An Investigation of the Expression of 2019 Novel Coronavirus Cell Receptor Gene ACE2 in a Wide Variety of Human Tissues

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Research Article

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Abstract

Background: The 2019 novel coronavirus (2019-nCoV) has affected more than 72,000 people worldwide and caused more than 1,800 deaths so far. 2019-nCoV uses the angiotensinconverting enzyme 2 (ACE2) as the cell receptor to invade the human host and primarily causes pneumonia. Thus, ACE2 is the key to understanding the mechanism of 2019-nCoV infection.

Methods: We compared ACE2 expression levels across 31 human normal tissues, between males and females, and between younger (ages <= 49 years) and older (ages > 49 years) persons in these tissues. We also investigated the correlations between ACE2 expression and immune signatures in various tissues.

Results: ACE2 expression levels were the highest in small intestine, testis, kidney, heart, thyroid, and adipose tissue, and were the lowest in blood, spleen, bone marrow, brain, blood vessel, and muscle. In lungs, colon, liver, bladder, and adrenal gland, ACE2 showed the medium expression levels. ACE2 was not differentially expressed between males and females and between younger and older persons in any tissue. In skin, digestive system, brain, and blood vessel, ACE2 expression levels were positively associated with immune signatures in both males and females. In thyroid and lungs, ACE2 expression levels were positively and negatively associated with immune signatures in males and females, respectively.

Conclusions: Our data provide potential cues for the 2019-nCoV epidemic may infect other tissues outside lungs, affect males and females and young and old persons equally, and old age and male are associated with higher mortality risk for 2019-nCoV infection.

Background

The recent outbreak and rapid spread of human 2019 novel coronavirus (2019-nCoV) infection in Wuhan of China have raised a global health emergency [1]. The patients infected with 2019-nCoV mainly displayed pneumonia-associated symptoms, including fever, cough, shortness of breath, sputum production, and myalgia or fatigue [2, 3], indicating that 2019-nCoV primarily infects the respiratory tract and causes acute respiratory disease. Moreover, 2019-nCoV infection may result in the symptoms of diseases associated with other tissues, such as digestive (*diarrhea, poor appetite,* nausea, and vomiting), nervous (confusion and headache), and cardiovascular (palmus, chest distress, and cardiac injury) systems [2, 3]. In addition, some studies indicated that person-to-person transmission of 2019-nCoV by the approach outside the respiratory tract is possible [4]. A study of 99 patients infected with 2019-nCoV showed that female was less susceptible to 2019-nCoV infection than male and that older males with comorbidities were more likely to be infected with 2019-nCoV [3]. Like SARS-related coronavirus (SARS-CoV) [5], 2019-nCoV uses the angiotensinconverting enzyme 2 (ACE2) as the host cell receptor [6–8]. A recent study uncovered that the ACE2-expressing lung cells were more abundant in Asian males [9],

potentially explaining the elevated susceptibility of males to 2019-nCoV infection. Nevertheless, the findings from that study are not sufficiently convincing due to a small number of samples being analyzed.

In this study, we analyzed the expression of ACE2 in various human normal tissues using the datasets from the Genotype-Tissue Expression (GTEx) project [10] and The Cancer Genome Atlas (TCGA) program (https://portal.gdc.cancer.gov/). We compared ACE2 expression levels across 31 human tissues, between males and females, and between younger and older persons in these individual tissues. Furthermore, we analyzed the correlations between ACE2 expression levels and immune signature enrichment levels in individual tissues. This study may provide new insights into the role played by ACE2 in the 2019-nCoV epidemic.

Results

ACE2 expression in various human tissues

Among the 31 GTEx human tissues, small intestine, testis, kidney, heart, thyroid, and adipose tissue had the highest ACE2 expression levels, while blood, spleen, bone marrow, brain, blood vessel, and muscle had the lowest ACE2 expression levels (Fig. 1A). In lungs, colon, liver, bladder, and adrenal gland, ACE2 showed the medium expression levels (Fig. 1A). These results suggest that ACE2 are expressed in a wide variety of human tissues in addition to lungs. Furthermore, the Human Protein Atlas (HPA) database (http://www.proteinatlas.org/) shows that the ACE2 protein has relatively high expression levels in duodenum, small intestine, gallbladder, kidney, testis, seminal vesicle, colon, rectum, and adrenal gland. The HPA database also shows that the gastrointestinal tract (duodenum, small intestine, colon, and rectum), kidney, gallbladder, and male tissues (testis and seminal vesicle) have high expression levels of both ACE2 gene and protein. Taken together, these data indicate that: i) 2019-nCoV may infect other human tissues in addition to lungs; ii) Males may be more susceptible to 2019-nCoV infection than females. These indications have been confirmed by recent publications [2–4]. For example, Holshue et al. uncovered that stool from a patient with 2019-nCoV infection was positive for 2019-nCoV, suggesting that 2019-nCoV may infect the gastrointestinal tract [5]. Huang et al. reported the virus-related cardiac injury in five patients with 2019-nCoV infection [2]. A study of 99 cases of 2019-nCoV pneumonia revealed the increased susceptibility of males to virus infection [3].

