Lung adenocarcinoma patients own higher risk of SARS-CoV-2 infection

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Abstract

Both lung adenocarcinoma and SARS-CoV-2 infection could cause pulmonary inflammation. Angiotensin-converting enzyme 2, not only as the functional receptor of SARS-CoV-2 but also play key role in lung adenocarcinoma. To study the risk of SARS-CoV-2 infection in lung adenocarcinoma patients, mRNA and miRNA profiles were obtained from TCGA and GEO databases followed by bioinformatics analysis. A regulatory network which regards angiotensin-converting enzyme 2 as the center would be structured. In addition, via immunological analysis about key factors in lung adenocarcinoma patients, to explore the essential reasons for the susceptibility of SARS-CoV-2. Compared with normal tissue, angiotensin-converting enzyme 2 was increased in lung adenocarcinoma patients. Furthermore, a total of 7 differently expressed correlated mRNAs (ACE2, CXCL9, MMP12, IL6, AZU1, FCN3, HYAL1 and IRAK3) and 5 differently expressed correlated miRNAs (miR-125b-5p, miR-9-5p, miR-130b-5p, miR-381-3p and miR-421) were screened followed by enrichment analysis. Interestingly, toll-like receptor signaling pathway with the most frequent occurrence was enriched by mRNA (IL6) and miRNA (miR-125b-5p) sets simultaneously. Finally through comprehensive analysis, it was assumed that miR-125b-5p-ACE2-IL6 axis in the structured regulatory network could alter risk of SARS-CoV-2 infection in lung adenocarcinoma patients.

Key words: lung adenocarcinoma, SARS-CoV-2, ACE2, miR-125b-5p, IL6.

Introduction

In December 2019, there was an outbreak of coronavirus pneumonia (COVID19) cause by SARS-CoV-2 in Wuhan, Hubei province in China. As SARS-CoV, SARS-CoV-2 also encodes spike protein and uses the same cell entry receptor and manner result in infection. Unlike many type I

fusion proteins, the S protein of coronaviruses is not cleaved in the virus-producing cell [1, 2]. However, two domains (S1 and S2) with different functions of processed coronaviruses can be defined [3]. The S1 domain mediates receptor association, whereas the S2 domain is membrane-associated and likely undergoes structural rearrangements that mediate membrane fusion. A discrete receptor-binding domain (RBD) of the S protein has been defined at residues 318-510 of the S1 domain. This RBD binds receptor with higher affinity than does the full S1 domain [4, 5].

Angiotensin-converting enzyme 2 (ACE2), a terminal carboxypeptidase, catalyzes the conversion of angiotensin II (Ang II) to angiotensin 1-7 (Ang 1-7). Ang II, the major effector molecule of the conventional renin-angiotensin system, is implicated in the pathogenesis of cardiovascular disorders, including hypertension, atherosclerosis, and myocardial infarction, whereas ACE2 and its product Ang 1-7 are thought to prevent the detrimental effects of angiotensin II [6]. In respiratory organs, ACE2 is the receptor for SARS-CoV [7, 8]. As a type I transmembrane protein, ACE2 is comprised of a short cytoplasmic domain, a transmembrane domain, and a large ectodomain [9]. A region of the ACE2 ectodomain that includes the first α-helix and lysine 353 and proximal residues of the N-terminus of β-sheet interacts with high affinity with the receptor-binding domain of the SARS-CoV S glycoprotein [10]. Furthermore, once SARS-CoV binds to ACE2, the abundance on the cell surface, mRNA expression and the enzymatic activity of ACE2 are significantly reduced. ACE2 has also been shown to attenuate inflammation and/or acute lung injury caused by SARS-CoV infection, influenza virus infection, or other etiologies mainly through inhibition of angiotensin II-mediated NF-κB signaling [11-15]. The loss of ACE2 function is implicated in SARS pathogenesis. ACE2 is predominantly localized on the apical surface of well-differentiated airway epithelia, especially ciliated cells, and also been identified with the pathology of various lung diseases like cancer and inflammatory lung disease [16-18].

Lung adenocarcinoma (LUAD) is the most common primary lung cancer which falls under the umbrella of non-small cell lung cancer (NSCLC) and has a strong association with smoking. LUAD usually evolves from the mucosal glands and represents about 40% of lung cancers. LUAD usually occurs in the lung periphery, and in many cases, may be found in scars or areas of chronic inflammation [19, 20]. The tumor microenvironment of LUAD may also regulate ACE2 expression level which alters the risk of SARS-CoV-2 infection. However, there are lots of factors could cause ACE2 level alteration which also leads to immunoregulation. Thus, through bioinformatics analysis

and network structuring, the potential mechanism of COVID19 in LUAD might be explored.

