].

In order to search for factors to predict susceptibility or severity of COVID-19 infection in cancer patients, the expression patterns of ACE2 and TMPRSS2 were compared across 36 different human healthy and cancer tissues with a focus on healthy lung and LUAD tissues and associated to the methylation pattern, sex, age, clinical parameters and smoking history using different bioinformatics tools.

Methods

Dataset selection

Datasets were collected from gene expression omnibus (GEO), The Cancer Genome Atlas (TCGA), and individual publications. The analysis of GEO was performed by GENT2 [31]. For datasets not been deposited into GEO, the selection was performed by a literature search and by referencing other commonly used databases.

Analysis of gene expression patterns

Expression data were downloaded from GENT2 system, a publicly accessible online cancer microarray database, which provides a landscape of gene expression profile across 72 normal and tumor tissues. The values of the ACE2 and TMPRSS2 mRNA expression were before analysis in normal and cancer tissues (log2-transformed) and then subjected to statistical comparison.

Meta-analysis

The Lung Cancer Explorer (LCE), an open-access web resource, is housing expression data and clinical data from more than 6700 patients in 56 studies [32]. Meta-analysis effectively combines the statistical strength from multiple data sets, which allows higher precision than using any of the single studies. Forest plots of ACE2 and TMPRSS2 were employed to summarize tumor – normal standardized mean difference for tumor vs normal meta-analysis.

UALCAN database analysis

UALCAN, a web resource for analyzing cancer OMICS data (<http://ualcan.path.uab.edu/index.html>) [33], used the TCGA assembler for obtaining 'Primary Solid Tumor' and 'Solid Tissue Normal' data for each cancer. It is built on PERL-CGI and can be used to assess the methylation levels of different genes. ACE2 and TMPRSS2 expression and promoter methylation profiles were studied using UALCAN. In addition, UALCAN obtained patient data for LUAD from Genomic Data commons (GDC) (<https://gdc.cancer.gov/>) using GDC data transfer tool. Using UALCAN, a stratified analysis based on patients' age, patients' sex, individual cancer stages, tumor grade, smoking status and nodal metastasis status was performed.

Comparative analysis

A function of the LCE database allows comparing gene expression from different tissue types or samples originated from patients with different clinical features. Based on the ACE2 expression of normal lung tissues of the LUAD dataset [34-40], clinical features such as sex,

aging and smoking were compared. ACE2 and TMPRSS2 expression levels for the above-selected groups were then visualized in a boxplot.

Gene expression across cancer stages

HCMDB (Human Cancer Metastasis Database) is an integrated database designed to store and analyze large scale expression data of cancer metastasis [41]. A total of 124 previously published transcriptome datasets were collected from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA). ACE2 and TMPRSS2 transcriptomes were compared between lung tissues with others in various cancer metastasis.

Co-expression analysis

The cBioPortal online platform [42, 43] was used to query the TCGA lung adenocarcinoma (LUAD). In total, 566 tumor samples (from the TCGA database) with messenger RNA (mRNA) next-generation sequencing data were employed.

Statistics

All statistical tests provided, such as a two-sample t-test, log-rank test, and meta-survival analysis, were implemented using R with Bioconductor plugins [31, 32]. Co-expressed genes were predicted by cBioPortal online analysis with the Pearson correlation coefficient.

Student's t test was used to compare ACE2 and TMPRSS2 gene expression levels between males and females, younger (< 60 years) and older (> 60 years) individuals and between smokers and non-smokers in healthy and tumor tissues. P-values of less than 0.05 were considered statistically significant.

