

Analysis the susceptibility of lung cancer patients to SARS-CoV-2 infection

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Abstract

Recent studies have reported that 2019 novel coronavirus disease (COVID-19) patients with lung cancer have a higher risk of severe events than patients without cancer. In this study, we investigated the expression of severe acute respiratory syndrome coronavirus 2 (SAR-CoV-2) receptor angiotensin I-converting enzyme 2 (ACE2) and the cellular protease transmembrane serine protease 2 (TMPRSS2) and their associations with prognosis in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). We found that there are significant differences in susceptibility to SAR-CoV-2 among each age stages of individuals with the expression of ACE2. ACE2 was also high expressed in LUAD and LUSC, and this suggests that COVID-19 patients with lung cancers are susceptible to SAR-CoV-2 infection. Our data showed the differential gene expression level and gene coexpression of ACE2 and TMPRSS2 among each subtypes and pathological stages of LUAD and LUSC and the data were verified by meta-analysis, gene expression omnibus (GEO) data and animal models results.

Introduction

Since the end of Dec 2019, a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has attacked Wuhan, China, and created a global epidemic. The World Health Organization (WHO) named the disease caused by this virus 2019 novel coronavirus disease (COVID-19). A total of 80,925 individuals have been confirmed to be COVID-19 patients, and 3140 have died (through March 10, 2020). The 25.2% of confirmed patients with more severe injury tended to exhibit some basic underlying disease, such as hypertension (30.0%), diabetes mellitus (12.1%), cancer (1%), chronic obstructive pulmonary disease (COPD), a smoking history, and older age (over 60) [1, 2]. Patients with lung cancer are more susceptible to infection than normal individuals [2, 3]. Some experts ascribe this to the reduced immunity that occurs after radiotherapy or chemotherapy, but no report has evaluated this hypothesis at the gene expression level.

The severe acute respiratory syndrome coronavirus (SARS-CoV) receptor angiotensin I-converting enzyme 2 (ACE2) and the cellular protease transmembrane serine protease 2 (TMPRSS2) have been recognized as participants in SARS-CoV-2 host cell entry. There is evidence that TMPRSS2 plays a

crucial role in regulating SARS-CoV-2 infection as an activator. TMPRSS2 activity seems to be required for viral spread, pathogenesis, immunopathology, and disease severity in the host. A lack of TMPRSS2 in the airways reduces the severity of lung pathology after infection by SARS-CoV [4].

In this study, we investigated the expression of ACE2 and TMPRSS2 and their associations with prognosis in two common lung cancers, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). We aimed to explore the expression differences in ACE2 and TMPRSS2 between these lung cancers and their relationships with SARS-CoV-2 infection.

Materials And Methods

Analysis of gene expression patterns in human lung development

The LungMAP website (<http://www.lungmap.net>) currently contains more than 6,000 high-resolution lung images and transcriptomic, proteomic, and lipidomic human and mouse data and provides scientific information to stimulate interest in research careers for young audiences [5, 6]. We used LungMAP to analyze the gene expression levels of ACE2 and TMPRSS2 in human lung development.

Compare the gene expression profiling in human lung cancers

Gene expression profiling interactive analysis 2 (GEPIA2, <http://GEPIA2.cancer-pku.cn>) is an updated web server for human cancer and normal gene expression profiling and interactive analyses [7]. We compared the mRNA expression levels of ACE2 and TMPRSS2 between human lung cancer samples and normal lung samples by GEPIA2. Descriptive data were generated in different stages of lung cancer to compare differential expression.

Meta-analyses of the roles of selected genes in lung cancers

Lung cancer explorer (LCE, <http://lce.biohpc.swmed.edu/lungcancer/>) is an open-access web portal for exploring gene expression and clinical associations in lung cancer data from over 6,700 patients in 56 studies. Meta-analysis is a unique tool provided by LCE to gain a quick overview of results for tumor vs normal tissue differential gene expression and expression-survival associations and visualize data as forest plots. Forest plots of tumor-normal standardized mean differences for tumor vs normal tissue meta-analysis ($p < 0.01$, $I^2 \geq 85\%$) summarize differences in heterogeneity. Comparative analysis was implemented to assess the associations between a selected gene and clinical factors within a specific dataset [8].

Statistical methods

All the analyses were performed with the tool's default values. Continuous variables were commonly described by the median and range. Differences between tumor and paired normal tissue samples were analyzed by a t test or analysis of variance (ANOVA). The cutoff of $|\text{Log}_2\text{FC}|$ was set as 1, the q-value was set as 0.01, the F-value was set as 3, and the data were always selected to match the cancer genome atlas (TCGA) normal data and the genotype-tissue expression (GTEx) data. We used $\log_2(\text{TPM}+1)$ to create a log scale when comparing three or more groups. Hazard ratios and the corresponding 95% confidence interval (CI) were determined by univariate and multivariate Cox proportional hazards models. All reported p values (abbreviate p-val) are two tailed, and $p < 0.05$ was considered statistically significant.

