

# Correlation between Level of Autophagy and Amount of CD8+T Cells in Chronic Obstructive Pulmonary Disease

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## Research article

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# Abstract

**Background:** This study aimed to shed light on the correlation between the amounts of CD8<sup>+</sup> T cells and autophagy level in COPD.

**Results:** The objects (n = 90) were divided into three groups: COPD group (patients in the stable phase; n = 30), SN group (healthy control of smoking with normal lung function group; n = 30), and NSN groups (healthy control of non-smoking with normal lung function group; n = 30). The amounts of CD8<sup>+</sup> (32.33 ± 4.23%), CD8<sup>+</sup> effector (25.63 ± 8.57%) and CD8<sup>+</sup>memory (11.94 ± 5.77%) T cell in the COPD group were significantly higher those in the other two groups, while the apoptotic rate was lower in the COPD group (P < 0.05). Significant linear correlations were found of P62/GAPDH (‰) with CD8<sup>+</sup>, CD8<sup>+</sup>effector, and CD8<sup>+</sup> memory- T cell amounts (P<0.001).

**Conclusions:** Autophagy level is positively and linearly associated with the amounts of CD8<sup>+</sup> T cells, suggesting that cell autophagy might be involved in COPD pathogenesis.

## Background

Chronic obstructive pulmonary disease (COPD) characterized by recurrent airway inflammation and progressive exacerbation of airflow, is a common chronic airway inflammatory disease. COPD has a high potential for severe disability, and is the third leading cause of death around globally. In China, its overall prevalence rate of adult above 40 years old is high (13.6%), while the long-term treatment of COPD is costly and irreversible, resulting in a huge economic burden on family and public health systems .(1) In clinical, airway inflammation and destructive emphysema are two major pathological features of COPD. (2) However, the pathogenesis of COPD is still poorly understood, and there are no definitive and effective cures by now. It is, therefore, of importance to explore the pathogenic mechanisms involved in COPD. Recent studies have shown that smoking is one of the major causes of COPD because the airway inflammation in lung tissue caused by smoking persist even after smoking cessation.(3, 4) Recently, some studies suggested that destruction of alveolar wall in smokers is mainly related to T-cell function. (5–7) CD8<sup>+</sup> T cells are the key subgroup of lymphocytes in infiltrating cells responsible for the chronic airway inflammation in COPD,(8–13) and largely infiltrate the airway in patients with stable COPD. However, the specific underlying mechanism has not been unveiled.

It was reported that tobacco extract (CSE) induces multiple autophagy(14, 15) and leads to emphysema. (16–19) Previous studies have found that the level of specific autophagy biomarkers are increased in the lung tissue of COPD patients.(16, 20) Autophagy promotes the survival of CD8<sup>+</sup>effect T cells and the formation of CD8<sup>+</sup> memory T cells.(21, 22) These studies indicated that there might be some association between cell autophagy and CD8<sup>+</sup> T cells in COPD. However, the mechanism by which CD8 + T cell amounts increase, and how autophagy is involved remain undefined. In this study, we assessed the expression levels of the autophagy related genes LC3 and p62 in the peripheral blood from the patients with stable COPD, and LC3II/I and P62 protein levels, as well as the amounts of CD8<sup>+</sup> T cells, including

CD8<sup>+</sup>effector and CD8<sup>+</sup> memory cells, and the apoptotic rate of CD8<sup>+</sup> T cells, to investigate the relationship between autophagy and the amounts of CD8<sup>+</sup> T cells, to reveal the potential pathogenic mechanisms of COPD.

## Results

### Baseline patient characteristics

Table 1–2 display the baseline patient characteristic. It is found that there was no statistically significant difference in age among the three groups. Pearson's chi square was 0.787, with  $p > 0.05$ , indicating that no significant difference was found in sex distribution among the three groups.

Table 1  
The gender and Age distribution in COPD, SN, and NSN groups

SEX	COPD(%)	SN(%)	NSN(%)	Total(%)
Female	6(40.0)	4(26.7)	5(33.3)	15(100.0)
male	24(32.0)	26(34.7)	25(33.3)	75(100.0)
Total	30(33.3)	30(33.3)	30(33.3)	90(100.0)

Table 2  
Multiple comparison of age in COPD, SN, and NSN three groups

Group	Group	Mean ± Standard error	Sig	95%Confidence intervals	
				lower limit	Upper limit
COPD	NSN	2.2 ± 1.284	0.09	-0.35	4.75
	SN	2.867 ± 1.284	0.082	0.31	5.42
SN	NSN	0.667 ± 1.284	0.605	-1.89	3.22

\* The significance level of the mean difference was 0.05.

### Subsets and apoptosis of CD8<sup>+</sup> T cells.

#### Subsets of CD8<sup>+</sup>T cells

As shown in Fig. 1, 2 and Table 3, The result of analysis of flow cytometry showed that the average percentage of CD8<sup>+</sup> T cells in peripheral blood from the COPD group was  $32.33 \pm 4.23\%$ , of which CD8<sup>+</sup>

effector and CD8<sup>+</sup> memory T cells were 25.63 ± 8.57% and 22.10 ± 6.706%, respectively. In the NSN group, the average percentage of CD8<sup>+</sup> T cells was 27.92 ± 4.18%. Among them, CD8<sup>+</sup>effector and CD8<sup>+</sup> memory T cells were 20.43 ± 6.62% and 11.94 ± 5.774%, respectively. In the SN group, the average amount of CD8<sup>+</sup> T cells was 25.82 ± 4.62%, with CD8<sup>+</sup> effector and CD8<sup>+</sup> memory T cells at 16.08 ± 5.55% and 7.35 ± 2.903%, respectively. The levels of CD8<sup>+</sup> T cells in the COPD group were significantly higher than those of the other two groups ( $p < 0.05$ ), and the SN group had higher values than the NSN group ( $p < 0.05$ ).