Methods

Sequencing Datasets

Dataset GSE74190 was acquired from GEO Dataset. All experiments were approved by the local ethics committee. Expression profiles of 723 human miRNAs were investigated in selected cancerous cell populations and normal cells derived from 36 LUAD and 44 adjacent normal tissues. ALL cells derived from 82 snap-frozen surgical specimens. The platform is Agilent-019118 Human miRNA Microarray V1 G4470A [miRBase release 9.1 miRNA ID version].

MiRNA target gene prediction and analysis of affected signaling pathway

The potential target genes of differentially expressed miRNAs in GCs were acquired from the widely used online databases TargetScanHuman 7.2 [21] and DIANA-microT [22]. In order to reduce false positives, the predicted target genes which appeared at both databases were accepted. Following this, the list of predicted target genes of individual miRNAs was imported to DIANA-mirPath, a miRNA pathway analysis web server. Lists of canonical pathways significantly affected by individual differentially expressed miRNAs were made a contrast and we obtained the common pathways of which elements were as targets of deregulation miRNAs. Following these canonical pathways were identified from the Kyoto Encyclopaedia of Genes and Genomes (KEGG) databases.

Microarray data and enrichment analysis

Total RNA from tissues was isolated using Trizol extractions (Invitrogen). The RNA quantity was assessed by NanoDrop®ND-1000 spectrophotometer and RNA 6000 NanoChips with the Agilent 2100 Bioanalyzer (Agilent, Palo Alto, USA). On the one hand, 100 ng of total RNA was amplified using the Ambion® WT Expression Kit (4411973, Life Technologies). Small RNA libraries were prepared using 1μg of total RNA according to the TruSeq Small RNA Sample Preparation Guide (Illumina, San Diego, CA). To generate count data, the raw sequences were compared to human mature miRNA sequences (from miRBase version 17) and non-coding RNA sequences (Rfam version 10) by MEGABLAST.

Background deletion, quantile normalization, and probe assembly were performed. Different expression miRNAs between normal vs. LUAD samples were detected by the R package DESeq [23]. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure [24]. MiRNAs with adjusted P-value of < 0.05 and logFC ≥ 2.0 were considered as differentially expressed. Gene and miRNA enrichment analysises were performed with DAVID version 6.7 and DIANA- mirPATH v.3 respectively. The enriched biological GO and pathway terms were identified [25]. The interaction network was drawn by Cytoscape. Some other databases used are listed in Table 1.

Table1. List of Database

Database ID	URL
GEO Dataset	https://www.ncbi.nlm.nih.gov/gds/?term=
TCGA	https://www.cancer.gov/
cBioportal of cancer genomics	https://www.cbioportal.org/
DSA	http://cancer.digitalslidearchive.net/
The Human Protein Atlas	https://www.proteinatlas.org/
Linked Omics	http://www.linkedomics.org/
Targetscan	http://www.targetscan.org/vert_72/
OncomiR	http://www.oncomir.org/oncomir/index.html
DAVID	https://david.ncifcrf.gov/
DIANA-mirPATH v.3	http://diana.imis.athena-innovation.gr/DianaTools/index.php
DIANA-microT	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index
TIMER	https://cistrome.shinyapps.io/timer/
STRING	https://string-db.org/
GEPIA	http://gepia.cancer-pku.cn/index.html
Pathview	https://pathview.uncc.edu/
TISIDB	http://cis.hku.hk/TISIDB/index.php

Statistical analyses

Results are presented as mean values ± standard error of the mean (SEM). Unless mentioned

otherwise, the statistical comparison between groups was performed by using t-test, a maximum of three comparisons were performed per panel, and robustness of statistical significance was verified after correction for multiple testing. Probability was considered to be significant at $p < 0.05$.

Result

The expression level of ACE2 in LUAD

Ang 1-7, the product of ACE2, was confirmed to inhibit the proliferation of human lung cancer cells through interaction with the Mas receptor (MasR) [26]. Ang1-7 not only reduces the size of human lung tumor xenografts in vivo but also markedly decreases their vessel density [27-29]. Thus, the expression level of ACE2 in LUAD would determine the cancer progression. To compare the ACE2 level of LUAD vs. normal, sequencing data of a total of 830 samples (347 normal tissues and 483 LUAD tissues) were obtained from TCGA database **(Figure 1)**. In LUAD patients, a higher expression level of ACE2 was detected when compared with normal tissues while there was no significant difference among various stages of cancer. Due to the function of combining with S glycoprotein, higher ACE2 level might lead to increased risk of SARS-CoV-2 infection.
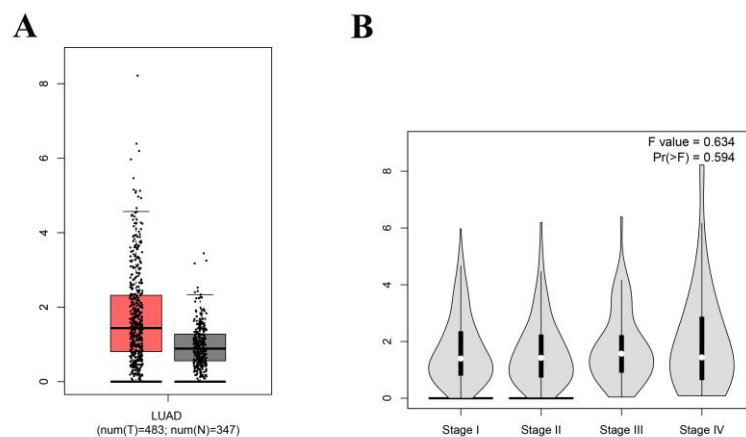