Results

Gene expression analysis of human lung development by LungMAP

The analysis of subject genes on the LungMAP website demonstrated that the ACE2 and TMPRSS2 gene expression levels were higher in epithelial cells (orange line) than in other cells. ACE2 gene expression was highest in 5-month-old babies, and the expression in young children was higher than that in adults. The expression level of ACE2 decreased with a wavy pattern as age increased (Fig 1A). The total trend for the TMPRSS2 gene expression level was similar to that of the ACE2 expression level, but TMPRSS2 expression was higher in 40-year-olds than in 24-year-olds (Fig 1B) and nearly 40 times higher than ACE2 expression. This may suggest that there are significant differences in susceptibility to SARS-CoV-2 among each age stages of individuals. The WHO has declared that everyone is assumed to be susceptible, although there may be risk factors that increase susceptibility to infection [9], including older age ($P < 0.0001$) [10]. The oldest COVID-19 patient is a 96-year-old woman, and the youngest is a 17-day-postbirth baby. These patients were both reported to be cured in China and conformed to the characteristics of the two gene expression patterns.

Gene expression analysis of human lung cancer pathological stages by GEPIA2

If lung cancer patients are susceptible to SARS-CoV-2, are there any differences in ACE2 and TMPRSS2 gene expression across the stages of lung cancer? By using GEPIA2, we profiled the expression of the ACE2 and TMPRSS2 genes in each pathological stage of two lung cancer types

(LUAD and LUSC) using box plots. ACE2 gene expression in each pathological stage of LUAD was not obviously different ($F\text{-val} = 0.634$, Fig 1C); likewise, in LUSC, it was also not obviously different ($F\text{-val} = 0.589$, Fig 1D). TMPRSS2 gene expression in each pathological stage of LUAD showed obvious differences ($F\text{-val} = 5.54$, Fig 1E), but in LUSC, it did not obviously vary ($F\text{-val} = 1.94$, Fig 1F). The data show that changes in the expression of these two genes had no statistical significance, which suggests that the ACE2 and TMPRSS2 genes are expressed consistently in each pathological stage of lung cancer, except for TMPRSS2 in LUAD. Furthermore, there are no significant differences in the susceptibility to SARS-CoV-2 among the pathological stages of LUAD and LUSC. TMPRSS2 maybe an tumor suppressor gene in LUAD, the downregulated expression level may decreased the susceptibility to SARS-CoV-2 for LUAD patients.

Gene expression analysis of human lung cancer subtypes by GEPIA2

With gene expression data, previous studies have classified LUAD into three subtype including terminal respiratory unit (TRU), proximal inflammatory (PI), proximal proliferative (PP) [11], and classified LUSC into four subtypes, including the primitive, classical, secretory and basal subtypes [12]. These subtypes have different survival outcomes and susceptible to drugs and viruses. We profiled the gene expression data for ACE2 and TMPRSS2 in LUAD, and LUSC subtypes.

ACE2 gene expression in each subtype of LUAD is all higher in lung cancers than normal lung tissues (Fig 2A), especially in proximal proliferative subtype. While in LUSC, they are similar, but ACE2 gene expression level in the primitive subtype is higher in normal lung tissues than lung cancers (Fig 2B). These data may show the susceptibility to SARS-CoV-2 among each subtypes of LUAD and LUSC. In converse, TMPRSS2 gene expression in each subtype of LUAD is all lower in tumors than normal lung tissues, but little difference in terminal respiratory unit subtype (Fig 2C). In LUSC, TMPRSS2 gene expression is much lower than LUAD (Fig 2D).

Gene expression meta-analysis of human lung cancers by LCE

In order to further understand the implications of the differential expression of the ACE2 gene between normal lung tissue and cancerous lung tissue, we compared ACE2 gene expression between these two tissue types in LCE, and the results are shown in a box plot of the two selected groups. The

data showed that the ACE2 gene was expressed at higher levels in LUAD tumor tissue than in normal lung tissue (Fig 2E, $p\text{-val} = 6.4e^{-06}$) and the ACE2 gene was expressed at nearly equal levels in LUSC tumor tissue and normal lung tissue (Fig 2F, $p\text{-val} = 0.11$). However, the data showed that the TMPRSS2 gene was expressed at higher levels in normal lung tissue than in LUAD tumor tissue (Fig 2G, $p\text{-val} = 3e^{-31}$) or LUSC tumor tissue (Fig 2H, $p\text{-val} = 4.1e^{-81}$).

Tissue-specific gene expression in lung cancers by GEPIA2

By using GEPIA2, we profiled the tissue-specific expression of the ACE2 and TMPRSS2 genes in two lung cancer types (LUAD and LUSC) using an interactive heat map. The expression of the ACE2 and TMPRSS2 genes in different lung cancer stages is illustrated in Fig 3A. The TMPRSS2 gene was expressed at higher levels in normal lung tissue than ACE2 and also more highly expressed in LUAD. We determined the ACE2 and TMPRSS2 gene coexpression in lung tissues. The ACE2 and TMPRSS2 genes were coexpressed in normal lung tissue (Fig 3B, $p\text{-val} = 7.1e^{-05}$) and lung cancer tissue (Fig 3C, $p\text{-val} = 0.0058$), but the differences were statistically significant ($p\text{-val} < 0.01$).

