Submitted: 13 March, 2023 Accepted: 19 April, 2023 Published: 30 June, 2023 DOI:10.22514/jomh.2023.044

ORIGINAL RESEARCH

JOMH Journal of Men's Health

LY6E level associated with smoking as risk for lung cancer patients susceptible to COVID-19

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Abstract

Studies revealed that cancer patients seemed more susceptible to COVID-19 and the clinical symptoms were serious. A recent study depicted that lymphocyte antigen 6 complex, locus E (*LY6E*) might inhibit coronavirus entry into cells by interfering with the membrane fusion process mediated through spike protein, and potently restricting the SARS-CoV-2 (Severe acute respiratory syndrome-associated coronavirus 2) infection. *LY6E* mRNA level in lung cancer was detected by publicly available datasets. Patient-specific features were used to analyze the potential factors that could affect *LY6E* level. Analysis of association between *LY6E* level and immune infiltration was also performed. In present study, it was found that smoker with lung adenocarcinoma showed lower *LY6E* level than non-smoker ($p < 0.05$). In LUSC (lung squamous cell carcinoma) patients, reformed smokers showed higher *LY6E* than smokers ($p < 0.05$). These results suggested that smoke can be a risk susceptible to COVID-19 in lung cancer patients. Further studies exhibited that *LY6E* was positively associated with immune cell infiltration in lung adenocarcinoma, indicating that *LY6E* may influence the infection severity of COVID-19 in lung cancer patients. In summary, smoke may downregulate *LY6E* level and exacerbate infection and deterioration of COVID-19 in lung cancer patients.

Keywords

LY6E; Smoke; Lung cancer; Susceptibility; COVID-19

1. Introduction

COVID-19 (Coronavirus disease) caused by SARS-CoV-2 has emerged as a worldwide threat and healthcare concern in 2020. A suppressed immune system may especially make it more vulnerable to COVID-19 in cancer patients. Multiple studies also depict that it is more likely to be diagnosed with COVID-19 in cancer patients, and symptoms are intense [1]. A study finds that most patients died from COVID-19 have active cancer [2]. Patients of non-Hodgkin lymphoma, leukemia and lung cancer show higher infection risk [3]. Compared to general population, a study reveals cancer patients having higher infection and mortality rates of COVID-19, and lung cancer patients are the most common type [4]. Several studies exhibit that the specific characteristics of lung cancer patients such as smoking status, patient age, hypertension history and chronic obstructive pulmonary disease (COPD), may impact the morbidity and severity of COVID-19 infection in cancer patients [5–7].

However, how these risks affect cancer patients is unclear. *LY6E* (lymphocyte antigen 6 complex, locus E) is a secreted or plasma membrane-related protein with characteristics of 18 conserved, three-finger folding motif [8]. *LY6E* is initially identified to distinguish immature subsets from mature thymocyte [9] and is related with immune modulation, particularly in regulating activation, proliferation and development of T cell [10]. Recent studies depict new role of *LY6E* in interaction of virus and host. Pfaender *et al*. [11] shows that *LY6E* may have role in restricting infection of multiple corona viruses, such as SARS-CoV-2. Further mechanistic studies have revealed that *LY6E* inhibits coronavirus entry by interfering with membrane fusion mediated by spike protein [12]. Respiratory tract is the main route for transmission of SARS-CoV-2, however, *LY6E* role in coronavirus infection process in lung cancer patients is unclear.

In this study, publicly available datasets were obtained and analyzed. First, *LY6E* mRNA level in lung cancer was detected. The patient-specific features were employed for analyzing the potential factor that could affect *LY6E* level. Moreover, the association between *LY6E* level and immune infiltration was also analyzed.

2. Material and methods

2.1 *LY6E* mRNA level in lung cancer based on public datasets

Differential *LY6E* mRNA level analysis in lung cancer was originated from UALCAN (The University of ALabama at Birmingham Cancer data analysis Portal) (http://ualcan.path.uab.edu). RNA levels in various cancers could be an-

alyzed using TCGA (The Cancer Genome Atlas) data through UALCAN. Relative *LY6E* mRNA levels in tumor and normal samples, as well as in tumor sub-groups such as patient's race, smoking habit or other clinic pathological features were also investigated by UALCAN. Expression level data were evaluated by one-way ANOVA (Analysis of Variance) or non-parametric testing.