Results

Heterogeneous expression profile of COVID receptor and COVID infection associated gene in healthy tissues and tumors

A tissue-wide gene expression profile of ACE2 and TMPRSS2 demonstrated a heterogeneous expression of both genes among 36 human healthy tissues using the GLP570 data. ACE2 was highly expressed in gallbladder (12.48 ± 0.54), testis (11.06 ± 0.35), kidney (10.44 ± 0.08), colon (8.9 ± 0.06) and small intestine (8.18 ± 0.84) (Figure 1A), whereas TMPRSS2 showed higher expression levels in colon (10.83 ± 0.06), stomach (9.13 ± 0.18), prostate (8.94 ± 0.46), lung (8.22 ± 0.07), gallbladder (8.15 ± 0.22) and small intestine (7.90 ± 0.40) (Figure 1B). Similar to the distinct expression patterns in normal tissues, tissues of different cancer types also exhibited a heterogeneous expression of ACE2. The overall analysis of 72 paired tissues of 5487 normal tissue samples and 35806 cancer tissues demonstrated significantly lower expression of ACE2 in cancer tissues when compared to normal tissues (Figure 2A; Table 1). However, detailed analysis revealed that ACE2 was overexpressed in some tumor entities, including lung adenocarcinoma (LUAD) with a 0.301-fold expression difference between tumor and normal tissues ($p < 0.001$; logFC -0.299). A heterogeneous gene expression pattern was also found for TMPRSS2 with a significant downregulation of TMPRSS2 in lung cancer ($p < 0.001$; logFC -0.962) (Figure 2B; Table 1).

Next, meta-analyses based on the lung cancer explorer were performed for ACE2 and TMPRSS2 expression using the data sets available. Meta-analysis of 17 studies demonstrated a statistically significant higher ACE2 expression in LUAD ($p = 4.7e-07$; logFC 0.48) (Figure 3A), while TMPRSS2 expression ($p = 0.072$; logFC -0.45) inversely correlated in the meta-analysis of 7 studies (Figure 3B). Interestingly, the ACE2 (Figure 4A) and TMPRSS2 (Figure 4C) expression in healthy lung tissues as well as in LUAD was inversely correlated to the methylation pattern of the ACE2 (Figure 4B) or TMPRSS2 promoter (Figure 4D), respectively.

Dependence of ACE2 expression in normal lung and LUAD tissues on sex, age and smoking history

The dependence of ACE2 expression on sex, age and smoking history was determined in healthy individuals as well as in LUAD patients. Using the TCGA data set, ACE2 expression was higher in male than in female normal lung tissues ($p = 0.17$), which was confirmed by Oklahoma and coauthors ($p = 0.23$) [44]. In contrast, there existed no sex-dependent ACE2 expression in LUAD patients (Supplementary Figure 1A).

Since an age-dependent incidence of COVID-19 infection was reported [45], the ACE2 expression was assessed in both healthy lung tissues and LUAD tissues from individuals of < 60 years and older. In healthy lung tissues, the ACE2 expression was slightly, but not statistically significantly lower in individuals < 60 years ($p = 0.32$) (Figure 5A), whereas a

statistical upregulation of ACE2 expression ($p = 0.0049$) was detected in LUAD patients over 60 years (Figure 5B), while there is no alteration ($p = 0.88$) in ACE2 expression between normal and LUAD of < 60 years (Figure 5C); there was a significant higher expression in LUAD patients > 60 years compared to healthy control samples (Figure 5D).

Besides, a link between ACE2 expression and smoking history was investigated in healthy and LUAD tissues. The ACE2 expression was altered in normal lung tissues of smokers vs. non-smokers with higher levels of ACE2 expression in smokers (TCGA: $p = 0.067$ (Figure 5E); Okayama 2012: $p = 0.043$ (Figure 5F)), while a decreased ACE2 expression was found in LUAD tissues (TCGA: $p = 0.0064$ (Figure 5G); Okayama 2012: $p = 0.16$ (Figure 5H)) of smokers compared to that of non-smokers. Furthermore, a combination of higher age (> 60 years) and smoking history was correlated with ACE2 expression in LUAD tissues ($p = 0.004$) (Supplementary Figure 2A).