Gene expression meta-analysis by LCE

Meta-analyses showed no significant correlation between ACE2 gene expression and LUSC (Fig 4A, $I^2 = 47\%$, $p\text{-val} = 0.0011$) or between ACE2 gene expression and LUAD (Fig 4B, $I^2 = 62\%$, $p\text{-val} = 3.8e^{-05}$). This suggests that ACE2 gene expression is not significantly different between normal lung tissue and cancerous lung tissue. TMPRSS2 gene expression had a significant association with negative expression outcomes in LUSC (Fig 4C, $I^2 = 88\%$, $p\text{-val} = 1.3e^{-12}$); however, there was no significant association in LUAD (Fig 4D, $I^2 = 86\%$, $p\text{-val} = 0.072$). This suggests that TMPRSS2 gene expression exhibits a meaningful difference between normal lung tissue and LUSC tissue but not between normal lung tissue and LUAD tissue.

Gene expression analysis in SARS-CoV infected animal models in vivo

In SARS-CoV-2-infected hACE2 transgenic mice, the typical histopathology was interstitial pneumonia with significant inflammatory cell infiltration around the bronchioles and blood vessels, and viral antigens were observed in bronchial epithelial cells and alveolar epithelial cells [13]. SARS-CoV infected hACE2 transgenic mice had severe pulmonary lesions, including interstitial hyperemia and

hemorrhage, monocytic and lymphocytic infiltration, protein exudation, and alveolar epithelial cell proliferation and desquamation [14]. SARS-CoV infected TMPRSS2-KO mice showed weakened inflammatory chemokine and/or cytokine responses to intranasal stimulation. TMPRSS2 deficiency affected the primary sites of infection and virus spread within the airways, which was accompanied by relatively less severe immunopathology [15].

Gene expression analysis in SARS-CoV infected human lung epithelial cells in vitro

The ACE2 and TMPRSS2 genes have obvious differential expression between SARS-CoV infected cells and Mock infected Calu-3 (subclone 2B4) cells, with p-val of 5.07×10^{-12} vs 4.65×10^{-45} , respectively, in GSE17400, which demonstrated that the SARS-CoV induced secretion of cytokines by epithelial Calu-3 cells could exacerbate or dampen host inflammatory and T cell responses [16]. However, in the lung cancer samples in GSE19804, the difference in TMPRSS2 gene expression was not obvious (Table 1, p-val = 2.37×10^{-01}). ACE2 gene expression was more reduced in Calu-3 cells infected with SARS-CoV than in lung cancer samples when compared with TMPRSS2 expression in average of 12, 24, 48 hours post infection (hpi), especially in 24 hpi (Fig 5A-B).

Table 1 ACE2 and TMPRSS2 differential gene expression in lung cells infected with SARS-CoV or lung cancer tissue.

GSE No.	Samples	Probe ID	Gene symbol	Adj. P value	P value
GSE17400	Calu-3 cells infected with SARS-CoV vs Mock infected cells	222257_s_at	ACE2	1.42×10^{-11}	5.07×10^{-12}
		211689_s_at	TMPRSS2	2.07×10^{-43}	4.65×10^{-45}
GSE19804	Lung cancer tissue vs normal lung tissue	222257_s_at	ACE2	2.87×10^{-08}	1.97×10^{-09}
		1570433_at	TMPRSS2	3.62×10^{-01}	2.37×10^{-01}

Discussion

The structural proteins of SARS-CoV-2 are genetically similar to those of SARS-CoV but not those of MERS-CoV [17]. The human airway epithelium is the initial site of SARS-CoV-2 infection. Recent studies have demonstrated that the SARS-CoV receptor ACE2 and the cellular protease TMPRSS2 are involved in the entry of SARS-CoV-2 into lung cells [18]. TMPRSS2 activates the spike protein of highly pathogenic human coronaviruses such as SARS-CoV and Middle East respiratory syndrome-related coronavirus (MERS-CoV). Lack of TMPRSS2 in the airways reduces the severity of lung pathology after

infection by SARS-CoV or MERS-CoV [19].

The gene expression level of ACE2 maybe indicate the susceptible to SARS-CoV-2 infection, and TMPRSS2 plays a supporting role. In this paper, we reported that ACE2 expressed in each age stages of the whole lifespan as a wave line. TMPRSS2 expression was higher in 40-year-old individuals than in 24-year-old individuals. Our data suggests that there are differences in susceptibility to SAR-CoV-2 among each age stages of individuals, but most individuals were susceptible to SARS-CoV-2 with the gene expression of ACE2.

There are little significant differences in the susceptibility to SARS-CoV-2 among the pathological stages of LUAD and LUSC, except the TMPRSS2 is decreased in LUAD (p-val = 0.00961). ACE2 expression was upregulated in LUAD samples but not in LUSC samples. TMPRSS2 expression was downregulated in LUAD (p-val = $3e^{-31}$) and LUSC (p-val = $4.1e^{-81}$) tissue samples. The TMPRSS2 gene was expressed at higher levels in normal lung tissue than was ACE2, and this difference was especially pronounced in LUAD tissue but had some statistically significant associations with pathological stages, and subtypes. Our data indicate that lung cancer patients are more susceptible to SARS-CoV-2 than normal individuals, especially LUAD.

In animal models, hACE2 transgenic mice infected with SARS-CoV-2 exhibited pathogenesis similar to that observed in COVID-19 patients. TMPRSS2-knockout mice exhibited weakened inflammatory chemokine responses to SARS-CoV. We also found that TMPRSS2 gene expression was lower in LUSC than in LUAD, which was further validated through meta-analysis ($P = 1.3e^{-12}$, $I^2 = 88\%$).