Figure 1. The expression level of ACE2. A, The box-plot showed the comparison of ACE2 mRNA level in normal tissues vs. LUAD tissues. The red samples represent tissues from LUAD patients and the gray ones represent tissues from health persons. **B,** The violin plot showed the expression level of ACE2 in different stages of LUAD. The parameters were listed in the upper right.

The regulatory network revolves around ACE2

The fluctuation of gene expression often involves complicated regulatory networks. To study the potential mechanism of ACE2 dysregulation, the relevant molecular around ACE2 were

identified. After screening and comparison, 7 differentially expressed correlative genes (DECGs) and 5 differentially expressed correlative miRNAs (DECMs) were detected. In addition, the DECGs (CXCL9, MMP12, IL6, AZU1, FCN3, HYAL1, IRAK3) were involved in multi defense processes of virus and the DECMs (miR-125b-5p, miR-9-5p, miR-130b-5p, miR-381-3p, miR-421) were predicted to target the 3' UTR of ACE2 **(Figure 2 and S1)**. Among them, CXCL9, MMP12, miR-9-5p, miR-130b-5p, miR-381-3p and miR-421 were up-regulated while IL6, AZU1, FCN3, HYAL1, IRAK3 and miR-125b-5p were down-regulated in LUAD patients. Meanwhile, AZU1, FCN3, HYAL1, IRAK3 and miR-125b-5p were positively correlated with ACE2 while CXCL9, MMP12, IL6, miR-9-5p, miR-130b-5p, miR-381-3p, and miR-421 were negatively correlated **(Figure 3)**. Notably, only miR-125b-5p own opposite expression trend with ACE2. Thus, miR-125b-5p was assumed to be the single upstream predicted regulator of ACE2. In addition, among the 7 DECGs, IL6 owns the highest mutation frequency (5%) in LUAD patients might indicate that IL6 could play key role in carcinogenesis **(Figure S2)**.
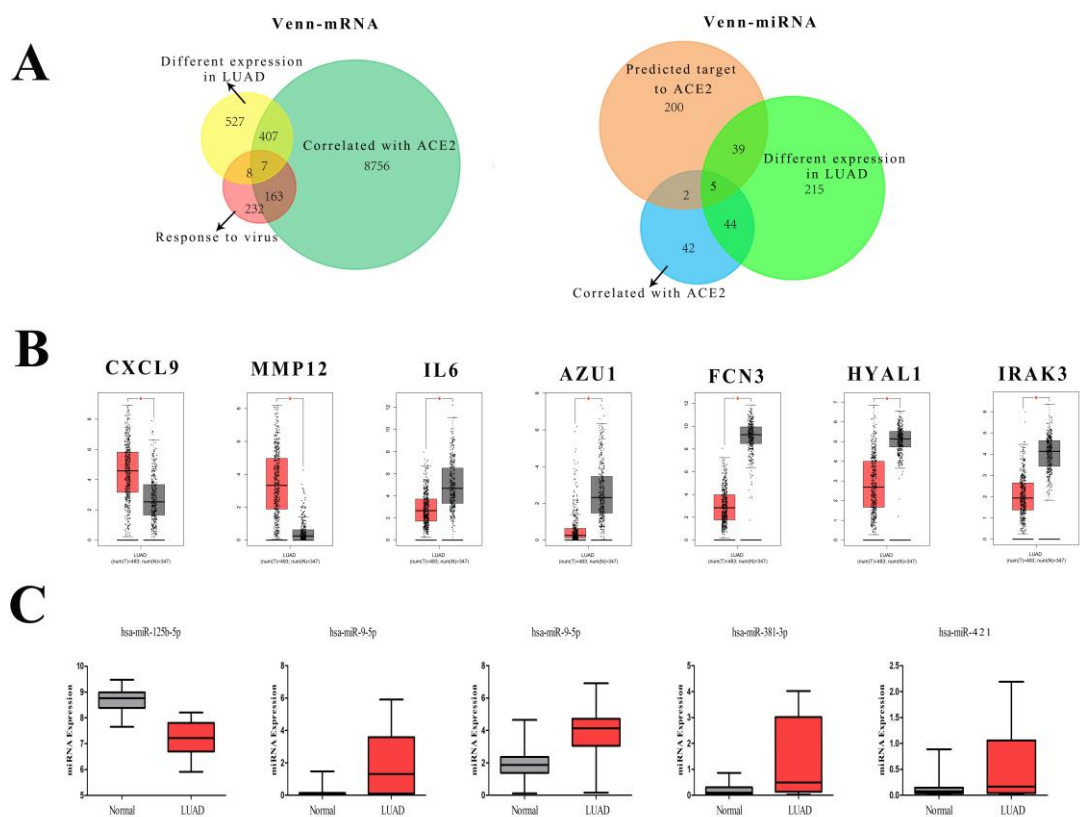