Table 3

The comparison of t-lymphocyte subsets and apoptosis in COPD, SN, and NSN three groups

Group	N	CD8 <sup>+</sup> T cells(%)	CD8 <sup>+</sup> Effector T cells(%)	CD8 <sup>+</sup> memory T cells(%)	CD8 <sup>+</sup> T apoptosis(%)
COPD	30	32.33 ± 4.23	25.63 ± 8.57	22.10 ± 6.71	23.51 ± 6.11
SN	30	27.92 ± 4.18	20.43 ± 6.62	11.94 ± 5.77	27.39 ± 7.21
NSN	30	25.82 ± 4.62	16.08 ± 5.55	7.35 ± 2.90	31.61 ± 4.98

As shown in Fig. 3 and Table 3, the average apoptotic rate of CD8<sup>+</sup> T cells of the COPD group (23.51 ± 6.11%) was lower than those of the other two groups (SN group: 27.39 ± 7.21%; NSN group: 31.61 ± 4.98%) ( $p < 0.05$ ). Apparently, the average apoptotic rate of the NS group was lower than that of the NSN group ( $p < 0.05$ ).

#### Expression levels of the autophagy related genes LC3 and P62 in CD8<sup>+</sup> T cells.

Three classic bands of RNA extracted by trizol, appeared in the 5th, 18th and 28th seconds respectively. The bands of 18 s and 28 s respectively represented two subunits of RNA, small and large, while the third 5 s band was clearly distinguished from electrophoresis because of the small length difference. When the RNA was explained, the 5 s bands were enhanced and blurred; However, the weaker the 5 s band, the clearer the 18 and 28 s band, which meanted no degradation of RNA and good purification. RNA in this experiment was highly purified, as shown in the Fig. 4.

Dissolution and amplification melting curve peaks for PCR products were at 88°C and 88°C, respectively and unimodal, without nonspecific bands (Figs. 5 and 6).

#### Relative mRNA levels of autophagy related genes:

The results showed  $F = 1.024$  for LC3 expression levels, indicating that there were no significant differences ( $P > 0.05$ ). The results showed that p62 gene expression levels in the COPD group were

markedly higher than those of the SN group, which in turn showed significantly higher amounts compared with the NSN group ( $p < 0.05$ ; Table 4–5).

Table 4  
qRT-PCR analysis of LC3 and p62 in COPD, SN, and NSN three groups

Group	N	LC3 RQ	P62 RQ
NSN	30	6.73 ± 3.58	16.08 ± 5.55
SN	30	7.78 ± 6.22	20.43 ± 6.62
COPD	30	6.08 ± 3.57	25.63 ± 8.57
The significance level of the mean difference was 0.05, $p > 0.05$			

Table 5  
multiple comparisons of qRT-PCR in COPD, SN, and NSN three groups

Group	Group	Mean Difference ±Standard	P	95%Confidence intervals	
				lower limit	lower limit
NSN	SN	-2.43 ± 0.73	0.032	-3.88	-0.99
	COPD	-6.82 ± 0.76	0.021	-8.26	-5.38
COPD	NSN	6.82 ± 0.75	0.028	5.38	8.26
	SN	4.39 ± 0.75	0.041	2.94	5.83
* the significance level of the mean difference was 0.05, $p < 0.05$					

### Expression levels of the autophagy related proteins LC3 II/I and P62 in CD8<sup>+</sup> T cells

To evaluate the protein expression levels of LC3 II/I and P62 in CD8<sup>+</sup> T cells, Western blot was utilized, and the results are presented in Fig. 7. We can find that there was no statistically significant difference among the samples in GAPDH levels, indicating that it could be used as an internal reference in this experiment. Immunoreactive bands are shown in Fig. 7A.B.

### Associations of LC3II/I and P62 expression levels with the amounts of CD8<sup>+</sup> T cells in COPD group

It is notable that there were moderate positive correlations between P62 expression and total amounts of CD8<sup>+</sup>T cells ( $r = 0.613, p < 0.05$ ), and amounts of CD8<sup>+</sup> effector T cell ( $r = 0.681, p < 0.05$ ). There was also a moderate positive correlation between p62 expression and the ratio of CD8 memory T cells ( $r = 0.745, p <$

0.05). No significant correlation was found between apoptosis of CD8<sup>+</sup> T cells and P62 expression. (Table 6)

Table 6  
Results of Pearson correlation analysis

Relative gray value (‰)		CD8 <sup>+</sup> T	CD8 <sup>+</sup> effector T	CD8 <sup>+</sup> memory T
LC3 $\beta$ / $\alpha$ /GAPDH	<i>r</i>	0.572	0.672	0.739
	<i>p</i>	0.001	0.000	0.000
	<i>n</i>	30	30	30
P62 /GAPDH	<i>r</i>	0.613	0.681	0.745
	<i>P</i>	0.000	0.000	0.000
	<i>N</i>	30	30	30
Note: at the level of 0.01, the correlation is significant				

### Regression analysis of autophagy and the number of CD8<sup>+</sup> T cells

When LC3 $\beta$ / $\alpha$ /GAPDH (‰) was regarded as an dependent variable, the linear regression model showed statistically significant linear correlations between the total amounts of CD8<sup>+</sup> T cells, CD8<sup>+</sup> memory T cell, and CD8<sup>+</sup> effector T cell,, respectively, and LC3 $\beta$ / $\alpha$ /GAPDH (‰) ( $P < 0.001$ ) (Table 7).