There is evidence that TMPRSS2 activates the SARS-CoV S protein for membrane fusion and reduces viral control mediated by the humoral immune response [20]. TMPRSS2 plays major roles in the early phases of SARS-CoV and MERS-CoV replication in the infected lungs and bronchioles. TMPRSS2-deficient mice intrinsically exhibit weakened or delayed inflammatory chemokine and cytokine responses induced via Toll-like receptor 3 (TLR3). Serine protease inhibitors such as camostat and aprotinin inhibit both influenza virus replication in human airway epithelial cells and the release of cytokines (IL-6 and TNF- α) into cell supernatants [21]. IL-6, TNF- α and IFN- γ are essential activators

of cytokine storm, which is often the cause of ARDS and has been observed in severe COVID-19 patients.

TMPRSS2 has been identified as an HA activator for some viruses, such as influenza virus and coronavirus [22]. TMPRSS4 and TMPRSS11A are also host cell proteases that, in addition to TMPRSS2, are able to activate the HA protein of influenza virus [23]. SARS-CoV and other respiratory viruses can be activated by TMPRSS2 [24]. TMPRSS2 plays crucial roles in viral spread within the airways of murine models infected by SARS-CoV or MERS-CoV and in the resulting immunopathology [25]. Mouse *Tmprss2* is expressed mainly in the prostate and kidneys; however, human TMPRSS2 is expressed in the prostate, colon, stomach, and salivary gland. Coexpression of ACE2 and TMPRSS2 is frequently found in the upper and lower aerodigestive tracts, with the exception of the vocal folds, epiglottis and trachea. TMPRSS2 and HAT are expressed by important influenza and SARS-coronavirus target cells and could thus support viral spread in the human host[26].

No reports on TMPRSS2 transgenic mice, which could serve as a SARS-CoV-2 animal model, have been published, but this approach should be considered. ACE2 gene expression is less elevated in Calu-3 cells infected with SARS-CoV than in lung cancer when compared to TMPRSS2 expression. Our study indicates that TMPRSS2 may be a useful therapeutic target in COVID-19, although the detailed mechanism still needs to be revealed. More attention has been paid to ACE2, and few studies have focused on TMPRSS2. This study attempts to call for researchers to seriously consider TMPRSS2, which may play a more crucial role together with ACE2 in COVID-19 patients.

Declarations

Acknowledgement

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Author contributions

Qi Kong designed the project and drafted the manuscript. Fei Geng reviewed the manuscript and provided some suggestions. Other authors were involved in the data analysis and interpretation.

Competing interests

We declare no competing interests.

