High Expression of *ACE2* and *TMPRSS2* at the Resection Margin Makes Lung Cancer Survivors Susceptible to SARS-CoV-2 with Unfavorable Prognosis

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Short Report

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Abstract

Coronavirus disease 2019 (COVID-19) has rapidly spread worldwide. Systematic analysis of lung cancer survivors at molecular and clinical levels is warranted to understand the disease course and clinical characteristics. We performed a retrospective study of 65 patients with COVID-19 from Wuhan Huoshenshan Hospital, of which 13 patients were diagnosed with lung cancer. Duringtreatment, lung cancer survivors infected with severe acute respiratory syndrome coronavirus 2 had a shorter median time from symptom onset to hospitalization (P=0.016) and longer clinical symptom remission time (P=0.020) than non-cancer individuals. No differences were observed among indicators such as time from symptom onset to hospitalization and symptom remission time between long-term and short-term survivors. The expression of *ACE2*(P=0.013) and *TMPRSS2*(P<0.001) was elevated in lung cancer survivors as compared with that in non-cancer individuals.

Results

Demographic and clinical characteristics of patients

Records of 3,057 patients with confirmed Coronavirus disease 2019 (COVID-19) infection were collected from the Wuhan Huoshenshan Hospital between February 4 and April 11, 2020. Thirteen patients (0.43%) who suffered from lung cancer and 52 matched patients were enrolled. The demographic and clinical features of these patients are shown in **Table 1**. The median (IQR) age of lung cancer patients was 65 (63-72) years, and 10 (76.9%) of them were men. The most common comorbidities were hypertension and diabetes observed in 30.8% patients. No significant differences were found in age, sex, and main symptoms and signs between the case and control groups. Further, the most prevalent symptom among the 65 enrolled patients was fever (n=50, 76.9%), followed by cough (n=41, 63.1%), fatigue (n=30, 46.2%), and shortness of breath (n=26, 40.0%).

Lung cancer patients were more likely to have dyspnea (15.4% vs. 1.9%; P=0.040) than the other groups (**Table S1)**. Five of these patients were long-term survivors, and none of them was diagnosed with stage IV cancer. All patients received at least one kind of antitumor treatment, including surgery (n=7, 53.8%), chemotherapy (n=6, 46.2%), epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) therapy (n=2, 15.4%), anti-angiogenesis therapy (n=1, 7.7%), and radiotherapy (n=2, 15.4%), while none of the long-term survivors received treatment within past 3 months. The percentage of long-term survivors who underwent surgery was four-fold higher than that of short-term survivors (100.0% vs. 25.0%, P=0.011). No difference was observed in comorbidities and symptoms between long- and short-term survivors (**Table S1**).

Clinical outcomes

During treatment, development of severe infection was more common among lung cancer patients (Wilcoxon's rank-sum test, *P*=0.064) (**Table 2**). The duration of symptoms before hospital admission in lung cancer patients was 10.5 (10.0-17.5) days, which was significantly shorter than that observed in other patients (30.0 [14.0-35.0]; Wilcoxon's rank-sum test, *P*=0.016) (**Table 2**). Moreover, the average time to clinical improvement in lung cancer patients was 12 (11.0-18.0) days, which was 4 days longer than that observed in non-cancer patients (5.8-14.0) (Wilcoxon's rank-sum test, *P*=0.020) (**Table 2**). The mortality (7.7%, 1/13) observed in our study was higher than that reported in the general population (2.3%) and lower than that (18.18%; 4/22) noted in a multicenter study[1,2]. There were no differences among indicators such as time from symptoms to hospitalization and symptom remission time between long-term and short-term survivors (**Table S2**). These findings suggested that lung cancer patients represent a highly vulnerable group to the current COVID-19 outbreak.