Table 7  
Multi-factor linear regression of LC3 $\beta$ / $\alpha$ /GAPDH (‰)

Mode	Variate	<i>P</i>	Standard error	<i>T</i>
1	(constant)	0.251	0.051	1.173
2	CD8 <sup>+</sup> T cells	0.001	0.002	3.686
	(constant)	0.001	0.026	4.202
3	CD8 <sup>+</sup> memory T cells	0.000	0.001	5.796
	(constant)	0.844	0.05	0.199
	CD8 <sup>+</sup> effector T cells	0.000	0.001	4.797

When P62/GAPDH (%) was regarded as the dependent variable, the linear regression model showed a statistically significant linear correlation between the total number of CD8<sup>+</sup> T cells and P62/GAPDH (%) ( $P < 0.001$ ). Significant linear correlations between the numbers of CD8<sup>+</sup> memory and CD8<sup>+</sup> effector T cells, respectively, and P62/GAPDH (%) were found (both  $P < 0.001$ ) (Table 8).

Table 8  
The multivariate linear regression of P62/GAPDH(%)

model	Variate	<i>P</i>	Standard error	<i>T</i>
1	(constant)	0.000	5.632	4.992
	CD8 <sup>+</sup> T cells	0.007	0.194	4.103
2	(constant)	0.008	2.887	12.086
	CD8 <sup>+</sup> memory T cells	0.000	0.095	5.909
3	(constant)	0.000	5.634	4.178
	CD8 <sup>+</sup> effector T cells	0.000	0.09	4.918

## Discussion

The pathogenic mechanism of COPD remains to be clarified. Viral infections(14) and increased production of autoantibodies.(23, 24) might be contributed to COPD. However, no specific definite virus or immune mechanism has been identified to date. In this study, it was found that LC3 gene expression had no significant difference between COPD group and healthy controls, indicating that the distribution of LC3 is not affected by COPD or smoking. However, p62 gene expression levels of COPD group were significantly higher than those of healthy control groups. The amount of LC3II/I and P62 were both increased in the COPD group, suggesting increased autophagy in the COPD group. Pearson correlation analysis showed that autophagy level and the amounts of CD8<sup>+</sup>T cells in COPD had a positive correlation.

The gene and protein expression levels of LC3 were equally almost the same among the three groups, while the amount of LC3II/I protein were significantly higher in the COPD group compared with the other two groups. LC3 is an autophagic biomarker gene for mammals and homologue to the yeast Atg8 (autophagy associated gene); It is widely distributed within mammalian cells and generally highly expressed during autophagy.(19, 25) LC3 has two formations, including LC3I and LC3II. LC3I is a precursor protein, which is distributed in the pulp. When autophagy occurs, LC3I is bound to phosphatidylethanolamine under the interaction of autophagy associated gene 7 (Atg7) and Atg12-Atg5-Atg16L, then hydrolyzes a small segment of the polypeptide to become LC3II, which is called autophagosome and binds to the autophagosome membrane. The molecular weight of LC3II is smaller

than that of LC3I. Therefore, occurrence of LC3II indicates autophagy. Higher LC3II expression indicates higher level of autophagy. Therefore, the LC3II/I ratio in cells is able to determine the degree of autophagy.(26) In this study, LC3II/I protein ratios in the COPD group were significantly increased, indicating that autophagy occurred in the COPD group under the circumstance of similar LC3 gene distribution. This result is consistent with the study reported by Sukkar et al.(27) We also found that both gene and protein expression levels of P62 were significantly increased in the COPD group. P62 protein, also known as sequestosome 1, is an ubiquitin-binding protein encoded by SQSTM1, and acts as an autophagic substrate during autophagy,(28) playing an important role in the autophagic process.(29) The previous studies of a Ubiquitin Related Area (UBA) in this protein revealed that P62/SQSTM1 has been associated with protein degradation.(30) After the formation of autophagosome LC3II, P62 can guide LC3 protein to link with the autophagosomal membrane and form autolysosomes, followed by degradation of P62. Usually, the production and degradation of P62 maintain an equilibrium at normal levels of autophagy. Excessive increase of autophagy and interruption of the degradation of autophagic products cause P62 to pile up.(31) P62, therefore, is often used to detect the patency of autophagosomes and autophagy flow, and P62 and LC3 are simultaneously used to assess autophagy level in cells.(32) Recent studies showed that occurrence of various diseases is related to P62 accumulation in cells.(33–36) Assessing the neurodegenerative changes, it was found that autophagy is inhibited, resulting in P62 accumulation and increased formation of P62 corpuscles.(32) As a consequence, the intracellular signal transduction and activation of Keap1 occurs, initiating the oxidative stress and tissue damage. P62 also activates the caspase8 apoptotic pathway, which initiates apoptosis.(37) Besides, several studies revealed that the expression of P62 is increased when the autophagy is elevated.(38–40) In addition, Shen et al. reported that P62 expression is negatively correlated with autophagy.(41)

In this study, the increased expression of LC3 in the COPD group supported autophagy elevation, and the pathways of downstream autophagy were smooth without obstruction. The increased P62 expression suggested that the excessive autophagy caused P62 accumulation., The increased autophagy in COPD patients in combination with the oxidative damage principle caused by P62 accumulation in the abovementioned diseases, led to elevated P62 accumulation. This may be contributed to the occurrence of alveolar oxidative damage.(11)