We further compared ACE2 expression levels between males and females in 22 individual human tissues using the GTEx datasets and found no statistically significant difference between males and females in any tissue with a threshold of adjusted p value < 0.05 and fold change > 1.5 (Fig. 1B). These results may indicate that like SARS-CoV [11], 2019-nCoV can affect males and females equally, although males are likely to have higher mortality risk than females with coronavirus infections [3, 12]. We also compared ACE2 expression levels between younger (ages < = 49 years) and older (ages > 49 years) groups and did not observe statistically significant difference between both groups in any tissue with a threshold of adjusted p value < 0.05 and fold change > 1.5 (Fig. 1B). These results indicate that like SARS-CoV [11],

2019-nCoV can attack young and old persons equally, although old age is associated with higher risk of death for patients with coronavirus infections [3, 13]. Likewise, in the TCGA datasets, ACE2 was not differentially expressed between males and females and between younger and older groups in any tissue (Fig. 1C), consistent with the results in GTEx. In TCGA, we also compared ACE2 expression levels between Asia and other races in five normal tissues (stomach, thyroid, breast, liver, and pancreas), and did not found significant difference between them in any tissue (Fig. 1C).

Association of ACE2 expression with immune signatures in various human tissues

Adaptive and innate immune responses play an important role in fighting off invading coronavirus, while they may induce cytokine storm to cause the immunopathological damage of patients with coronavirus infections [14]. We analyzed the correlations between ACE2 expression levels and immune signature (CD8+ T cells, interferon response, B cells, and NK cells) enrichment levels in various human tissues for males and females, respectively. In skin, digestive system (esophagus, stomach, colon, and pancreas), brain, and blood vessel, we observed significant positive correlations between ACE2 expression levels and CD8+ T cell enrichment levels in both males and females (Pearson's correlation test, adjusted $p < 0.05$, $0.27 < r < 0.78$) (Fig. 2A). In addition, in thyroid, lungs, adrenal gland, liver, and kidney, ACE2 expression levels showed significant positive correlations with CD8+ T cell enrichment levels solely in males ($0.20 < r < 0.68$). However, in thyroid and lungs, ACE2 expression levels were negatively correlated with CD8+ T cell enrichment levels in females ($r = -0.36$). In heart, ACE2 expression levels had a negative and a positive correlation with CD8+ T cell enrichment levels in males ($r = -0.23$) and females ($r = 0.32$), respectively. Likewise, the interferon response signature had significant positive correlations with ACE2 expression levels in skin, digestive system (esophagus, stomach, liver, and pancreas), brain, and blood tissue in both males and females ($0.14 < r < 0.75$) (Fig. 2A). In thyroid, lungs, kidney, adrenal gland, colon, and bladder, ACE2 expression levels had significant positive correlations with the interferon response signature solely in males ($0.32 < r < 0.82$). In contrast, in thyroid, lungs, and colon, ACE2 expression levels were negatively correlated with the interferon response signature in females ($-0.26 < r < -0.20$). In heart, ACE2 expression levels had a negative and a positive correlation with the interferon response signature in males ($r = -0.18$) and females ($r = 0.54$), respectively. Similar results were observed for B cells and NK cells (Fig. 2A). Collectively, these results demonstrate the commonality and distinction in the association of ACE2 expression with immune signatures between males and females. Interestingly, the inflammation of the lungs (pneumonia) is the most common disease caused by coronavirus, while the correlations between its receptor expression and immune signatures in lungs are adverse between males and females. This indicates that the host immune response to coronavirus infections could be different between males and females, partially explaining why males and females have markedly distinct clinical outcomes of coronavirus infections [3, 12].

We further analyzed the correlations between ACE2 expression and immune signatures in the younger and older groups, respectively. In most individual tissues, the correlations between ACE2 expression levels

and immune signature enrichment levels displayed consistent trends between the younger and older groups. However, in lungs they had a positive and a negative correlation in the older and younger groups (Fig. 2B). Again, these results suggest the potential difference in the host immune response to coronavirus infections between young and old persons.