Figure 2. The definition of DECGs and DECMs. A, Two venn-plots showed the sifting process of DECGs (left) and DECMs (right). The yellow circle represents the differentially expressed mRNA in LUAD. The green circle represents the genes correlated with ACE2 in LUAD. The carnation circle represents the gene set involved in virus

defense. The orange circle represents the miRNAs which could bind to 3'-UTR of ACE2. The aqua circle represents

the differentially expressed miRNAs in LUAD. The blue circle represents the miRNAs correlated with ACE2 in

LUAD. **B and C,** The box-plots showed the comparison of DECGs or DECMs level in normal tissues vs. LUAD

tissues. The red samples represent tissues from LUAD patients and the gray ones represent tissues from health
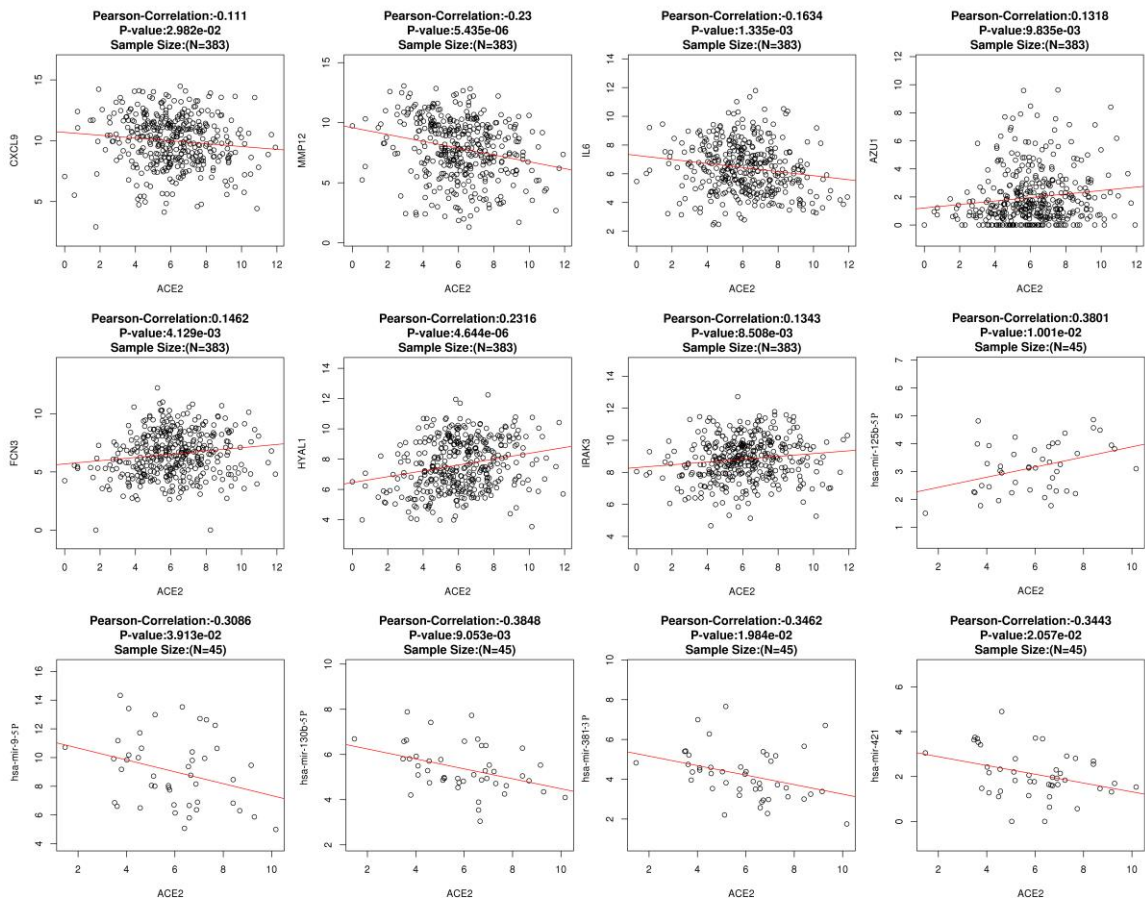
persons.

Figure 3. The correlation between ACE2 and DECGs or DECMs. Each hollow circle represents a single sample.

The red line represents the correlation. A total of 383 and 45 samples were obtained to calculated correlation between

ACE2 and DECGs or DECMs, respectively.