Next, we used flow cytometry to investigate CD8<sup>+</sup> T cell subsets, and found that patients with stable COPD had significantly higher amounts of CD8<sup>+</sup> T cells in peripheral blood than that in the SN group and NSN groups. The numbers of effector and memory T cells were also significantly increased. Meanwhile, the apoptotic rate of CD8<sup>+</sup> T cells was reduced. Current studies have found that CD8<sup>+</sup> T cells infiltrated in alveolar walls, the airway epithelium and the lung tissue are in an elevated state during remission from COPD.(11–13, 42, 43)

Then, we performed a Pearson correlation analysis of LC3II/I and P62 levels and the numbers of CD8<sup>+</sup> T cells, including CD8<sup>+</sup> effector and memory T cells, in stable COPD patients. There was a moderate positive correlation between increased autophagy level and elevated amounts of CD8<sup>+</sup> T cells. CD8<sup>+</sup> T

cell autophagy was increased in patients with stable COPD, promoting the survival of CD8<sup>+</sup> effector T cells and the formation of memory T cells, therefore, the overall cell number increased.(21, 22) These result demonstrate that autophagy may be one of the main factors that increased the amounts of CD8<sup>+</sup> T cells in peripheral blood in patients with stable COPD.

Of not, it was also found that CD8<sup>+</sup> T cell autophagy in patients with stable COPD was increased, yet the apoptosis was reduced. Autophagy and apoptosis are both cell death processes, which are different from necrosis. However, the causes and mechanisms of autophagy and apoptosis are different. Autophagy is mainly used to eliminate waste cell organelles, and forms autophagy lysosomes with no typical characteristics of apoptosis such as nuclear condensation, nuclear rupture, cell shrinkage, and formation of apoptotic bodies. It is therefore called autophagic cell death, representing a new type of programmed cell death. To distinguish it from apoptosis, autophagy is termed Type I cell death, while the apoptosis and necrosis are regarded as Types II and III cell death, respectively. Autophagy is associated with both cell survival and death. In some cases, it is involved in cell death, while the autophagy promotes cell survival in other cases.(44) The relationship between autophagy and apoptosis is relatively complex, and the specific mechanism has not been fully understood. Under the normal conditions, the autophagy rarely occurs in cells unless there are predisposing factors such as starvation, the lack of growth factors, ischemia and hypoxia, microbial infections, etc. In some cases, autophagy is an adaptive mechanism under stress conditions; cells remove impaired mitochondria by autophagy, increase the ability of anti-hypoxia, and prevent the necrosis and apoptosis. Hence, under reasonable stress conditions, autophagy exerts a protective effect on cells. However, the excessive autophagy may damage cells and cause cell death. Previous studies reported that the increased autophagy promoted the the formation of CD8<sup>+</sup> effector and memory T cells.(6, 7) In this study, autophagy level, the total number of CD8<sup>+</sup> T cells, as well as the amounts of effector and memory T cell, were increased in patients with COPD, while the apoptotic rate was decreased. We considered that the autophagy of CD8<sup>+</sup> T cells was increased in patients with COPD, repairing organelle damage and preventing cell necrosis or apoptosis. As a result, the overall rate of cell survival increased, and the apoptotic rate decreased. The Autophagy promoted the survival of CD8<sup>+</sup> effector T cells and the generation of memory T cells, and the overall number of cells increased. Therefore, there is no contradiction between the autophagy and apoptosis. These results suggested that the number of CD8<sup>+</sup> T cells was positively correlated with autophagy level in these cells. However, the irrespective of apoptotic level, indicated that the amounts of long-term CD8<sup>+</sup> T cells increased in stable COPD patients may because of the elevated autophagy levels rather than the decreased apoptotic rate.

Furthermore, we found that the amounts of CD8<sup>+</sup> T cells and the expression levels of the autophagy associated proteins LC3II/I and P62 in SN group were higher than those of NSN group. Currently, several studies reported that smoking is in relation to the amounts of CD8<sup>+</sup> T cells. Compared with non-smokers, smokers showed increased amounts of CD8<sup>+</sup> T cells infiltrated into the bronchial epithelium and the lung tissue.(5, 7) Previous studies also found that smoking are able to promote autophagy(16, 17), resulting in emphysema.(15–19) Yang et al. found that in nicotine-controlled human periodontal ligament cells, autophagic level is significantly increased.(45) All of these study demonstrated that smoking promotes

autophagy. It is likely that smoking promotes the survival of effector T cells and the formation of memory T cells through increase of autophagy, leading to a long-term increase in amounts the CD8<sup>+</sup> T cells and maintaining such increase even after smoking quitting.(3, 4) Next, we plan to treat cells with tobacco extracts, and observe the dynamic changes of autophagy levels and amounts of T-cells, to further explore the correlation between smoking and autophagy.

At the beginning, this study was designed as a controlled study of COPD and non-COPD patients. The COPD group was not further divided into acute and remission stage groups, mainly because that acute infection can greatly induce autophagy. However, after reviewing the study, a limitation of this study was that if the COPD group was divided into acute and remission stage groups, we could detect changes of autophagy levels and amounts of CD<sup>+</sup>8 and CD<sup>+</sup>4 cells between both phases, which might provide additional insights. The other one limitation of this study is that the indicators used to detect autophagy were relatively simple. Only the ubiquitous autophagy associated protein LC3 and the autophagy degradation protein P62 were utilized, and a number of proteins that reflect the autophagy level were not included due to limited research funds. Future studies will address the these issues.