2.2 Analysis of *LY6E* and immune infiltration by TIMER database

Data of immune infiltration in lung cancer were downloaded from Tumor Immune Estimation Resource (TIMER) databases (https://cistrome.shinyapps.io/timer/). 10,897 samples of 32 cancer types from TCGA were included by the TIMER, and six tumor-infiltrating immune cells subsets (B cells, CD4 (Cluster of Differentiation 4) T cells, CD8 T cells, macrophages, neutrophils, and dendritic cells) were used for estimating the immune infiltration across diverse cancer types. In present study, the correlations of *LY6E* mRNA levels with abundance of tumor-infiltrating immune cells such as $CD8^+$ T cells, $CD4^+$ T cells, neutrophils, macrophages, B cells and dendritic cells, were investigated. Moreover, the tumor purity in lung cancer patients was also analyzed. Correlation analysis was carried out by spearman test.

2.3 Association analysis of *KRAS* and *LY6E* in lung adenocarcinoma

Lung adenocarcinoma often harbored mutations in rat sarcoma (*RAS*) genes which might lead to cancer development and progression. The commonly mutated *RAS* isoform Kirsten rat sarcoma (*KRAS*) was chosen to explore the relationship of *RAS* mutation and *LY6E*. *KRAS* mRNA level based on patients' smoking habits was analyzed through UALCAN public datasets. Correlation between *KRAS* mutation and immune cells infiltration was determined through online TIMER databases. Finally, correlation between *KRAS* and *LY6E* mRNA level was evaluated by TIMER databases.

3. Results

3.1 Expression level of *LY6E* in lung cancer patients

The results depicted that *LY6E* was upregulated in lung adenocarcinoma (LUAD) tissue than in normal (Fig. 1). *LY6E* was lower in LUAD patients of African Americans than of Asians. However, the difference in lung squamous cell carcinoma (LUSC) was not significant. *LY6E* mRNA level differences between men and women were also explored where the level was higher in men than in women with LUAD, but there was no significance in LUSC (data not shown).

3.2 *LY6E* level associated with smoking

Further analyses revealed that whether it was LUAD or LUSC, smoking was an important factor associated with *LY6E* level in cancer patients. Smoker with LUAD exhibited lower *LY6E* level than non-smoker ($p = 0.01$), and it was even lower than normal control ($p = 0.01$). *LY6E* was significantly lower in reformed smoker (meantime >15 years) with LUAD than non-smoker with LUAD ($p = 0.01$). In LUSC patients, reformed smokers (meantime <15 years, and meantime >15 years) depicted higher *LY6E* mRNA level than smokers having lung cancer ($p = 0.04$ and $p = 0.02$, respectively). These results suggested that smoke could be a risk for lung cancer patients being susceptible towards COVID-19.

3.3 *LY6E* mRNA level associated with immune infiltration in lung cancer patients

Further investigations revealed that *LY6E* mRNA level in LUAD was positively associated with $CD4^+$ T cells and dendritic cell infiltration, and positively associated with $CD4^+$ T cells, neutrophil and dendritic cells infiltration in LUSC ($p < 0.0001$, Fig. 2).

3.4 *KRAS* associated with smoking in LUAD

KRAS mRNA level was higher in smoking patients than those of non-smoker with LUAD ($p < 0.05$, Fig. 3A). In LUAD patients, reformed smokers (meantime <15 years, and meantime >15 years) depicted higher *KRAS* mRNA level than non-smokers patients ($p < 0.01$ and $p < 0.05$, respectively). However, the difference of *KRAS* mRNA level in smokers and reformed smokers was not significant ($p > 0.05$). Next, the correlation analysis of *KRAS* mutation with immune cells infiltration was performed where *KRAS* mutation was negatively correlated with B cells and dendritic cells ($p < 0.05$ respectively, Fig. 3B). Finally, the correlation between *KRAS* and *LY6E* mRNA level was evaluated and results exhibited that *KRAS* mRNA level was negatively correlated with *LY6E* mRNA level ($p < 0.05$, Fig. 3C).