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Figures

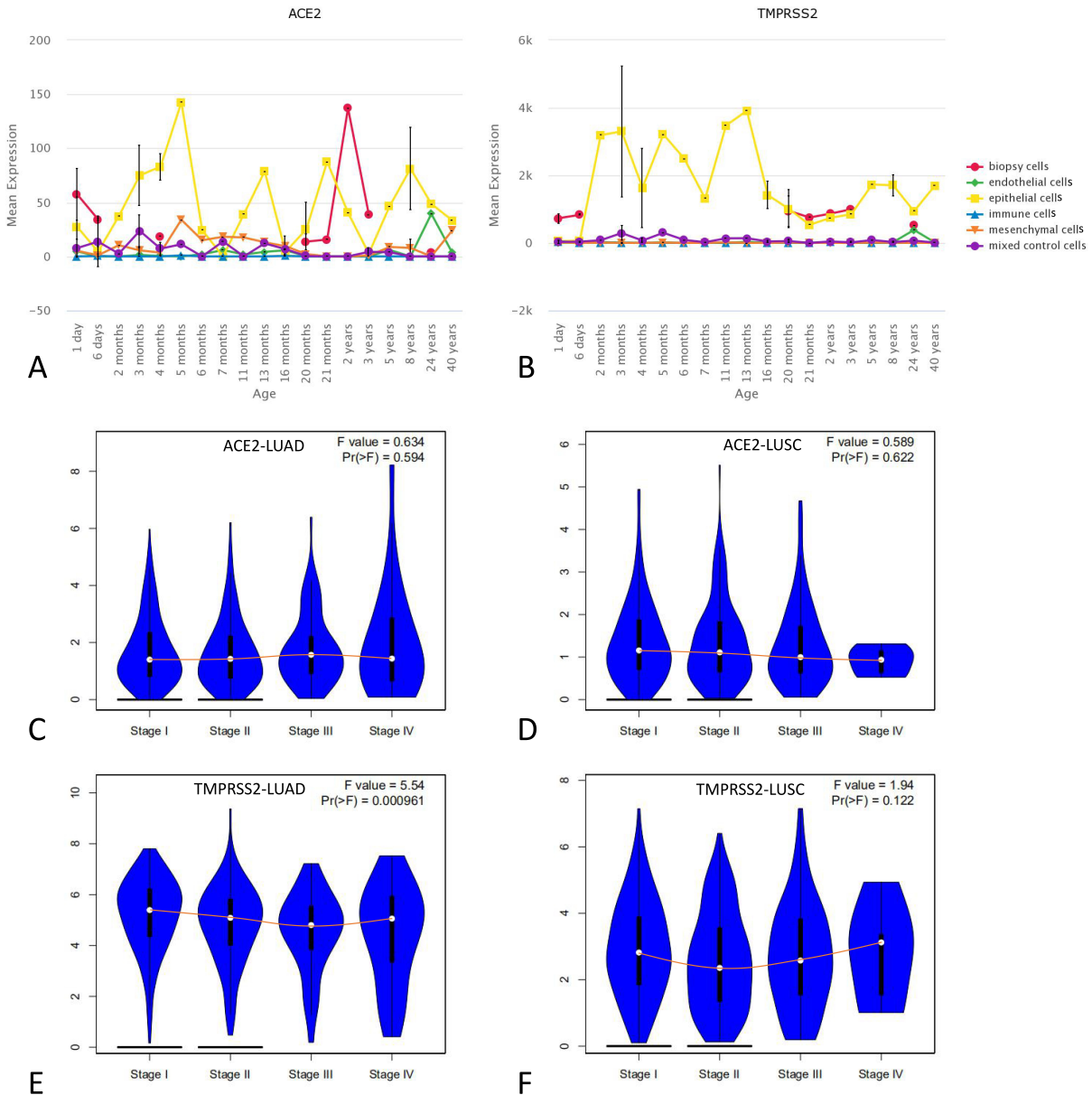


Figure 1

The expression level trends of the ACE2 and TMPRSS2 genes in lung tissues. A. ACE2 gene expression level trends in lung tissues determined by RNA-seq methods; B. TMPRSS2 gene expression level trends in lung tissues determined by RNA-seq methods; C. ACE2 gene expression level trends in different LUAD pathological stages; D. ACE2 gene expression level trends in different LUSC pathological