Dependence of TMPRSS2 expression in normal lung and LUAD tissues on sex, age and smoking history

Next to ACE2, the TMPRSS2 expression levels were analyzed in non-tumorous lung and LUAD tissues and compared to sex, age and smoking history. The TMPRSS2 expression was lower in females compared to males with a p-value of 0.24 in healthy tissues (Supplementary Figure 1C), while only slightly higher TMPRSS2 expression levels were seen in female LUAD tissues ($p = 0.0012$) (Supplementary Figure 1D). Concerning age dependence, in normal tissues the expression of TMPRSS2 was not altered during the age but increased in LUAD tissues with aging ($p = 0.071$) (Figure 6A, 6B). On the other hand, irrespective of age categories (0-59: $p = 1.4e-11$; >60 : $p = 8.2e-21$), a lower TMPRSS2 expression was found in LUAD compared to normal lung (Figure 6C, 6D).

Furthermore, an association of TMPRSS2 expression with the smoking history was found with a slight, but not significantly lower expression of TMPRSS2 expression in non-tumorous lung tissues (TCGA: $p = 0.71$ (Figure 6E); Okayama 2012: $p = 0.66$ (Figure 6F)), which was more pronounced when LUAD tissues of non-smokers vs smokers were compared (TCGA: $p = 0.0011$ (Figure 6G); Okayama 2012: $p = 0.0044$ (Figure 6H)). Interestingly, a combination of higher age (> 60 years) and smoking history was correlated with higher TMPRSS2 expression in LUAD patients ($p = 0.06$) (Supplementary Figure 2B).

Clinical relevance of ACE2 and TMPRSS2 expression in LUAD

In order to address whether the higher ACE2 expression in LUAD tissues might be of clinical relevance, the ACE2 expression was determined in different stages of LUAD using the UALCAN database. ACE2 was not differentially expressed (Figure 7A) or methylated (Figure 7B) in stage I tumors ($n = 277$) compared to stage III ($n = 85$) and stage IV ($n = 28$) tumors.

Furthermore, no significant differences in the ACE2 expression/methylation levels of lymph node metastasis (N1-N3) (Figure 7C, Figure 7D) and primary tumor LUAD without metastasis (NO) was detected. In contrast, ACE2 expression was higher in lung metastasis of other tumor entities, e.g., breast, prostate and liver carcinoma, than in metastasis of these malignancies in other locations, e.g., lymph node, brain, and liver (Table 2 and Supplementary Figure 3). Thus, the ACE2 expression levels were more pronounced in lung metastasis independent of the tumor type. In contrast to ACE2, TMPRSS2 was downregulated during disease progression from stage I to stage IV (Figure 7E), which was directly associated with enhanced promoter methylation of TMPRSS2 (Figure 7F). Likewise, the expression (Figure 7G) of TMPRSS2 in LUAD was more reduced coupled with increased methylation (Figure 7H) in metastasis compared to primary tumors.

Correlation of ACE2 and TMPRSS2 expression with patients' survival

To determine whether the level of ACE2 expression was associated with the overall survival (OS) of LUAD patients, meta-analyses were performed from 19 studies. No correlation of ACE2 expression with the OS of LUAD patients (HR: 0.95; $p = 0.26$) was found (Figure 8A). In contrast, a meta-analysis of TMPRSS2 expression from 21 studies demonstrated a significantly reduced OS with an HR of 0.77, $p < 0.01$ (Figure 8B) suggesting that TMPRSS2 might be a more suitable biomarker for OS than ACE2.

Discussion

Although it has been suggested that patients with cancer have a higher likelihood of being infected by COVID-19 [22], the current data available are insufficient to conclude due to the low number and heterogeneity of samples. In addition, most COVID-19 infected patients have mild disease [25]. Based on the risks of cancer progression, it is controversially discussed to withdraw cancer treatment causing immune suppression or decrease their dosages [46, 47]. Thus, there is an urgent need to identify predictive biomarkers and to improve the understanding of the different outcomes of COVID-19 infection in cancer patients.