4. Discussion

Only the upper respiratory tract can generally be infected by coronaviruses and the clinical symptoms are relatively mild [13]. However, SARS-CoV-2 may infect and replicate in the lower respiratory tract and induce pneumonia [14]. Macrophages, dendritic cells, and neutrophils as the body first line defense have immune role after contracting infection. Some studies found macrophages infiltrated bronchial mucosa in COVID-19 patients [15]. Thus, SARS-CoV-2 can trigger hyper-inflammation in pulmonary tissues through immune system hyperactivation and uncontrolled release of cytokines, known as "cytokine storm" [16]. Immunity and inflammation are the two crucial characteristics of tumor microenvironment in tumor evolution. The interactions imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines create pro-tumorigenic microenvironment. Conversely, tumors with poor prognosis depict significant increase in pro-inflammatory activity [17]. Furthermore, exosomes released by cancer cells also promote pro-inflammatory mediators production through cancer-associated fibroblasts (CAF) [18]. It can thus be hypothesized that tumor patients are more prone to severe inflammatory reactions after contracting virus infection.

Previous studies exhibited cancer patients having increased risk of COVID-19 infection, mainly owing to tumor induced

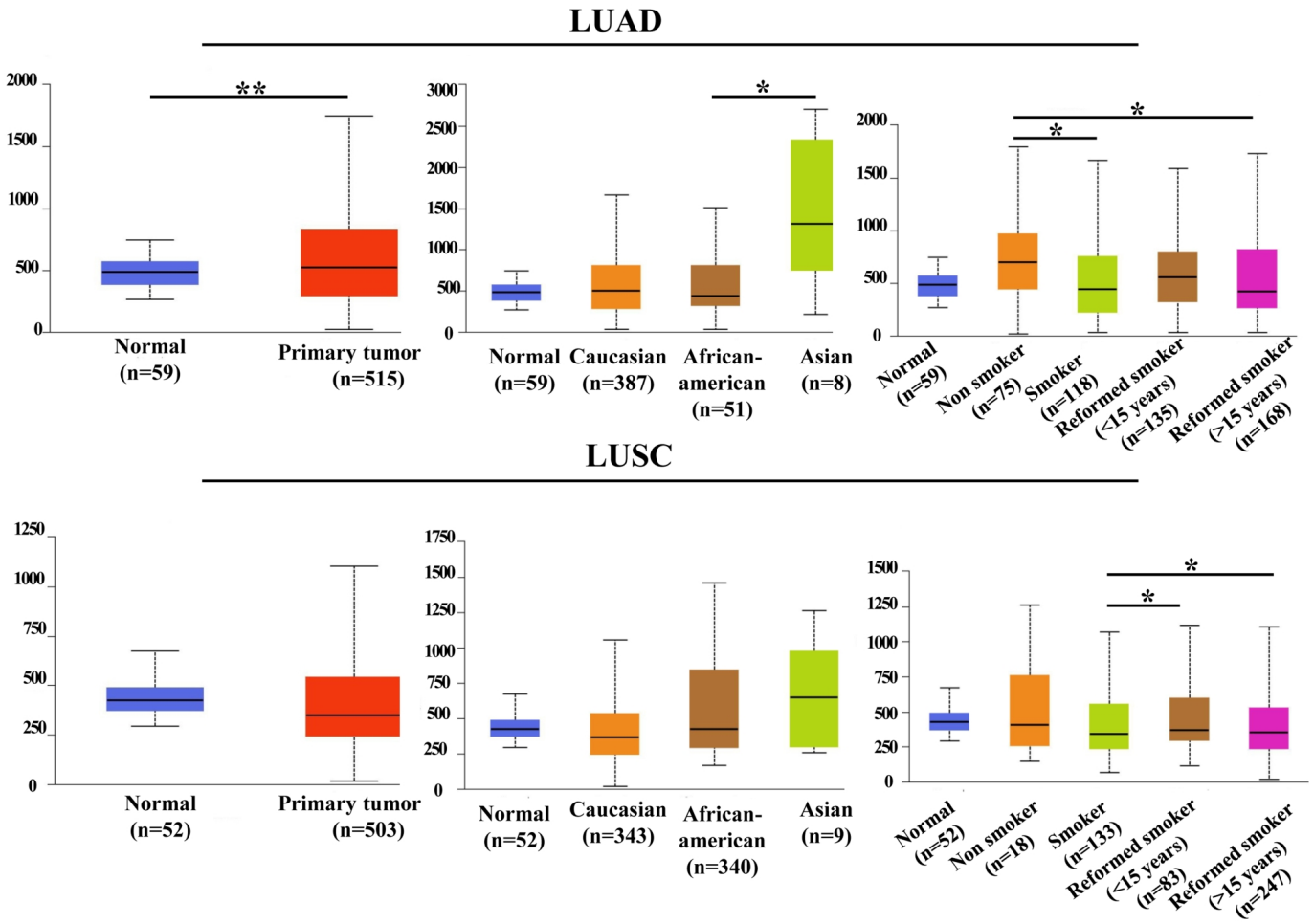

