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

Ying-Yu Ma1,2,3, Jing-Xing Si3, Xiao-Zhou Mou3, Hai Zou1,*

1 Department of Critical Care, Fudan University Shanghai Cancer, 200032 Shanghai, China
2 Cancer Center, Key Laboratory of Gastroenterology of Zhejiang Province, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, 310014 Hangzhou, Zhejiang, China
3 Clinical research institute, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, 310014 Hangzhou, Zhejiang, China

*Correspondence zouhai@fudan.edu.cn
(Hai Zou)

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 [3]. 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. Pfäender 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 (mean time >15 years) with LUAD than non-smoker with LUAD ($p = 0.01$). In LUSC patients, reformed smokers (mean time <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 (mean time <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.
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 immunoregulatory 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 HZ—designed the research study, wrote the final manuscript. YYM and JXS—performed the research.

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