ACE2 and *TMPRSS2* are overexpressed at resection margins of lung cancer patients

Several studies have shown that coronaviruses enter cells via binding of the viral spike (S) proteins to cellular receptors *ACE2* and following S protein priming by host cell proteases. *TMPRSS2* as a transmembrane protease can induce the virus-plasma membrane fusion[3,4]. Hoffmann and coworkers recently demonstrated that Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses the SARS-CoV receptor *ACE2* for entry and the serine protease *TMPRSS2* for S protein priming[5,6]. *ACE2* is broadly expressed in epithelial cells[7]. We then evaluated if resection margins of lung cancer harbor more epithelial cells. We collected three resection margin tissues from lung cancer and three other non-cancerous lung tissues (**Table S3**). We performed IHC to investigate the population of epithelial cells among the two groups, and found that the percentage of the stained area was much higher in the resection margin tissues than in the non-cancerous tissues (**Figure 1A, B**).

We analyzed single-cell sequencing data from the existing research[8] and found that the epithelial cells at the resection margin of lung cancer were more likely to highly activate the genes related to lung cancer, such as *KRAS*, *MET*, and *EGFR* (**Figure 1C, D**). The genomic instability of these cells inferred by inferCNV in the resection margin was much higher than that in the normal tissues **(Figure 1E**), and these cells had stronger capability for invasion and infiltration (**Figure 1F**). These findings suggested that the cells at the margin of resection were more likely to be a sort of tumor-like cells.

The proportion of epithelial cells expressing *ACE2* and *TMPRSS2* was higher at the resection margin of lung cancers than in normal tissues **(Figure 1G**), suggesting that the resection margins of lung cancer tissues were still more susceptible to COVID-19 infection. We also analyzed the expression of *ACE2* in resection margin tissues of lung cancer survivors and normal lung tissues from general individuals using The Cancer Genome Atlas (n=110) and GTEx (n=288) databases and found that the mRNA expression of *ACE2* was higher in lung cancer patients than in general individuals (Wilcoxon's rank-sum test, *P*=0.013) (**Figure 1H**). The expression of *TMPRSS2*, the co-factor of *ACE2*, was also significantly higher in lung cancer patients than in the general population (Wilcoxon's rank-sum test, *P*<0.001) (**Figure 1H**), suggesting that patients with lung cancer were more likely to be susceptible to COVID-19.

Conclusions

This study revealed the high expression of the SARS-CoV-2 receptors, *ACE2* and *TMPRSS2*, at resection margins of lung cancer survivors and its possible relationship with the higher susceptibility of these patients to COVID-19. Clinical data revealed that lung cancer patients, including long-term survivors, diagnosed with COVID-9 infection may have worse outcomes and should be carefully considered.

Abbreviations

COVID-19: Coronavirus disease 2019

SARS-COV-2: Severe acute respiratory syndrome coronavirus 2

ACE2: Angiotensin-converting enzyme 2

TMPRSS2: Transmembrane protease serine 2

IQR: Interquartile range

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Ethical Committee of Wuhan Huoshenshan Hospital (HSSLL011), and the Ethical Committee of Nanjing Medical University (2020-511). Written informed consent was obtained from each patient.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

Software and resources used for the analyses are described in supplementary file.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

QW, XX, ZZ, and RG had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. QW, LL, TQ, and LW contributed equally. Concept and design: QW, XX, ZZ, and RG. Data collection: XX, QW, TQ, and ZZ. Data analysis and interpretation: LL, LW, KL, ZW, MZ, BH, WW, MW, and RD. Drafting of the manuscript: QW, LL, TQ, and LW. All authors approved the final manuscript.

Availability of supporting data

Not applicable.

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Authors' information

Qianqian Wang, Liangyu Li, Lingxiang Wu, and Tianyu Qu contributed equally to this work.