To understand the functional involvement of the DECGs and DECMs in LUAD, enrichment

analysis was performed **(Figure 4)**. A total of 41 pathways and 129 GO functions were enriched

based on DECGs and DECMs respectively. Interestingly, both DECGs (CXCL9, IL6) and DECMs

(miR-125b-5p, miR-9-5p, miR-130b-5p, and miR-421) were enriched in toll-like receptor signaling

pathway **(Figure S3)**. In the pathway, CXCL9 would take part in chemotactic effects of T cells and

IL6 participates in proinflammatory effects. According to the relations between DECGs, DECMs and virus-related processes, an interaction network was constructed **(Figure 5)**. Notably, not only ACE2 but IL6 were predicted as the targets of miR-125b-5p. Similarly, both ACE2 and CXCL9 were regarded as the predicted target of miR-9-5p. However, due to the expression trend of CXCL9 and miR-9-5p were not suitable with ACE2, it was suggested that both CXCL9 and miR-9-5p may not play a decisive role in ACE2 expression.
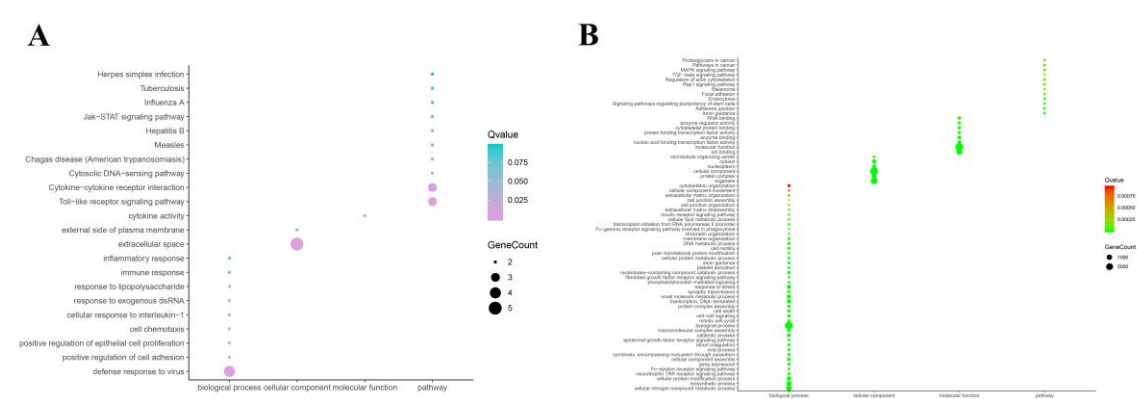

Figure 4. Enrichment analysis of DECGs (A) and DECMs (B). Each point represents the enriched pathway or GO item. The color gradient represents significance and the point size represents the count of mRNAs which were involved in each pathway or GO item.
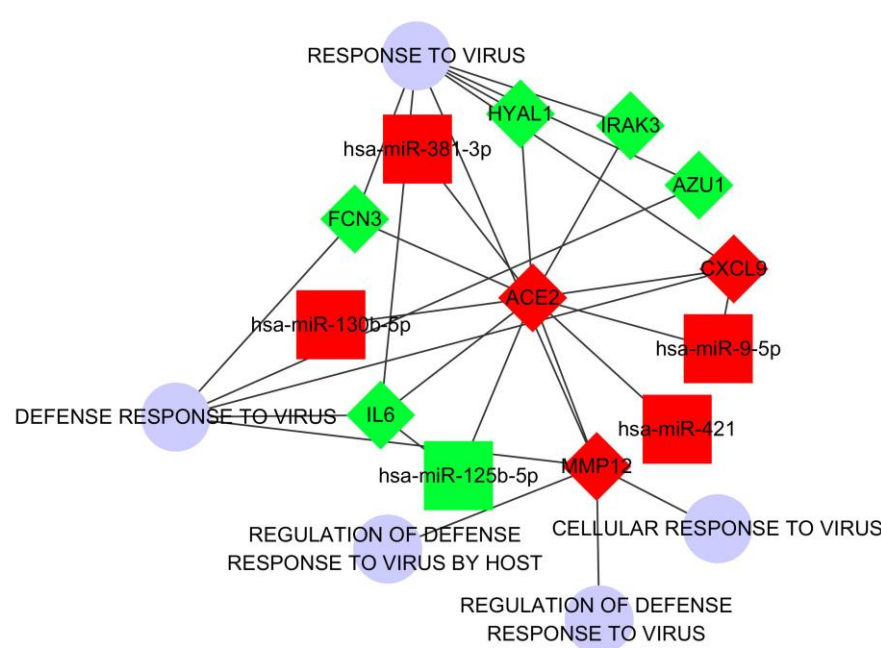
Figure 5. The regulatory network which regards ACE2 as the center. The squares represent DECMs. The rhombus represents DECGs. The blue circles represent biological processes correlated with virus defense. The red nodes represent up-regulation of DECGs or DECMs. The green nodes represent down-regulation of DECGs or DECMs. The edges between every 2 nodes represent subordination or interactive relationship.