stages; E. TMPRSS2 gene expression level trends in different LUAD pathological stages; and F. TMPRSS2 gene expression level trends in

different LUSC pathological stages.

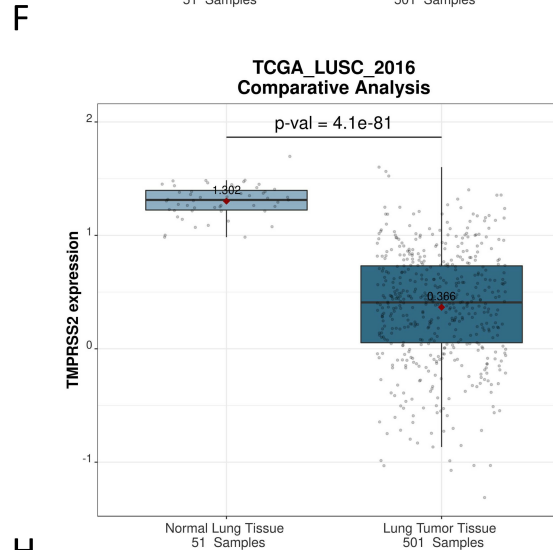
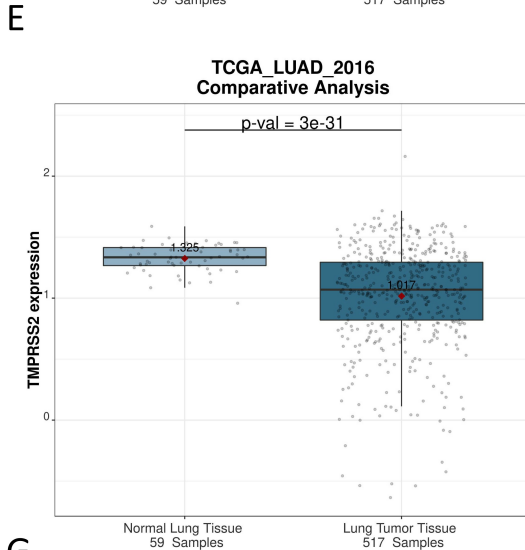
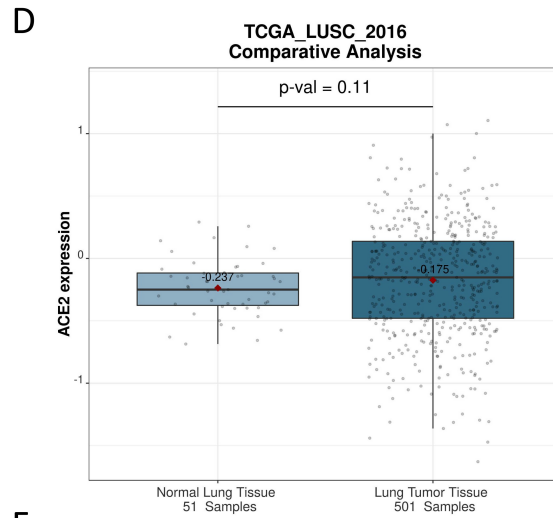
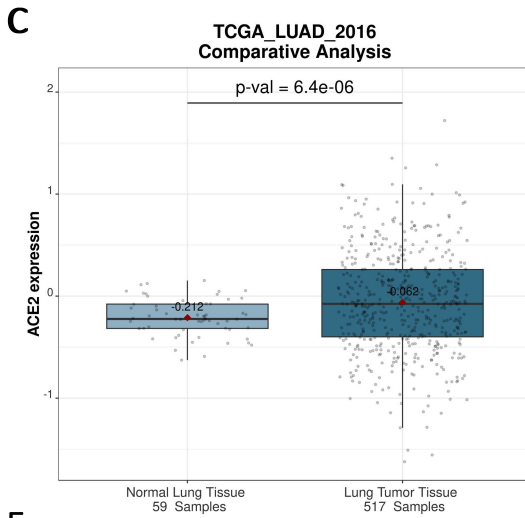
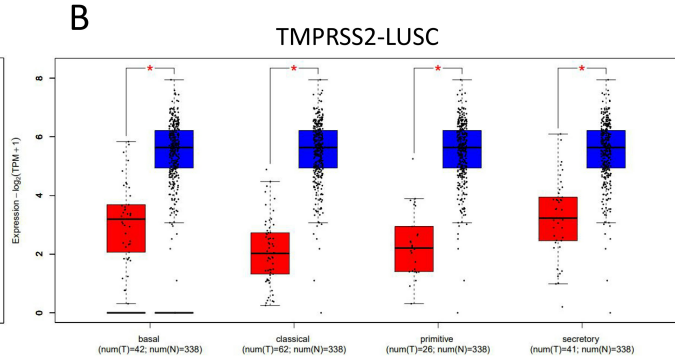
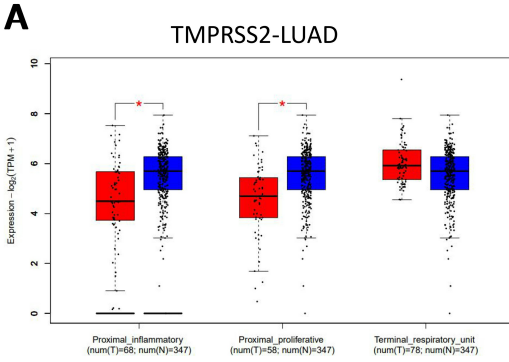
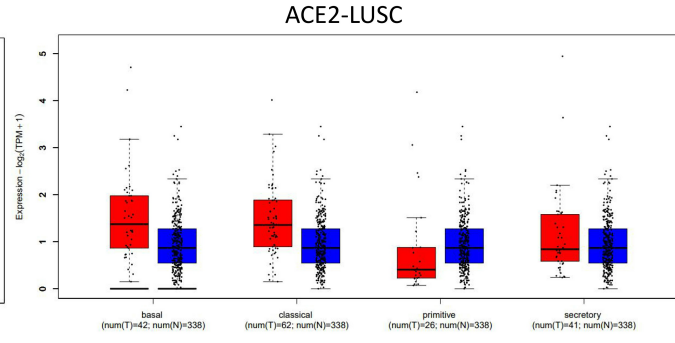
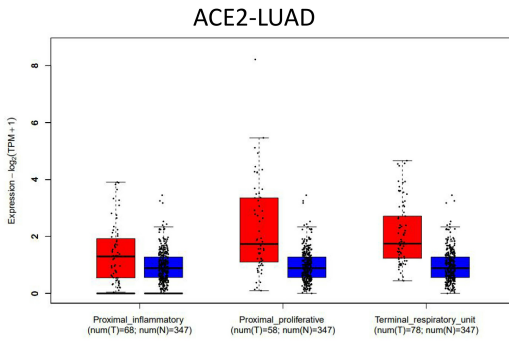