Methods

Datasets

We downloaded the GTEx RNA-Seq gene expression profiling datasets (RSEM normalized) for 31 human normal tissues from the UCSC Xena project (https://xenabrowser.net/datapages/), and downloaded the TCGA RNA-Seq gene expression profiling datasets (RSEM normalized) for 12 human normal tissues from the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov/). All gene expression values were added 0.001 and then log2-transformed before analysis.

Evaluation of the immune signature enrichment levels in tissue

We defined the enrichment level of an immune signature in a tissue as the mean expression levels of marker genes of the immune signature in the tissue. We analyzed two immune signatures, including CD8+ T cells, interferon response, B cells, and NK cells. The CD8+ T cell marker genes included CD2, CD247, CD28, CD3D, CD3E, CD3G, CD8A, ICAM1, ITGAL, ITGB2, PTPRC, and THY1 [15]; the interferon response marker genes included IFIT1, IFIT2, IFIT3, IRF7, ISG20, MX1, MX2, RSAD2, TNFSF10, GPR146, SELP, and AHR [16]; the B cell marker genes included BACH2, BANK1, BLK, BTLA, CD79A, CD79B, FCRL1, FCRL3, HVCN1, and RALGPS2 [16]; the NK cell marker genes included KLRC1 and KLRF1 [16].

Statistical analyses

We used Pearson's correlation test to calculate the correlations between ACE2 expression levels and immune signature enrichment levels in individual tissues, and Student's t test (two-sided) to compare ACE2 expression levels between males and females, and between younger (ages < = 49 years) and older (ages > 49 years) persons in individual tissues. The adjusted p value estimated by the Benjamini and Hochberg (BH) method [17] was used to adjust for multiple tests.

Discussion

We analyzed the expression of ACE2, the gene encoding human host cell receptor of 2019-nCoV and SARS-CoV, across 31 human normal tissues. To the best of our knowledge, this is the first study investigating ACE2 expression across a wide variety of human tissues. We found that although the inflammation of the lungs is the primary symptom of patients with 2019-nCoV infection, the ACE2 expression levels in lungs are medium among all tissues. This indicates that 2019-nCoV may affect other

tissues in addition to lungs. In fact, many cases of 2019-nCoV have confirmed the symptoms outside pneumonia, e.g., *diarrhea,* nausea, vomiting, confusion, headache, and cardiac injury [2–4]. In addition, the predominant symptoms in the lungs with 2019-nCoV infection could also be attributed to the fact that the respiratory tract is the readiest transmission approach for virus. Furthermore, we found that ACE2 expression levels showed no significant difference between males and females, between younger and older persons, as well as between Asian and other races. This indicates that the infection risk of 2019-nCoV and SARS-CoV may have no a significant association with sex, age, or race. This indication has been established for SARS-CoV [11], while it needs more data to be proved for 2019-nCoV. Nevertheless, the mortality risk for 2019-nCoV and SARS-CoV infections appears to be significantly associated with sex and age with higher risk for old versus young persons and for males versus females [3, 11].

It has been shown that the lung cells highly expressing ACE2 are more readily infected by SARS-CoV [18]. We found that the correlation between ACE2 expression and immune signatures was negative in females and in younger persons while it was positive in males and in older persons in lung tissue. Collectively, it means that: i) if the lungs highly expressing ACE2 are infected by SARS-CoV in females or young persons, they will have weaker immune signatures; ii) if the lungs highly expressing ACE2 are infected by SARS-CoV in males or old persons, they will have stronger immune signatures. This suggests that the *excessive* activation of immune response (cytokine storm or immunopathological damage) could be more likely to occur in males and old persons with SARS-CoV or 2019-nCoV infection.

Our study has several limitations. First, the findings from the bioinformatics analysis need to be further validated by experimental and clinical data. Second, our data cannot explain the epidemic divergence between 2019-nCoV and SARS-CoV in that 2019-nCoV is less virulent while more infectious than SARS-CoV. An in-depth analysis of genomic and protein structures of both coronaviruses would warrant for exploring the mechanism underlying the epidemic divergence between 2019-nCoV and SARS-CoV.

Conclusions

Our data may provide cues for understanding the associations of symptoms, sex, age, and race with 2019-nCoV infection.

List Of Abbreviations

2019-nCoV: 2019 novel coronavirus; **ACE2:** angiotensinconverting enzyme 2; **GTEx:** Genotype-Tissue Expression; **TCGA:** the Cancer Genome Atlas; **BH:** Benjamini and Hochberg; **HPA:** Human Protein Atlas.

Declarations

Ethics approval and consent to participate

Ethical approval was waived since we used only publicly available data and materials in this study.