FIGURE 1. ***LY6E*** **expression level was associated with race and smoking in LUAD and with smoking in LUSC.** Smoking patients depicted lower *LY6E* expression than non-smokers or reformed smokers in LUAD and LUSC. *$p < 0.05$, **$p < 0.01$. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

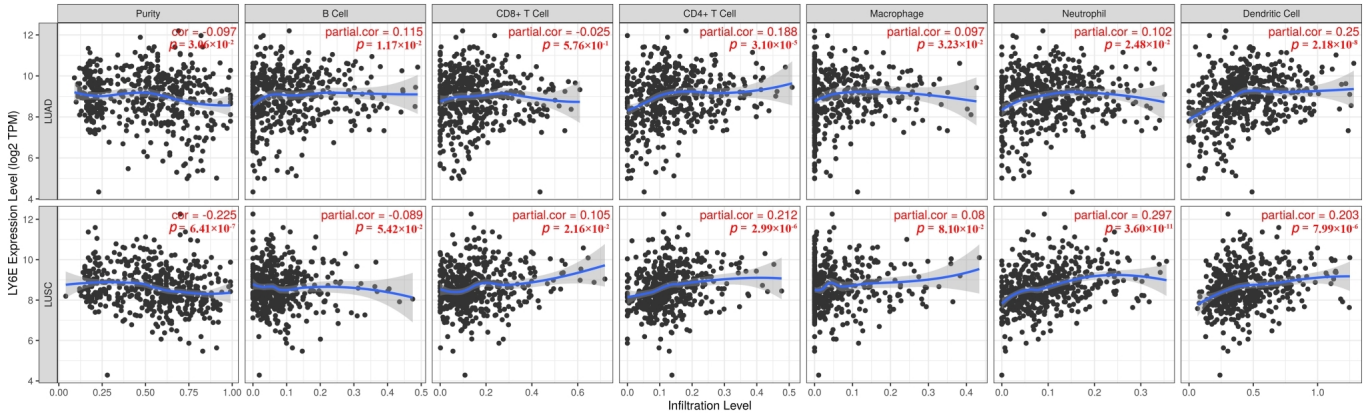

FIGURE 2. ***LY6E*** **expression was associated with CD4$^+$ T cells and dendritic cell infiltration in LUAD, and with CD4$^+$ T cells, neutrophil and dendritic cells infiltration in LUSC.** *LY6E*, lymphocyte antigen 6 complex, locus E. CD, Cluster of Differentiation; TPM, Transcripts Per Million.

systemic immunosuppression and anti-cancer therapy [19], especially in lung cancer patients [3, 20]. Recent research had focused on series of immune biomarkers for the prognosis of COVID-19 patients, as per the characteristics of immune response to coronavirus infection. *LY6E* as an antiviral protein was reported to inhibit corona virus entry. Mar *et al.* [21] revealed that cell intrinsic antiviral effects by *LY6E* were attained by the distinct cellular compartments, thus providing resistance to mice against diseases caused by coronavirus.

Zhao *et al.* [22] revealed that *LY6E* overexpression in human non-small cell lung cancer cell lines A549 inhibited HCoV-OC43 (Human coronavirus organ cultures 43) infection. The downregulation of *LY6E* expression increased its susceptibility to HCoV-OC43 infection in hepatocellular carcinoma cells