## Conclusions

Collectively, the results of this study demonstrated that the autophagic level of CD8<sup>+</sup> T cells and the total amounts of CD8<sup>+</sup> T cells in patients with stable COPD were increased. In addition, there was a positive correlation between the autophagy level and the amounts of CD8<sup>+</sup> T cells. These indicated that the increased CD8<sup>+</sup> T cell autophagy in patients with COPD may be one of the factors caused long-term increase of CD8<sup>+</sup> T cells, and smoking might be a driver of autophagy. The increased CD8<sup>+</sup> T cell autophagy was involved in COPD associated with chronic airway inflammation. This opens new avenues for exploring the pathogenesis of COPD. Animal models and cell experiments should be used to further investigate the correlation between autophagy level and CD8<sup>+</sup> T cell amounts. Autophagy blocking may have a great potential in the therapy of COPD.

## Methods

### Patients and samples collection

This study included 30 patients with stable COPD at the Respiratory Outpatient of Shenzhen Longgang Center Hospital during January 2017 and August 2017. The study was performed according to the Helsinki Declaration and conformed to medical ethics.

All cases were diagnosed according to the standard diagnostic guideline of chronic obstructive pulmonary disease (2013 edition, Chinese version). All the patients were investigated in stable state without acute exacerbation within the recent 2 weeks. None of the participants had chronic hunger, malnutrition, acute infection, chronic viral hepatitis, herpes simplex virus infection, autoimmune disease, coronary artery sclerosing heart disease, diabetes, other organ failure, pulmonary encephalopathy,

psychosis, acute hypoxia or mental stress. All patients of mixed gender were 50–70 years old. The subjects were random Han nationals, participated voluntarily and provided signed informed consent.

Age, sex, and ethnicity-matched healthy controls were recruited during the same period. According to the smoking history. The healthy controls were divided into smoking with normal lung function group (SN group, n = 30) and non-smoking with normal lung function group (NSN group, n = 30). A smoker was defined as an individual who currently smoked more than one cigarette per day without giving up, at least one year before enrolment.

### **Pulmonary Function Test**

Pulmonary function test was carried out for all subjects, who inhaled a bronchodilator agent (salbutamol 400 µg), followed by lung function measurement. The percentage of forced expiratory volume occupied by vital capacity in the first second and FEV1 in the first second were recorded. The FEV1 ratio of the first second forced expiratory volume to the predicted value was assessed. The pulmonary function was measured on a Medisofthyp Air type pulmonary function instrument.

### **Autophagy and CD8<sup>+</sup>T cell assessments**

A FACSAria III flow cytometer (BD, San Jose, CA) was used for identification and separation of CD8<sup>+</sup>T cells. Next, real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was employed to detect the expression levels of the autophagy associated genes LC3 and p62 by using MAX<sup>™</sup> System (BD, San Jose, CA). Western blot was utilized to determine the protein expression levels of LC3II/LC3I and P62. The flow cytometer was used to assess the distribution of CD8<sup>+</sup> T cell subsets, including the ratios of CD8<sup>+</sup>, memory and effector T cells, and apoptosis in CD8<sup>+</sup> T cells.

### **Statistical methods**

Data were plotted and analyzed by using GraphPad Prism. Data are presented as mean ± standard deviation (SD). All statistical analysis was performed using the SPSS 21 software (SPSS, USA). Single factor analysis of variance (ANOVA) was employed to compare the three groups; group pairs were tested by LSD. Pearson's method or nonparametric Spearman's method analysis was used to assess the correlation between autophagy level (expression levels of protein LC3II/I and P62) and the amounts of CD8<sup>+</sup> T cells. Standard  $p > 0.01$  was eliminated by multi-factor linear regression, and the introduction method was ENTER.  $P < 0.05$  was used to indicate statistical significance.

## **Declarations**

### **Ethics approval and consent to participate**

The study was approved by the Institutional Ethic Committee of Center for Clinical Studies at Long Gang Central Hospital of Shenzhen (LGCHZ-2-16-0020). This study was performed according to the Helsinki

Declaration and conformed to medical ethics. The subjects were random Han nationals, participated voluntary and provided signed informed consent.

### **Consent for publication**

All authors read and approved the final manuscript for publication.

### **Competing interests**

The authors declare no conflict of interest.

### **Funding**

No funding was received

### **Availability of data and materials**

The datasets used and analyzed in this study are available from the corresponding author on reasonable request

### **Authors' contributions**

HZ and SMZ carried out the studies, participated in collecting data, and drafted the manuscript. WGZ, YS and XQX performed the statistical analysis and participated in its design. NL and SDC helped to draft the manuscript.