Figure 2

Comparative analysis of the tissue-specific differential expression of the ACE2 and TMPRSS2 genes in lung tissues performed with GEPIA2 and LCE. A. ACE2 gene expression in each subtypes of LUAD; B. ACE2 gene expression in each subtypes of LUSC; C. TMPRSS2 gene expression in each subtypes of LUAD; D. TMPRSS2 gene expression in each subtypes of LUSC; E. ACE2 in normal lung tissue and lung cancer tissue samples from TCGA_LUAD_2016 (p-val = 6.4×10^{-6}); F. ACE2 in normal lung tissue and lung cancer tissue samples from TCGA_LUSC_2016 (p-val = 0.11); G. TMPRSS2 in normal lung tissue and lung cancer tissue samples from TCGA_LUAD_2016 (p-val = 3×10^{-31}); and H. TMPRSS2 in normal lung tissue and lung cancer tissue samples from TCGA_LUSC_2016 (p-val = 4.1×10^{-81}).

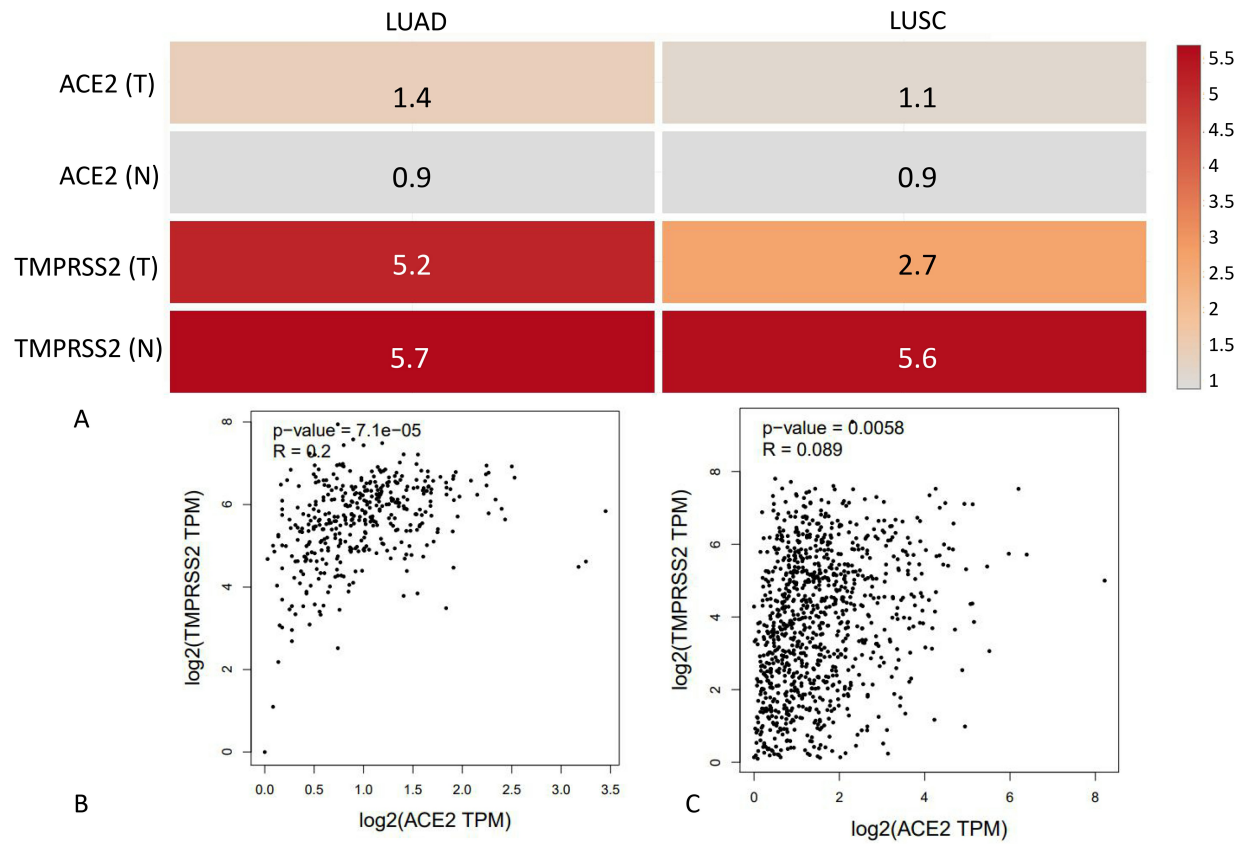
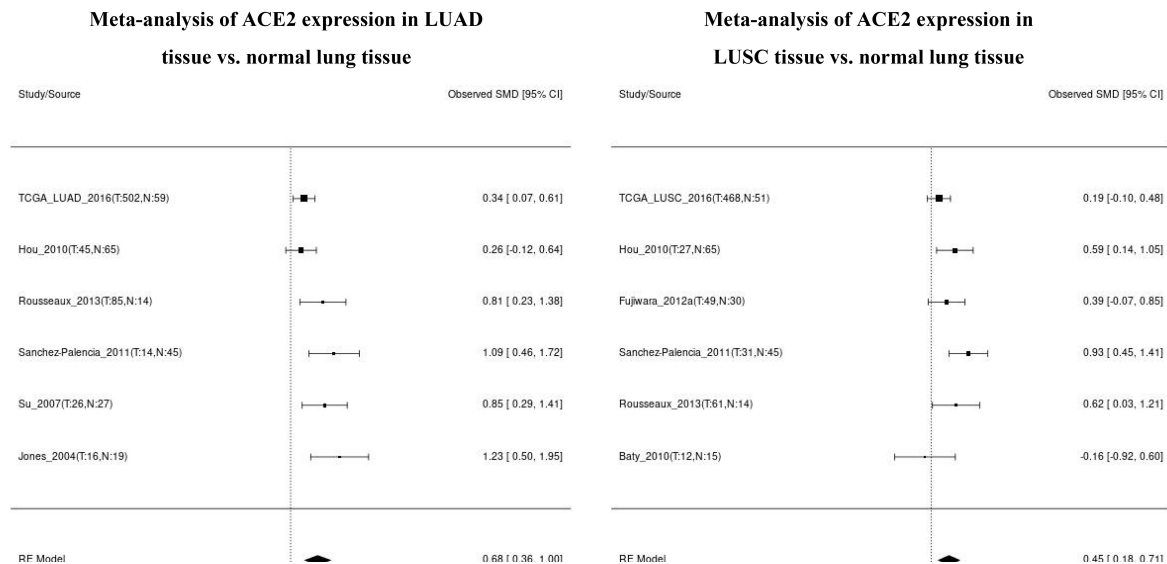


Figure 3

The tissue-specific expression and correlations of the ACE2 and TMPRSS2 genes in LUAD and LUSC. A. Comparison of ACE2 and TMPRSS2 gene expression levels in lung cancer tissues (T) and lung normal tissues (N); B. ACE2 and TMPRSS2 gene coexpression in lung normal tissues (p-val=7.1e-05); and C. ACE2 and TMPRSS2 gene coexpression in lung cancer tissues (p-val=0.0058).