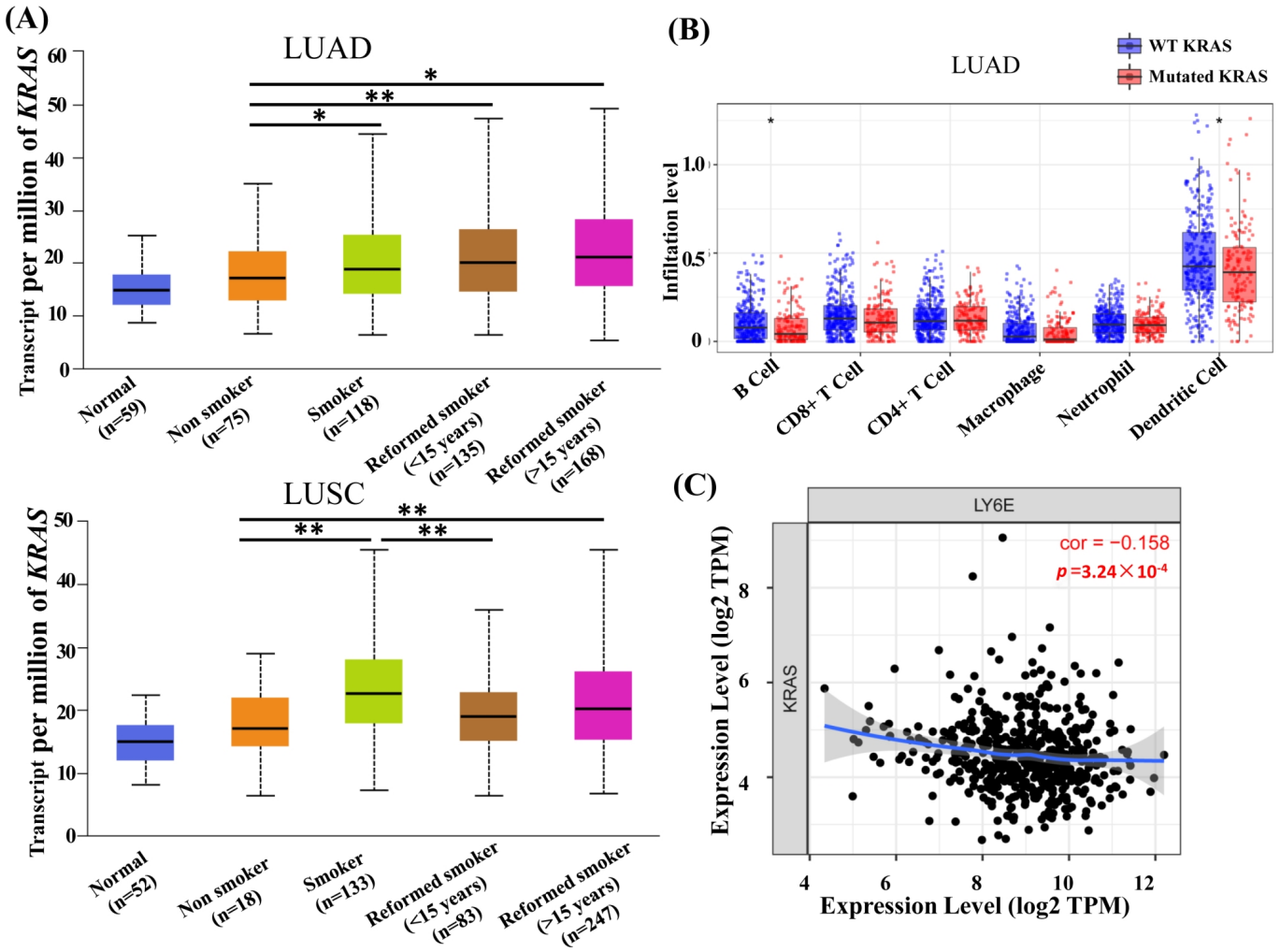

FIGURE 3. *LY6E* level was associated with *KRAS* mutation. (A) *KRAS* mRNA level was associated with smoking in LUAD and LUSC. (B) *KRAS* mutation was negatively correlated with B cells and dendritic cells infiltration. (C) *KRAS* mRNA level was negatively correlated with *LY6E* level. *$p < 0.05$, **$p < 0.01$. LUAD, lung adenocarcinoma; KRAS, Kirsten rat sarcoma; LUSC, lung squamous cell carcinoma; *LY6E*, lymphocyte antigen 6 complex, locus E. CD, Cluster of Differentiation; TPM, Transcripts Per Million.

HepG2 (Hepatoma HepG2 cell line). In present study, it was found that smoker with lung adenocarcinoma exhibited lower *LY6E* level than non-smoker, and in LUSC patients, reformed smokers depicted higher *LY6E* than smokers. These results implied that smoke could be a risk for lung cancer patients susceptible to COVID-19.