Immunological function of ACE2 and IL6

In conclusion, the reduced miR-125b-5p might be the primary regulator of ACE2 in LUAD. Once ACE2 was dysregulated, IL6 in the toll-like receptor signaling pathway might influence the immune system as the downstream effector. No matter defense of SARS-CoV-2 or pneumonia in LUAD, the altered immunoreaction was the primary cause. Thus, the ability to regulate immune system of ACE2 and IL6 was evaluated based on lymphocyte, immune-inhibitor, immune-stimulator, MHC molecule, chemokine, and chemokine receptor (**Figure 6**). It was confirmed that the expression level, copy number and methylation of ACE2 and IL6 would regulate immunoreaction. Interestingly, CXCL9 could mediate immunoreaction as a chemokine which also is influenced by ACE2 and IL6 (expression level and methylation). Therefore, even CXCL9 might not participate in regulating ACE2 level, it would like to mediate immune-regulation of ACE2 and IL6.
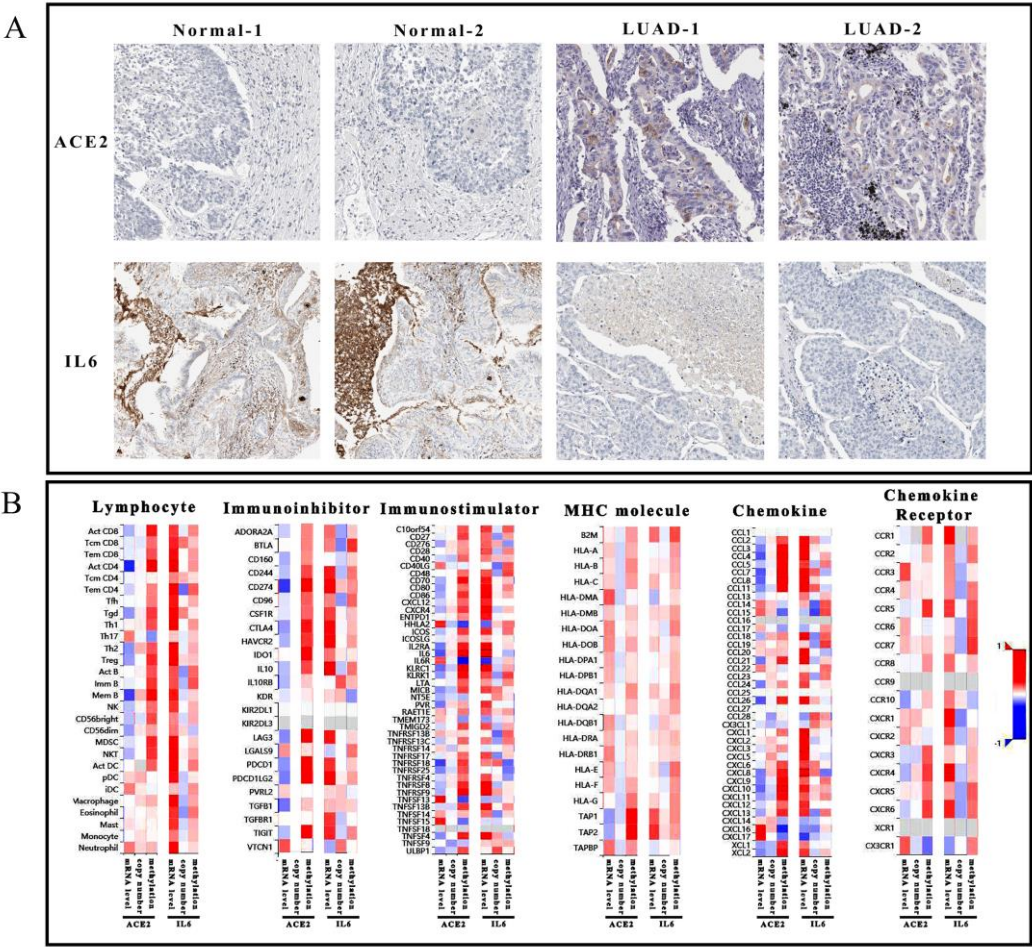
Figure 6. Immunological analysis of ACE2 and IL6. A, The pathological sections of tumor tissue and normal tissue which was stained by ACE2 and IL6 antibody. Each group has 2 duplicate samples. **B,** The heatmap showed the correlation of 6 immunological factors (lymphocyte, immune-inhibitor, immune-stimulator, MHC molecule, chemokine, and chemokine receptor) vs. mRNA expression, copy number and methylation of ACE2 and IL6.