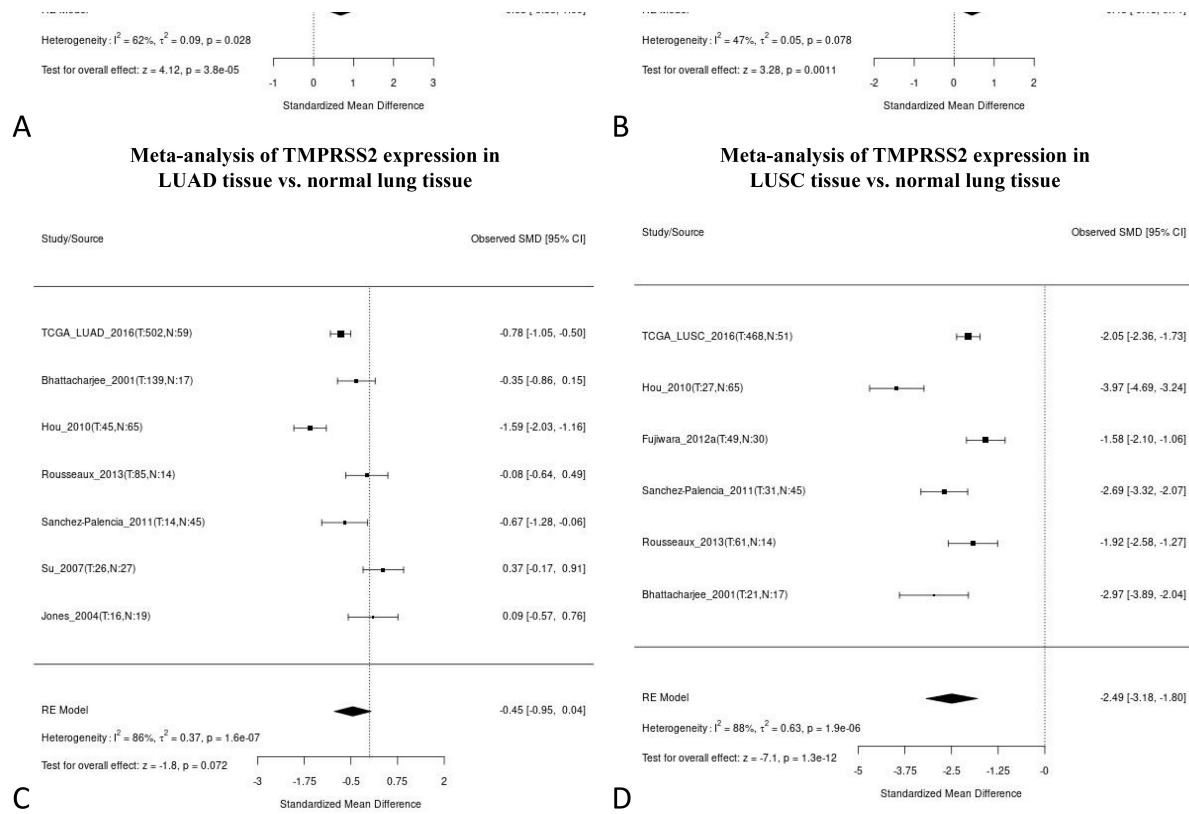


Figure 4

The graph represents meta-analyses of tumor vs. normal tissue data for ACE2 and TMPRSS2 gene expression. A. Meta-analysis of ACE2 expression in LUAD tissue vs. normal lung tissue(p -val= $3.8e-05$, $I^2=62\%$); B Meta-analysis of ACE2 expression in LUSC tissue vs. normal lung tissue (p -val= 0.0011 , $I^2=47\%$); C. Meta-analysis of TMPRSS2 expression in LUAD tissue vs. normal lung tissue (p -val= 0.072 , $I^2=86\%$); and D. Meta-analysis of TMPRSS2 expression in LUSC tissue vs. normal lung tissue (p -val= $1.3e-12$, $I^2=88\%$).

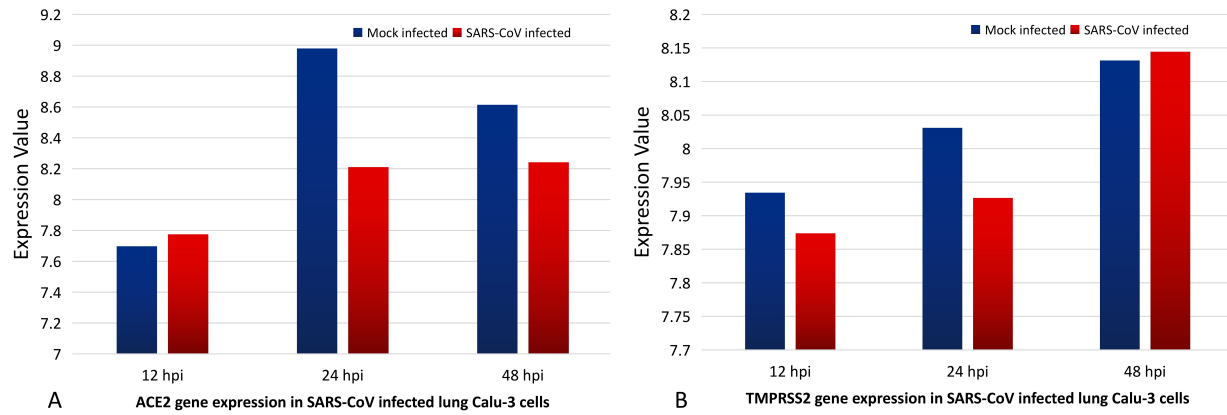


Figure 5

ACE2 (A) and TMPRSS2 (B) differential genes expression in human airway bronchial epithelial cells (Calu-3, a non-small-cell lung cancer cell line) infected with SARS-CoV.