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## Figures

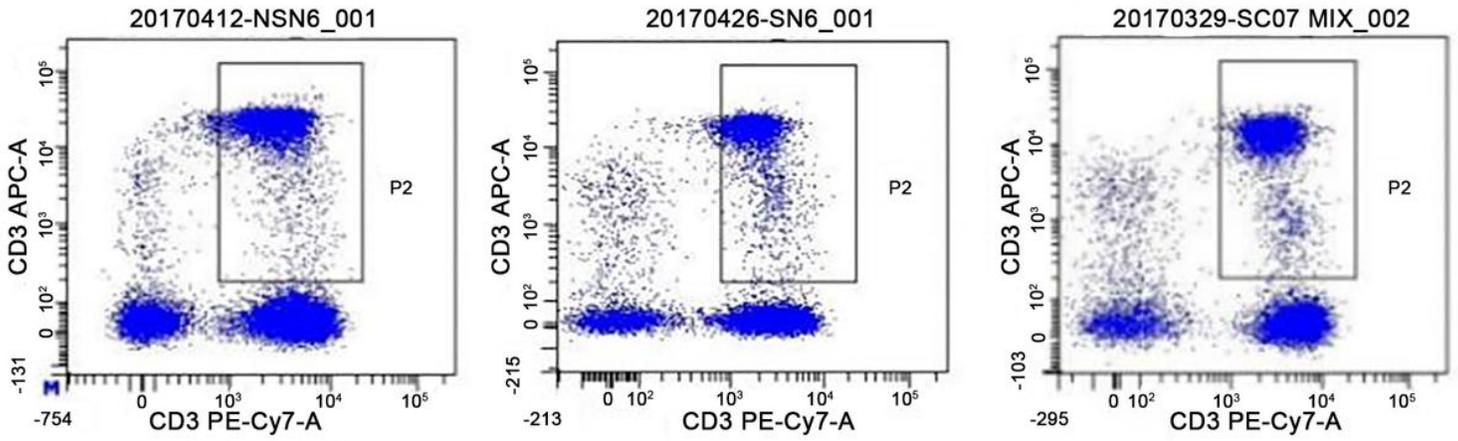


Figure 1

Flow cytometric analysis of CD8+T cells

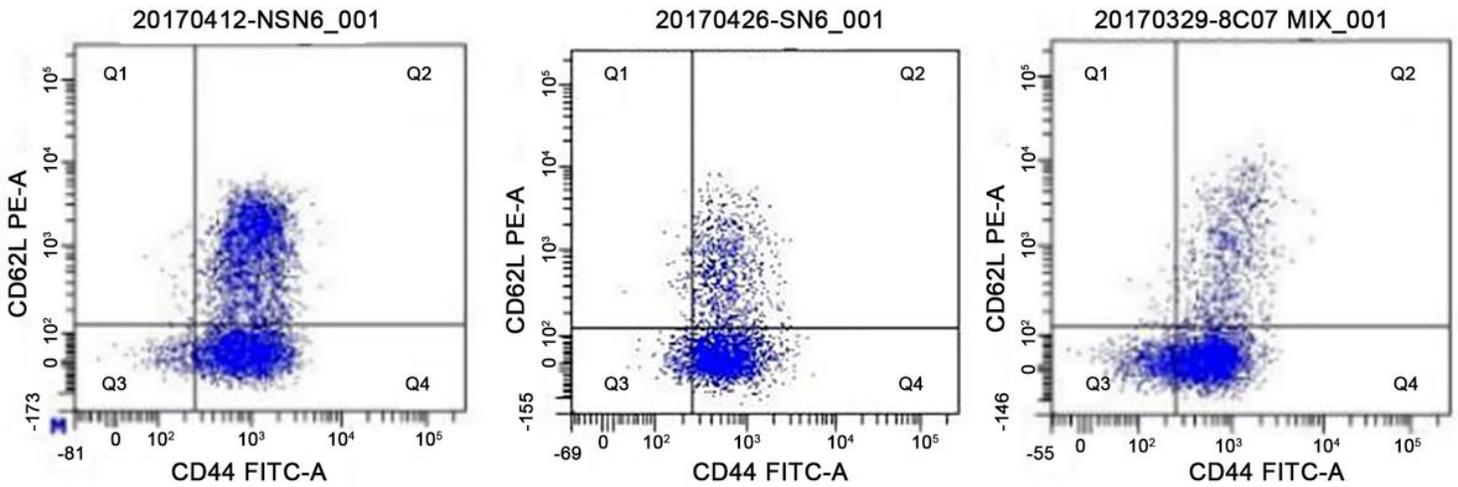


Figure 2

Flow-cytometric analysis of CD8+effector and memory T cells

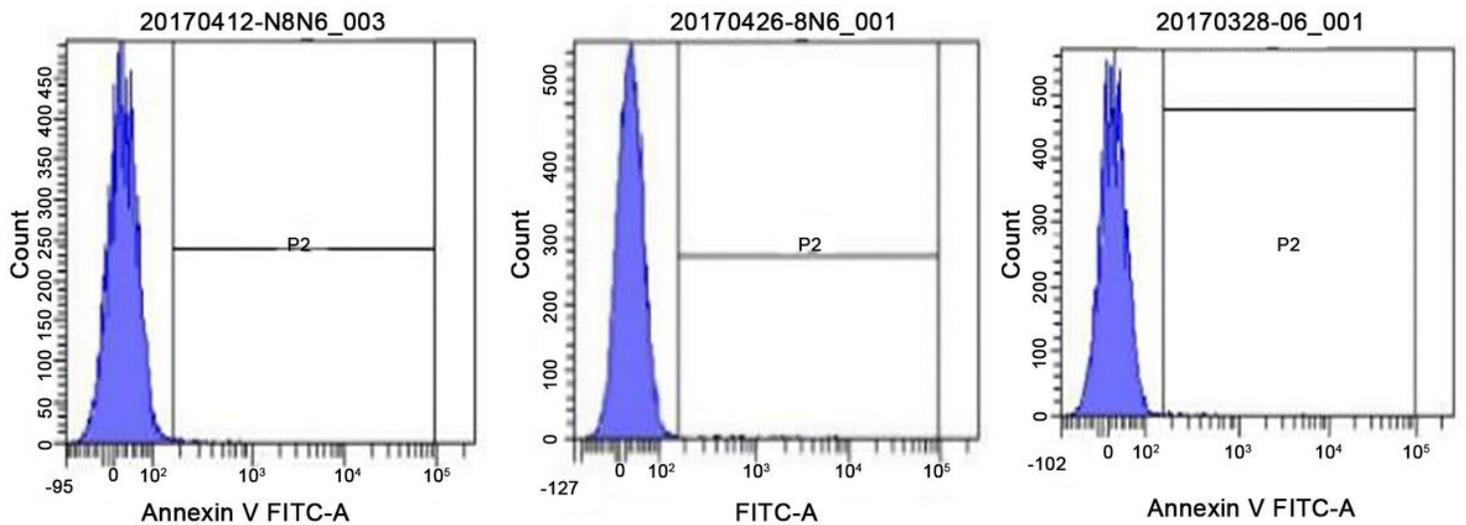