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Tables

Table 1. Characteristics of patients with COVID-19

Characteristic	All patients (*n*=65)	Lung cancer (*n*=13)	Non-lung cancer (*n*=52)	*P* value
Age , years	66(56-72)	65(63-72)	66(56-72)	0.761
Sex				0.779
Male	48(73.8%)	10(76.9%)	38(73.1%)	
Female	17(26.2%)	3(23.1%)	14(26.9%)	
Comorbidities				
Any	38(58.5%)	8(61.5%)	30(57.7%)	0.803
Hypertension	22(33.8%)	4(30.8%)	18(34.6%)	0.795
Diabetes	17(26.2%)	4(30.8%)	13(25.0%)	0.674
Chronic obstructive pulmonary disease	3(4.6%)	1(7.7%)	2(3.8%)	0.557
Coronary heart disease	3(4.6%)	1(7.7%)	2(3.8%)	0.557
Cerebrovascular disease	5(7.7%)	1(7.7%)	4(7.7%)	1.000
Chronic liver disease	4(6.2%)	3(23.1%)	1(1.9%)	0.005
Symptoms and signs				
Fever	50(76.9%)	9(69.2%)	41(78.8%)	0.465
Chill	13(20.0%)	2(15.4%)	11(21.2%)	0.644
Chest pain	1(1.5%)	0(0.0%)	1(1.9%)	0.617
Cough	41(63.1%)	8(61.5%)	33(63.4%)	0.257
Fatigue	30(46.2%)	4(30.8%)	26(50.0%)	0.217
Shortness of breath	26(40.0%)	5(38.5%)	21(40.4%)	0.126
Chest tightness	7(10.8%)	3(23.1%)	4(7.7%)	0.112
Expectoration	10(15.4%)	3(23.1%)	7(13.5%)	0.394
Dyspnoea	3(4.6%)	2(15.4%)	1(1.9%)	**0.040**
Diarrhea	3(4.6%)	1(7.7%)	2(3.8%)	0.557
Headache	3(4.6%)	0(0.0%)	3(5.8%)	0.379
Myalgia	12(18.5%)	3(23.1%)	9(17.3%)	0.634
Nausea	1(1.5%)	0(0.0%)	1(1.9%)	0.617
Vomiting	2(3.1%)	0(0.0%)	2(3.8%)	0.476

Table 2. Outcome of lung cancer patients and general population

Characteristic	All (n=65)	Lung cancer (n=13)	Non-lung cancer (n=52)	P value
Hospital stay (days)	11.0(8.0-18.0)	13.0(11.0-18.0)	10.0(7.0-17.3)	0.178
Most critical type during hospitalization				0.064
Mild / Moderate	29(44.6%)	3(23.1%)	27(51.9%)	
Severe / Critical	35(53.8%)	10(76.9%)	25(48.1%)	
Time from symptoms to hospitalization (days)	19.0(10.0-35.0)	10.5(10.0-17.5)	30.0(14.0-35.0)	**0.016**
Clinical symptoms remission time (days)	9.0(6.0-15.0)	12.0(11.0-18.0)	8.0(5.8-14.0)	**0.020**
Admission to intensive care unit				0.433
Yes	4(6.2%)	2(15.4%)	2(3.8%)	
No	61(93.8%)	11(84.6%)	50(96.2%)	
ICU stay (days)	16.5(13.5-21.5)	13.5(11.3-15.8)	23.5(19.3-27.8	
Clinical outcomes				**0.046**
Discharge from hospital	64(98.5%)	12(92.3%)	52(100.0%)	
Death	1(1.5%)	1(7.7%)	0(0.0%)	
Time from diagnosis to death (days)	18.0(18.0-18.0)	18.0(18.0-18.0)	--	--