Viral pathogenesis was reported to exacerbate with the loss of liver immune cells and higher splenic viral burden in *LY6E* knockout mice [11]. Therefore, *LY6E* may influence infection severity of COVID-19 due to immunosuppression in patient having lung cancer. So, the association between *LY6E* mRNA level and immune cell infiltration was further analyzed wherein *LY6E* was positively associated with immune cell infiltration in lung adenocarcinoma, suggesting that *LY6E* can influence COVID-19 infection severity in patient having lung cancer.

RAS is the frequently mutated oncogene in cancer, and *KRAS* is commonly mutated *RAS* isoform in human lung adenocarcinoma [23]. Smoking is related to *KRAS* mutations in lung cancer development. *KRAS* mutation rate reaches 20–40%, especially in LUAD [24]. There was significant difference between smokers (30%) and non-smokers (11%) regarding these mutations [25]. In present study, it was found that *KRAS* mRNA level was associated with patients' smoke habits. LUAD patients with history of smoking depicted higher *KRAS* level than non-smokers. It was also shown that *KRAS* mutation was negatively correlated with B cells and dendritic cells infiltration, suggesting that *KRAS* mutation may impact immune regulatory function in LUAD. Further analysis exhibited that *KRAS* mRNA level was negatively correlated with *LY6E* mRNA level. Based on results, it was supposed that lung cancer smoke patients with *LY6E* deficiency may induce susceptibility to COVID-19. Conversely, induced increase in *KRAS* by *LY6E* may promote lung cancer development and progression through immunosuppression.

The present study has limitations such as small number of patients, lacking conclusive clinical evidence, and insufficiency of *in vitro* and *in vivo* experimental verifications. The obtained data provide potential insights into how COVID-19 impacts cancer patients clinical management, especially in lung cancer patients.

5. Conclusions

In summary, *LY6E* was upregulated in lung adenocarcinoma tissue, and its level was associated with smoking in cancer patients. Further study revealed that *LY6E* was positively linked with immune cell infiltration and negatively with *KRAS*. These results indicated that smoking may downregulate *LY6E* level and is a risk to lung cancer patients regarding the susceptibility to COVID-19.

ABBREVIATIONS

COVID-19, Coronavirus disease 19; SARS-CoV-2, Severe acute respiratory syndrome-associated coronavirus 2; *LY6E*, Lymphocyte antigen 6 complex, locus E; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; HCoV-OC43, Human Coronavirus-organ cultures 43; CD4, Cluster of differentiation 4; CD8, Cluster of differentiation 8.

AVAILABILITY OF DATA AND MATERIALS

The gene expression profiles and clinical data of *LY6E* can be found at UALCAN (http://ualcan.path.uab.edu). The infiltrating immune cells data can be downloaded from TIMER (https://cistrome.shinyapps.io/timer/).

AUTHOR CONTRIBUTIONS

YYM and HZ—designed the research study, wrote the manuscript. YYM and JXS—performed the research. XZM—provided help and advice on data analysis. YYM and XZM—analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

We thank the peer reviewers for their opinions and suggestions.

FUNDING

This research was funded by Natural Science Foundation of Zhejiang Province, grant number LBY22H180006 and LY21H160048.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Sidaway P. COVID-19 and cancer: what we know so far. Nature Reviews Clinical Oncology. 2020; 17: 336–336.

[2] Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA. 2020; 323: 1775–1776.

[3] Wang Q, Berger NA, Xu R. Analyses of risk, racial disparity, and outcomes among us patients with cancer and COVID-19 infection. JAMA Oncology. 2021; 7: 220.

[4] Liang W, Guan W, Chen R, Wang W, Li J, Xu K, *et al*. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. The Lancet Oncology. 2020; 21: 335–337.

[5] Luo J, Rizvi H, Preeshagul IR, Egger JV, Hoyos D, Bandlamudi C, *et al*. COVID-19 in patients with lung cancer. Annals of Oncology. 2020; 31: 1386–1396.

[6] Passaro A, Bestvina C, Velez Velez M, Garassino MC, Garon E, Peters S. Severity of COVID-19 in patients with lung cancer: evidence and challenges. Journal for ImmunoTherapy of Cancer. 2021; 9: e002266.