While all types of malignancies seem to be associated with high COVID-19 prevalence, morbidity and mortality, in particular lung cancer has different cumulative risk factors for COVID-19 complications, including age, significant cardiovascular and respiratory comorbidities, smoking-related lung damage as well as treatment-related immune suppression. Out of 1524 patients with cancer, 228 (14.96%) patients had non-small cell lung carcinoma (NSCLC) with a higher incidence of COVID-19 infection in NSCLC patients older than 60 years [25].

Recently, ACE2 and TMPRSS2 have been postulated as candidate biomarkers and therapeutic targets of COVID-19 infection [48]. Analysis of ACE2 expression in normal human tissues demonstrated a moderate expression of both genes across cell tissues, but in particular in the lung, intestinal tract and male tissues [49]. This was confirmed in our study and extended to TMPRSS2. Furthermore, an inverse correlation between ACE2 and TMPRSS2 expression and methylation exists. The difference between ACE2 and TMPRSS2 expression levels in normal lung epithelium between males and females, younger and older individuals indicates that the infection might be associated with gender and age.

Using TCGA data, our study demonstrated a significantly decreased expression of ACE2 and TMPRSS2 in many cancer types compared to normal adjacent tissues (Figure 2; Table 1). However, ACE2 has been shown to be overexpressed in some cancers, including cervical, pancreatic, renal and lung carcinoma [50]. Detailed analysis revealed that ACE2 was overexpressed in LUAD, while TMPRSS2 expression was downregulated. The clinical relevance of the discordant expression of TMPRSS2 and ACE2 has not yet been identified. However, in the context of COVID-19 infection, LUAD patients with higher expression of ACE2 might have a higher risk to be infected. This is in line with a recent report demonstrating that lung tissues expressing higher ACE2 were more likely to be infected by COVID-19 [51]. In contrast to the human lung cancer samples, ACE2 overexpression attenuates the metastasis of lung cancer in a mouse model [52]. Interestingly, TMPRSS2 downregulation, but not ACE2 upregulation was more pronounced in metastasis and correlated with a reduced LUAD

patients' survival suggesting that the level of TMPRSS2 expression plays an important role for the outcome of LUAD patients independently of COVID-19 infection compared to ACE2.

For COVID-19-infected patients, a regular surveillance including monitoring oxygen saturations, occurrence of infection, chemotherapy-induced neutropenia and hospital admission should be provided [25]. Intriguingly, patients with cancer co-infected with HIV-1 and hepatitis B did not have viral reactivation during chemotherapy [53], suggesting no treatment stop, although data may be distinct for different viruses [25]. In cancer patients infected with COVID-19, excessive immune cell activation and cytokine production might be important for the outcome of patients [54, 55].

Observations from clinical centers dealing with large cohorts of patients with COVID-19 infection showed compelling evidence that the patients' mortality was directly related to aging, smoking history and probably also sex [26]. However, a meta-analysis of ACE2 expression demonstrated a low prevalence of individuals with smoking history in COVID-19 infected patients [56].

Conclusions

Despite this bioinformatics study provides novel information on the role of ACE2 and TMPRSS2 expression in LUAD patients for COVID-19 infection and its association with clinical behavior and smoking history, it has several limitations: (i) The data need to be validated in a larger cohort of patients. (ii) The potential mechanisms of interaction between COVID-19, ACE2 and TMPRSS2 has to be identified and (iii) the role of the discordant expression of ACE2 and TMPRSS2 in LUAD and upon COVID-19 infection has to be clarified in this disease.

Abbreviations:

ACE2, angiotensin-converting enzyme 2; CoV, coronaviruses; GEO, gene expression omnibus; HCMDB, Human Cancer Metastasis Database; IHC, immunohistochemistry; LCE, lung cancer explorer; LUAD, lung adenocarcinoma; MERS, Middle East respiratory syndrome; NSCLC, non-small cell lung carcinoma; OS, overall survival; SARS, severe acute respiratory syndrome; TCGA, The Cancer Genome Atlas; TMPRSS2, transmembrane protease serine 2; OS overall survival; VEGF, vascular endothelial growth factor

Declarations

- Ethics approval and consent to participate

not applicable

- Consent for publication

All authors give their consent for publication.