Figure 3

# Flow-cytometric analysis of apoptosis in CD8+ T cells

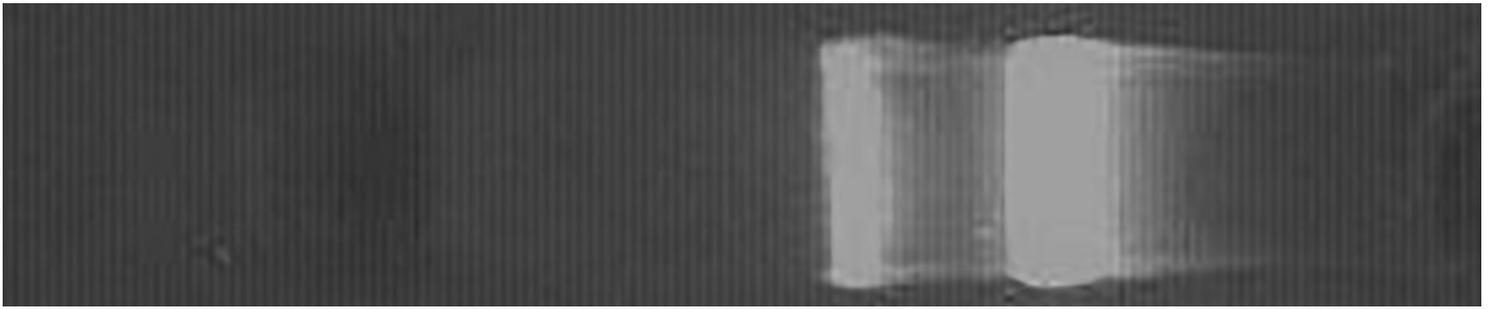


Figure 4

Total RNA extracted by the Trizol method and assessed by electrophoresis

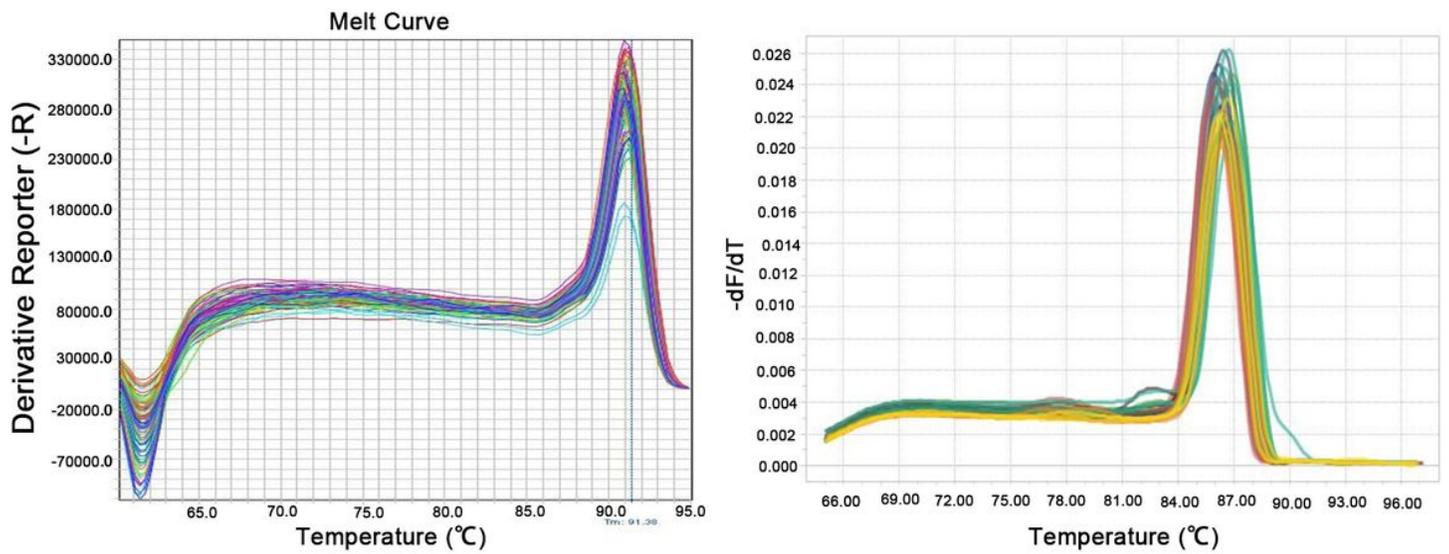
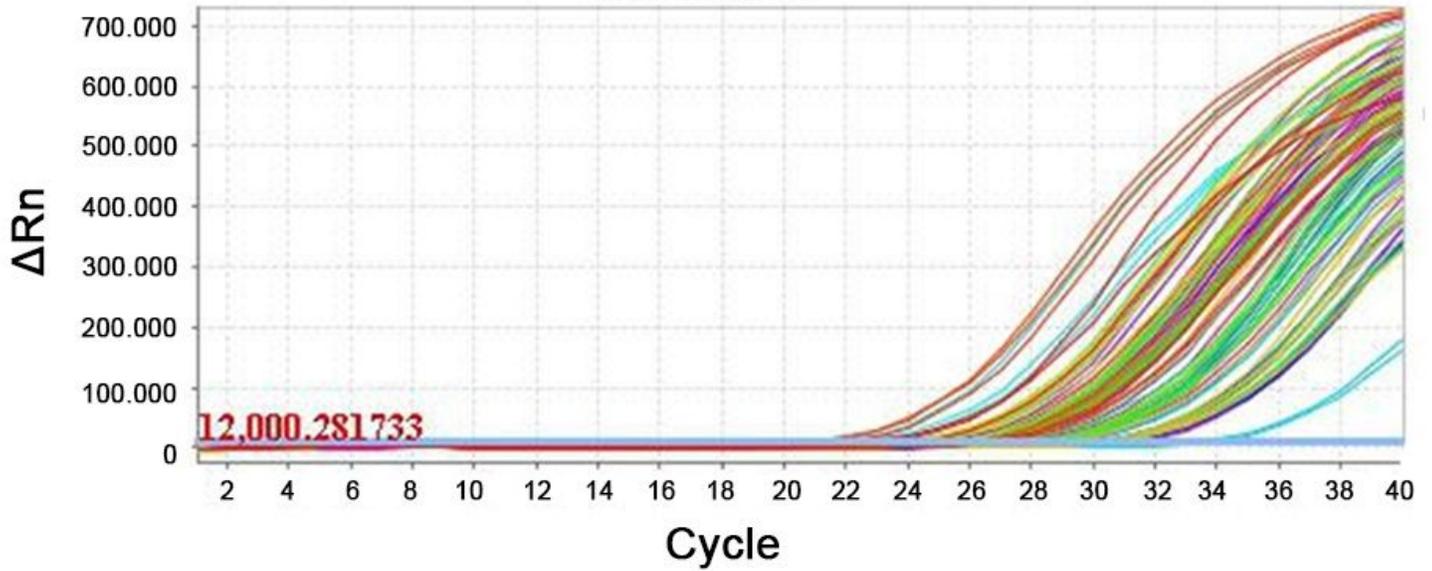