Conclusion

Once infected with SARS-CoV-2, the pathogenic T cells were activated rapidly followed by immune response and virus eliminating. However, the fatal cause of viral pneumonia was the uncontrolled inflammation. ACE2, not only as the functional receptor of coronavirus, has emerged as a potent negative regulator of renin-angiotensin system (RAS) which maintaining homeostasis of blood pressure and inflammatory responses [30]. Altered activation of the RAS is attributed to the pathogenesis of many diseases such as hypertension, myocardial infarction and inflammatory lung disease [31, 32]. In murine ARDS models, it was confirmed that the lack of ACE2 expression in the lung resulted in attenuated vascular permeability, enhanced lung edema, neutrophil infiltration,

and further deteriorated lung function [33].Thus, though higher ACE2 level would increase the risk of SARS-CoV-2 infection, the normal level of ACE2 would also improve prognosis of patients with acute lung injury.

The RAS contained ACE2 play role in maintaining homeostasis of inflammatory responses. However, once SARS-CoV-2 binds to ACE2, the abundance on the cell surface, mRNA expression and the enzymatic activity of ACE2 are significantly reduced and lead to the inflammatory storm. Coincidentally, IL6 was the main factor which could induce inflammatory storm. Once infected with SARS-CoV-2, the activated pathogenic T cells could produce granulocyte-macrophage colony-stimulating factors (GM-CSF) and IL6. The GM-CSF could further activate CD14+CD16+ inflammatory monocytes followed by more cytokines production include IL6 via positive feedback loop. A large concentration of immune cells and tissue fluid in the lungs can block gas exchange between alveoli and capillaries, leading to acute respiratory distress syndrome. Once a cytokine storm forms, the immune system would kill lots of normal cells in the lung while eliminating the virus, severely damaging the lung's ventilation function. In a word, similar to ACE2, IL6 play key role in defense of SARS-CoV-2 with normal expression level. Once deregulated, IL6 will become a lethal factor for patients.

Conventionally, the LUAD patients have lower immunity than health persons. The increased ACE2 level in lung might lead to susceptibility to infection. Nevertheless, the pathology of COVID19 is complicated, except miRNA introduced in present study, transcription factor, lncRNA and ceRNA may participate in mechanism of COVID. The result of our study may provide a reference for further research.

Reference

[1] Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS. The SARS-CoV S glycoprotein: expression and functional characterization. Biochemical and biophysical research communications. 2003;312(4):1159-1164.

[2] Moore MJ, Dorfman T, Li W, Wong SK, Li Y, Kuhn JH, Coderre J, Vasilieva N, Han Z, Greenough TC, Farzan M, Choe H. Retroviruses pseudotyped with the severe acute respiratory syndrome coronavirus spike protein efficiently infect cells expressing angiotensin-converting enzyme 2. Journal of virology. 2004;78(19):10628-10635.

[3] Gallagher TM, Buchmeier MJ. Coronavirus spike proteins in viral entry and pathogenesis. Virology. 2001;279(2):371-374.

[4] Wong SK, Li W, Moore MJ, Choe H, Farzan M. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. The Journal of biological

chemistry. 2004;279(5):3197-3201.

[5]　Babcock GJ, Esshaki DJ, Thomas WD, Jr., Ambrosino DM. Amino acids 270 to 510 of the severe acute respiratory syndrome coronavirus spike protein are required for interaction with receptor. Journal of virology. 2004;78(9):4552-4560.

[6]　Jiang F, Yang J, Zhang Y, Dong M, Wang S, Zhang Q, Liu FF, Zhang K, Zhang C. Angiotensin-converting enzyme 2 and angiotensin 1-7: novel therapeutic targets. Nature reviews Cardiology. 2014;11(7):413-426.

[7]　Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. The Journal of pathology. 2004;203(2):631-637.

[8]　Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nature medicine. 2005;11(8):875-879.

[9]　Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. The Journal of biological chemistry. 2000;275(43):33238-33243.

[10] Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, Wong SK, Huang IC, Xu K, Vasilieva N, Murakami A, He Y, Marasco WA, Guan Y, Choe H, Farzan M. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. The EMBO journal. 2005;24(8):1634-1643.

[11] Jia HP, Look DC, Tan P, Shi L, Hickey M, Gakhar L, Chappell MC, Wohlford-Lenane C, McCray PB, Jr. Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. American journal of physiology Lung cellular and molecular physiology. 2009;297(1):L84-96.

[12] Liu Z, Huang XR, Chen HY, Penninger JM, Lan HY. Loss of angiotensin-converting enzyme 2 enhances TGF-beta/Smad-mediated renal fibrosis and NF-kappaB-driven renal inflammation in a mouse model of obstructive nephropathy. Laboratory investigation; a journal of technical methods and pathology. 2012;92(5):650-661.

[13] Meng Y, Yu CH, Li W, Li T, Luo W, Huang S, Wu PS, Cai SX, Li X. Angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas axis protects against lung fibrosis by inhibiting the MAPK/NF-kappaB pathway. American journal of respiratory cell and molecular biology. 2014;50(4):723-736.