- Availability of data and material

All data generated or analyzed during this study are included in this published article.

- Competing interests

There exists no financial and non-financial competing interests.

- Funding

not applicable

- Authors contribution

K. Subbarayan and B. Seliger designed the study and wrote the article, K. Ulagappan performed the data analyses, C. Wickenhauser discussed the data and revised the manuscript.

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Figure 1. Heterogeneous expression pattern of ACE2 and TMPRSS2 across 36 healthy tissues

The expression levels of ACE2 and TMPRSS2 were analyzed in 36 different normal tissues using the NCBI GEO public database and detected by the GENT2 system and ranked according to the expression levels.

A, B: ACE2 (A) and TMPRSS2 (B) expressions in 36 healthy tissues

Figure 2. Comparison of ACE2 and TMPRSS2 expression in different cancers and corresponding normal tissues. Red indicates the boxplots of cancer samples. Blue indicates the boxplots of corresponding normal samples.

C, D: Boxplots of ACE2 (C) and TMPRSS2 (D) expression across 72 paired tissues

Figure 3. Expression pattern and methylation of ACE2 and TMPRSS2 in LUAD vs. non-tumorous

Box-whisker plots showing the mRNA expression and promoter methylation aberration in normal and LUAD samples for ACE2 (A, B) and TMPRSS2 (C, D)

Figure 4. Meta-analysis of the expression pattern of ACE2 and TMPRSS2

In the forest plot, the name of each study is followed by the number of tumor and normal samples.

A: ACE2 expression

B: TMPRSS2 expression

SMD: standardized mean difference

Figure 5. Impact of aging and smoking history on ACE2 in healthy lung and LUAD tissues

A, B: Box plots comparing ACE2 expression between two groups dichotomized among normal lung (A) or LUAD (B) tissues on an aging variable: 0-59 vs. above 60

C, D: Box plots comparing ACE2 expression between two groups dichotomized among 0-59 (C) or above 60 (D) age categories on a single variable: normal vs. tumor

E, F, G, H: Box plots comparing ACE2 expressions between non-smokers and smokers: normal (TCGA: E, Okayama 2012: F) and tumor (TCGA: G, Okayama 2012: H) lung tissues. Data collected from TCGA and Okayama 2012 datasets.

Figure 6. TMPRSS2 expression in normal and LUAD tissues regarding aging and smoking history

A, B: Box plots comparing TMPRSS2 expression between two groups dichotomized among normal lung (A) or LUAD (B) tissues on an aging variable: 0-59 vs. above 60

C, D: Box plots comparing TMPRSS2 expression between two groups dichotomized among 0-59 (C) or above 60 (D) age categories on a single variable: normal vs. tumor

E, F, G, H: Box plots comparing TMPRSS2 expressions between non-smokers and smokers: normal (TCGA: E, Okayama 2012: F) and tumor (TCGA: G, Okayama 2012: H) lung tissues. Data collected from TCGA and Okayama 2012 datasets.

Figure 7. Expression, promoter methylation and survival meta-analysis of ACE2 and TMPRSS2 in individual cancer stages and nodal metastasis statuses

Box plots showing relative expression (A: stages, C: metastasis) and promoter methylation (B: stages, D: metastasis) of ACE2

Box plots showing relative expression (E: stages, G: metastasis) and promoter methylation (F: stages, H: metastasis) of TMPRSS2

Figure 8. Meta-analysis of the expression pattern ACE2 and TMPRSS2 on survival in LUAD. In the forest plot, the name of each study is followed by the number of total tumor samples.

C, D: Survival meta-analysis of ACE2 (C) and TMPRSS2 (D)

TE estimated treatment effect, seTE standard error of treatment effect, HR hazard ratio, CI confidence interval

Table 1: Log fold-change and p values of ACE2 and TMPRSS2 expression in different cancers and corresponding normal tissues.

Table 2: ACE2 expression on different metastasis sites from GEO and TCGA datasets, which were analyzed using the HCMDB database