Figures

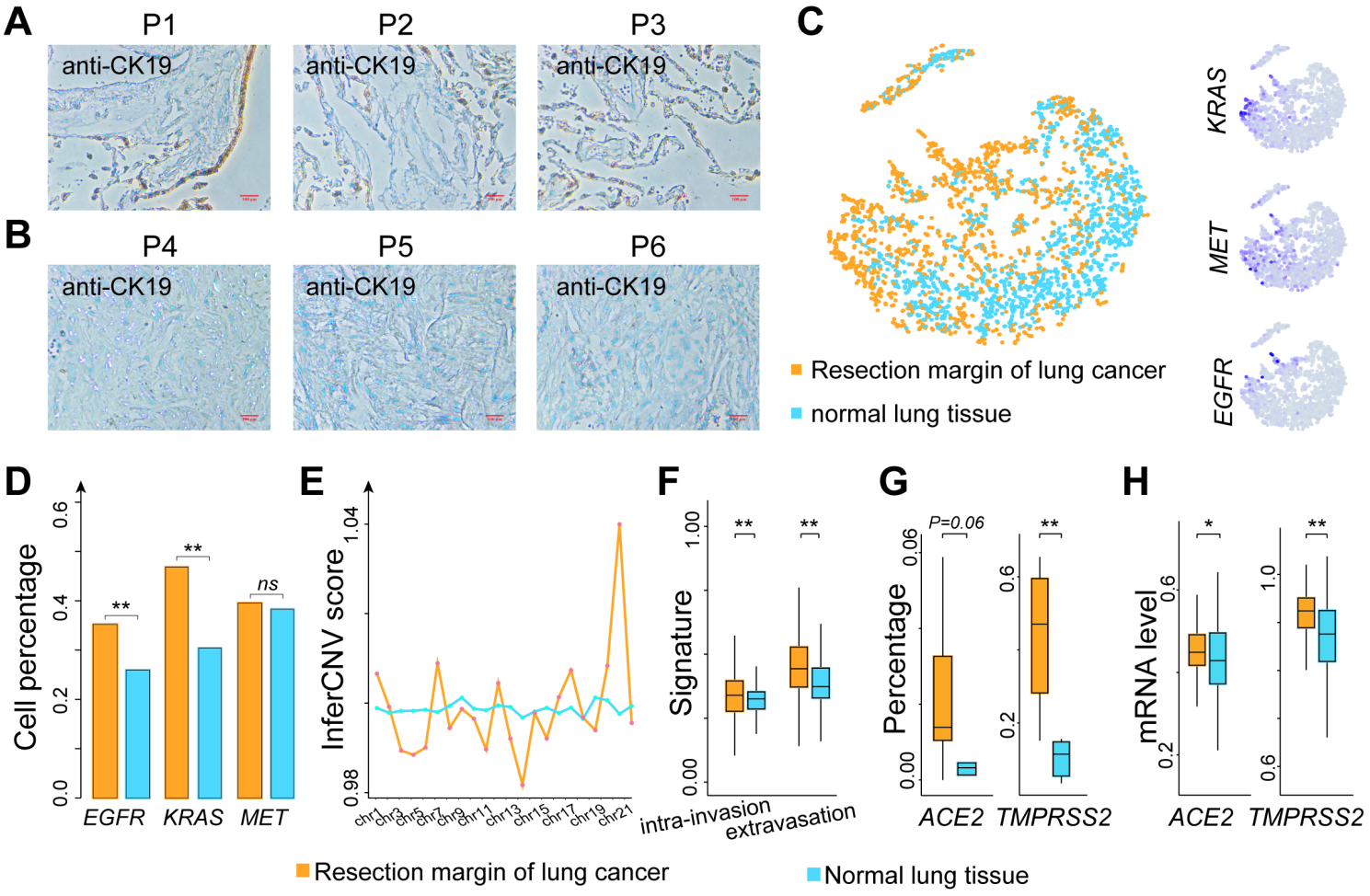

Figure 1

ACE2 and TMPRSS2 are highly expressed at resection margins of lung cancer patients. (A) Immunohistochemistry images of resection margin tissues from lung cancer patients using anti-CK19 antibody. (B) Immunohistochemistry images of non-cancer samples using anti-CK19 antibody. (C) Left: t-SNE plot of epithelial cells from the resection margins of lung cancers and normal lung tissues. Right: Distribution of the indicated cell marker genes overlaid on a 2D-tSNE plot. (D) Comparison of the percentage of indicated genes expressed in epithelial cells between the resection margin of lung cancers and normal lung tissues. (E) Comparison of inferCNV scores of cells between the resection margin of lung cancers and normal tissues across indicated chromosomes. (F) Comparison of intra-invasion and extravasation signature scores between indicated groups. (G) Comparison of the percentage of ACE2 and TMPRSS2 expressed in cells between indicated groups. (H) Comparison of mRNA levels of ACE2 and TMPRSS2 between indicated groups.

Supplementary Files

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- supplementaryMethods.docx

- tableS1.xlsx
- tableS2.xlsx
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