Consent for publication

Not applicable.

Availability of data and materials

The GTEx and TCGA gene expression profiling datasets for human normal tissues were downloaded from the UCSC Xena project (https://xenabrowser.net/datapages/) and the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov/), respectively.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

ML performed data analyses and edited the manuscript. LL performed data analyses. YZ performed data analyses and edited the manuscript. XW conceived of the research, designed the methods, and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

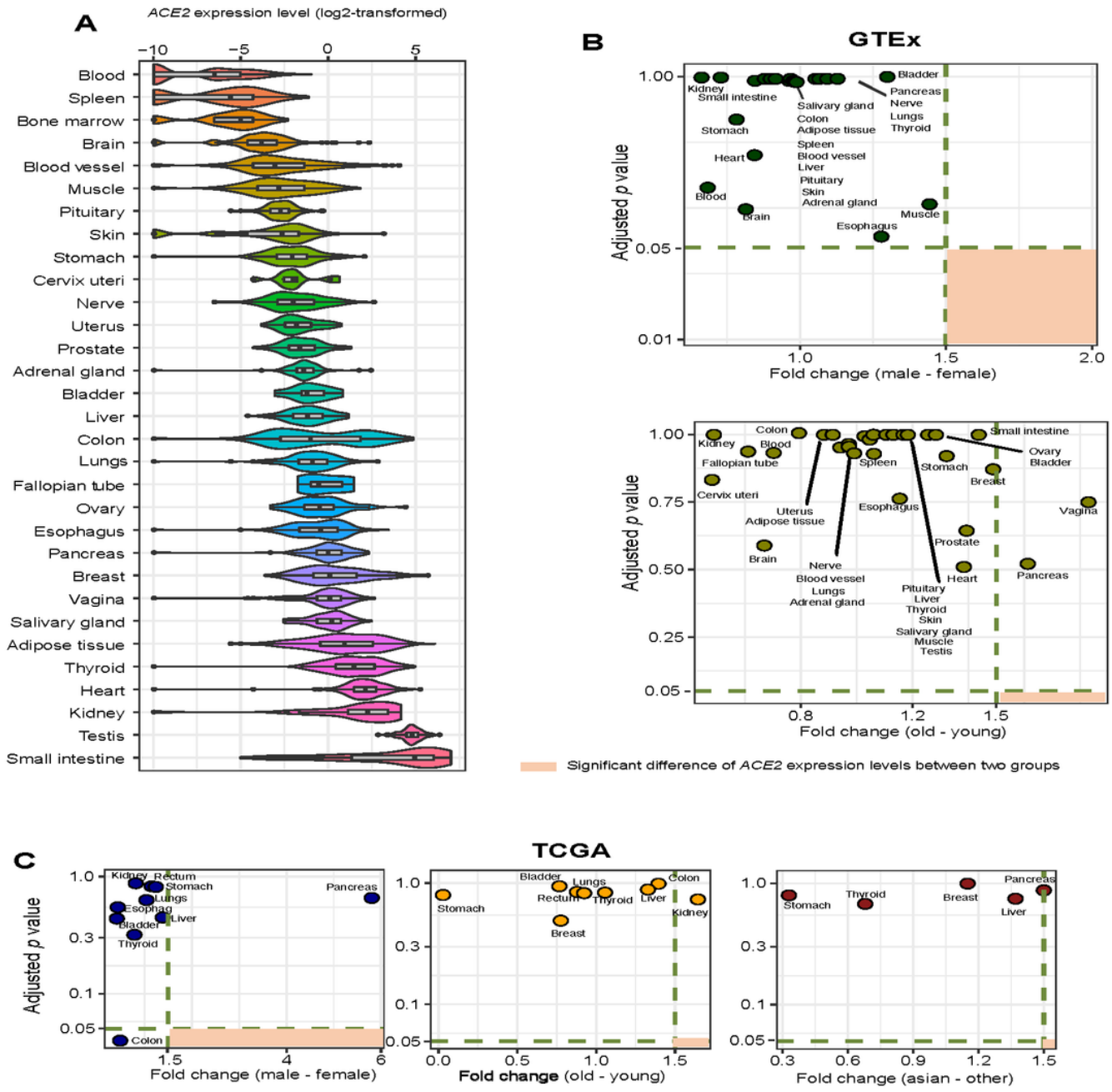

Figure 1

ACE2 expression in various human tissues. (A) Comparison of ACE2 expression levels across 31 human tissues in GTEx [10]. Comparison of ACE2 expression levels between males and females and between younger (ages <= 49 years) and older (ages > 49 years) groups in individual human tissues in GTEx (B) and TCGA (C). In TCGA, a comparison of ACE2 expression levels between Asia and other races in five normal human tissues was also performed (C). Two-sided Student's t test was used in (B, C). The adjusted p value was calculated by the Benjamini and Hochberg (BH) method [17].