[7] Pathania AS, Prathipati P, Abdul BA, Chava S, Katta SS, Gupta SC, *et al*. COVID-19 and cancer comorbidity: therapeutic opportunities and challenges. Theranostics. 2021; 11: 731–753.

[8] Lee PY, Wang J, Parisini E, Dascher CC, Nigrovic PA. Ly6 family proteins in neutrophil biology. Journal of Leukocyte Biology. 2013; 94: 585–594.

[9] Godfrey DI, Masciantonio M, Tucek CL, Malin MA, Boyd RL, Hugo P. Thymic shared antigen-1. A novel thymocyte marker discriminating immature from mature thymocyte subsets. Journal of Immunology. 1992; 148: 2006–2011.

[10] Saitoh S, Kosugi A, Noda S, Yamamoto N, Ogata M, Minami Y, *et al*. Modulation of TCR-mediated signaling pathway by thymic shared antigen-1 (TSA-1)/stem cell antigen-2 (Sca-2). Journal of Immunology. 1995; 155: 5574–5581.

[11] Pfaender S, Mar KB, Michailidis E, Kratzel A, Boys IN, V'Kovski P, *et al*. LY6E impairs coronavirus fusion and confers immune control of viral disease. Nature Microbiology. 2020; 5: 1330–1339.

[12] Nandi S, Roy H, Gummadi A, Saxena AK. Exploring spike protein as potential target of novel coronavirus and to inhibit the viability utilizing natural agents. Current Drug Targets. 2021; 22: 2006–2020.

[13] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Coronaviruses. Methods in Molecular Biology. 2015; 1282: 1–23.

[14] Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. The Lancet Respiratory Medicine. 2020; 8: e46–e47.

[15] Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 autopsies, Oklahoma, USA. American Journal of Clinical Pathology. 2020; 153: 725–733.

[16] Hu B, Huang S, Yin L. The cytokine storm and COVID-19. Journal of Medical Virology. 2021; 93: 250–256.

[17] Nestler T, Dalvi P, Haidl F, Wittersheim M, von Brandenstein M, Paffenholz P, *et al*. Transcriptome analysis reveals upregulation of immune response pathways at the invasive tumour front of metastatic seminoma germ cell tumours. British Journal of Cancer. 2022; 126: 937–947.

[18] Strnadová K, Pfeiferová L, Přikryl P, Dvořánková B, Vlčák E, Frýdlová J, *et al*. Exosomes produced by melanoma cells significantly influence the biological properties of normal and cancer-associated fibroblasts. Histochemistry and Cell Biology. 2022; 157: 153–172.

[19] Addeo A, Obeid M, Friedlaender A. COVID-19 and lung cancer: risks, mechanisms and treatment interactions. Journal for ImmunoTherapy of Cancer. 2020; 8: e000892.

[20] Robilotti EV, Babady NE, Mead PA, Rolling T, Perez-Johnston R, Bernardes M, *et al*. Determinants of COVID-19 disease severity in patients with cancer. Nature Medicine. 2020; 26: 1218–1223.

[21] Mar KB, Van Dyke MC, Lopez AH, Eitson JL, Fan W, Hanners NW, *et al*. LY6E protects mice from pathogenic effects of murine coronavirus and SARS-CoV-2. bioRχiv. 2023. [Preprint].

[22] Zhao X, Zheng S, Chen D, Zheng M, Li X, Li G, *et al*. LY6E Restricts entry of human coronaviruses, including currently pandemic SARS-CoV-2. Journal of Virology. 2020; 94: e00562-20.

[23] Prior IA, Hood FE, Hartley JL. The frequency of Ras mutations in cancer. Cancer Research. 2020; 80: 2969–2974.

[24] Martin P, Leighl NB, Tsao M, Shepherd FA. KRAS mutations as

prognostic and predictive markers in non-small cell lung cancer. Journal of Thoracic Oncology. 2013; 8: 530–542.

[25] Adderley H, Blackhall FH, Lindsay CR. KRAS-mutant non-small cell lung cancer: converging small molecules and immune checkpoint inhibition. EBioMedicine. 2019; 41: 711–716.

How to cite this article: Ying-Yu Ma, Jing-Xing Si, Xiao-Zhou Mou, Hai Zou. *LY6E* level associated with smoking as risk for lung cancer patients susceptible to COVID-19. Journal of Men's Health. 2023; 19(6): 20-25. doi: 10.22514/jomh.2023.044.