[14] Zou Z, Yan Y, Shu Y, Gao R, Sun Y, Li X, Ju X, Liang Z, Liu Q, Zhao Y, Guo F, Bai T, Han Z, Zhu J, Zhou H, Huang F, Li C, Lu H, Li N, Li D, Jin N, Penninger JM, Jiang C. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. Nature communications. 2014;5:3594.

[15] Tao L, Qiu Y, Fu X, Lin R, Lei C, Wang J, Lei B. Angiotensin-converting enzyme 2 activator diminazene aceturate prevents lipopolysaccharide-induced inflammation by inhibiting MAPK and NF-kappaB pathways in human retinal pigment epithelium. Journal of neuroinflammation. 2016;13:35.

[16] Yamaguchi M, Hirai S, Sumi T, Tanaka Y, Tada M, Nishii Y, Hasegawa T, Uchida H, Yamada G, Watanabe A, Takahashi H, Sakuma Y. Angiotensin-converting enzyme 2 is a potential therapeutic target for EGFR-mutant lung adenocarcinoma. Biochemical and biophysical research communications. 2017;487(3):613-618.

[17] Qian YR, Guo Y, Wan HY, Fan L, Feng Y, Ni L, Xiang Y, Li QY. Angiotensin-converting enzyme 2 attenuates the metastasis of non-small cell lung cancer through inhibition of epithelial-mesenchymal transition. Oncology reports. 2013;29(6):2408-2414.

[18] Jia H. Pulmonary Angiotensin-Converting Enzyme 2 (ACE2) and Inflammatory Lung Disease.

Shock (Augusta, Ga). 2016;46(3):239-248.

[19] Li C, Lu H. Adenosquamous carcinoma of the lung. OncoTargets and therapy. 2018;11:4829-4835.

[20] Myers DJ, Wallen JM. Cancer, Lung Adenocarcinoma.   StatPearls. Treasure Island (FL): StatPearls Publishing

StatPearls Publishing LLC.; 2020.

[21] Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003;115(7):787-798.

[22] Kiriakidou M, Nelson PT, Kouranov A, Fitziev P, Bouyioukos C, Mourelatos Z, Hatzigeorgiou A. A combined computational-experimental approach predicts human microRNA targets. Genes & development. 2004;18(10):1165-1178.

[23] Anders S, Huber W. Differential expression analysis for sequence count data. Genome biology. 2010;11(10):R106.

[24] Klipper-Aurbach Y, Wasserman M, Braunspiegel-Weintrob N, Borstein D, Peleg S, Assa S, Karp M, Benjamini Y, Hochberg Y, Laron Z. Mathematical formulae for the prediction of the residual beta cell function during the first two years of disease in children and adolescents with insulin-dependent diabetes mellitus. Medical hypotheses. 1995;45(5):486-490.

[25] Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols. 2009;4(1):44-57.

[26] Raizada MK, Ferreira AJ. ACE2: a new target for cardiovascular disease therapeutics. Journal of cardiovascular pharmacology. 2007;50(2):112-119.

[27] Bernardi S, Zennaro C, Palmisano S, Velkoska E, Sabato N, Toffoli B, Giacomel G, Buri L, Zanconati F, Bellini G, Burrell LM, De Manzini N, Fabris B. Characterization and significance of ACE2 and Mas receptor in human colon adenocarcinoma. Journal of the renin-angiotensin-aldosterone system : JRAAS. 2012;13(1):202-209.

[28] Gallagher PE, Tallant EA. Inhibition of human lung cancer cell growth by angiotensin-(1-7). Carcinogenesis. 2004;25(11):2045-2052.

[29] Menon J, Soto-Pantoja DR, Callahan MF, Cline JM, Ferrario CM, Tallant EA, Gallagher PE. Angiotensin-(1-7) inhibits growth of human lung adenocarcinoma xenografts in nude mice through a reduction in cyclooxygenase-2. Cancer research. 2007;67(6):2809-2815.

[30] Balakumar P, Jagadeesh G. A century old renin-angiotensin system still grows with endless possibilities: AT1 receptor signaling cascades in cardiovascular physiopathology. Cellular signalling. 2014;26(10):2147-2160.

[31] del Castillo Rueda A, Guerrero Sanz JE, Escalante Cobo JL, Grau Carmona T, de Portugal Alvarez J. [Serum and pulmonary angiotensin converting enzyme as a marker of acute lung injury in an experimental model of adult respiratory distress syndrome]. Anales de medicina interna (Madrid, Spain : 1984). 1999;16(5):229-235.

[32] Gonzalez NC, Allen J, Schmidt EJ, Casillan AJ, Orth T, Wood JG. Role of the renin-angiotensin system in the systemic microvascular inflammation of alveolar hypoxia. American journal of physiology Heart and circulatory physiology. 2007;292(5):H2285-2294.

[33] Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436(7047):112-116.