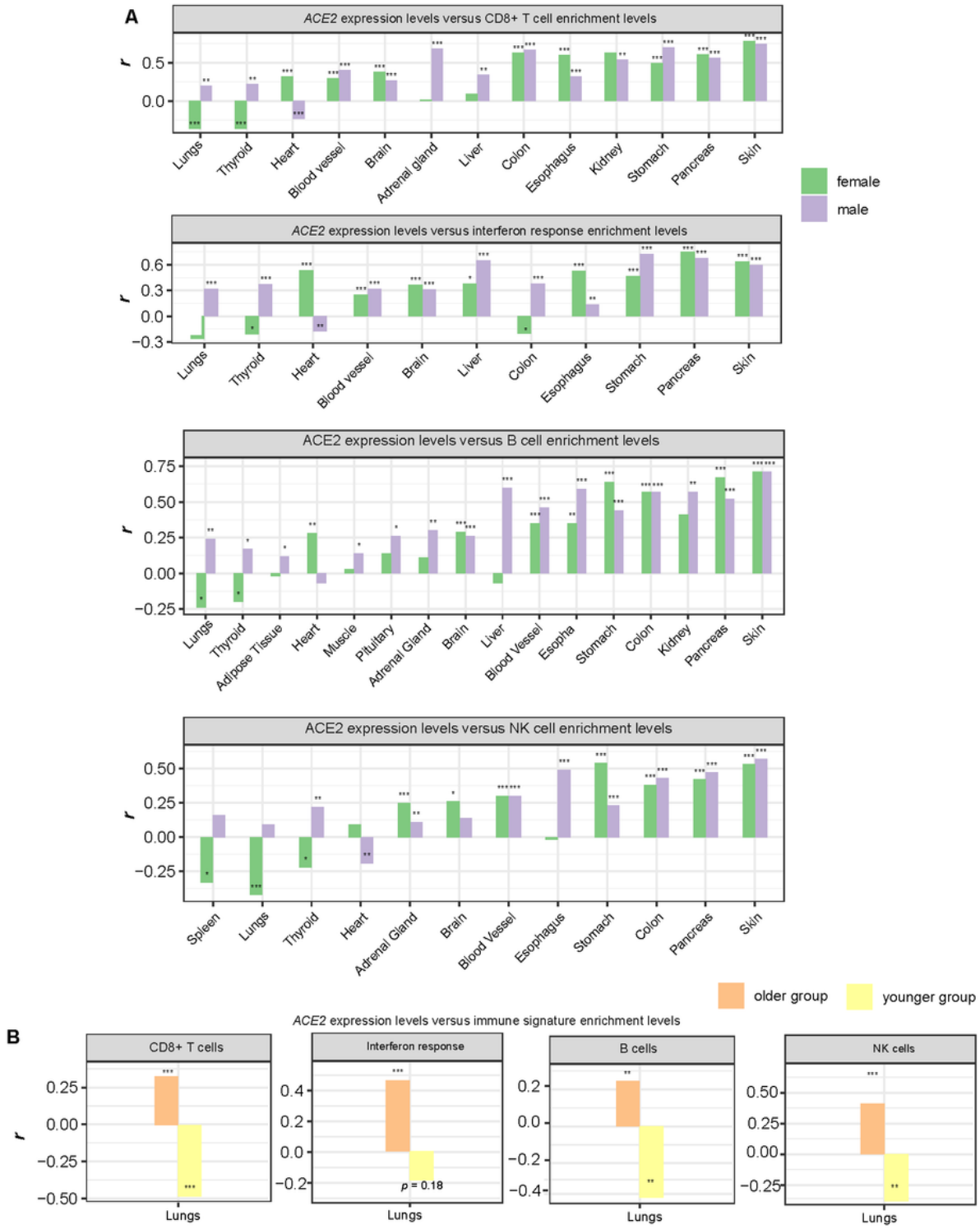

Figure 2

Association of ACE2 expression with immune signatures in various human tissues. (A) Correlation between ACE2 expression levels and immune signature enrichment levels in various human tissues of males and females. (B) Correlation between ACE2 expression levels and immune signature enrichment levels in lungs and thyroid of the older (ages > 49 years) and younger (ages <= 49 years) groups.

Pearson’s correlation test was used to calculate the correlation coefficient (r) and p value in (A, B). *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$.