Figure 5

Dissolution curves of the LC3 and P62 genes

## Amplification Plot



## Amplification Plot

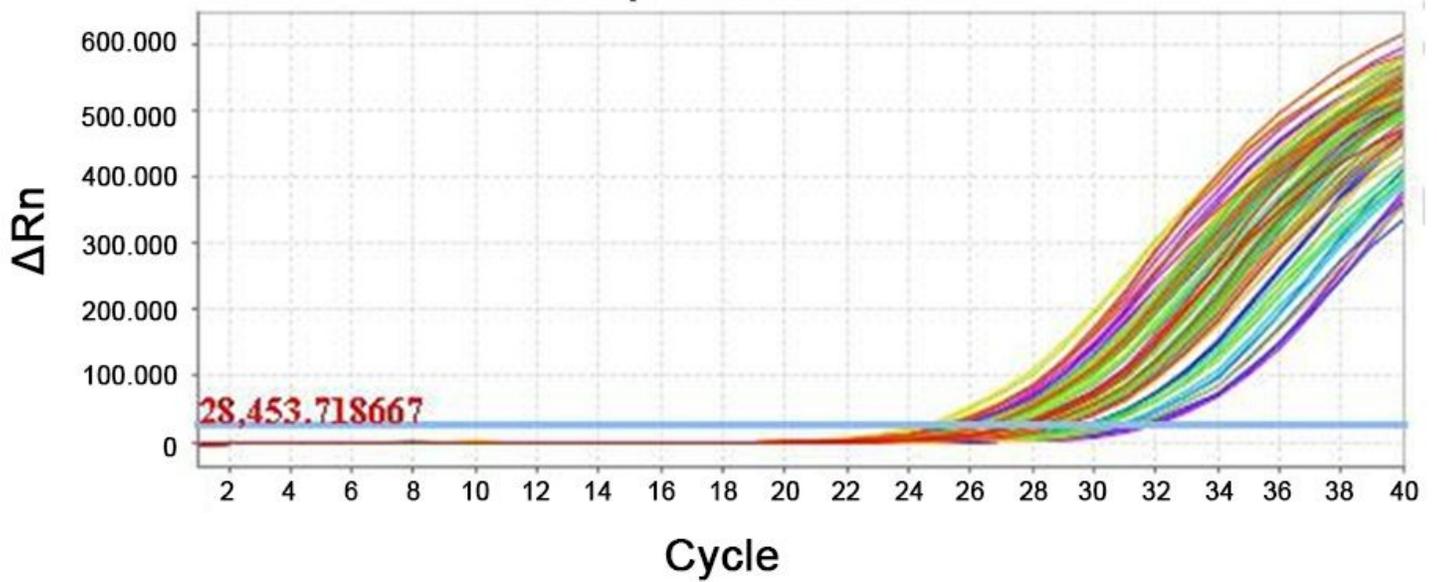
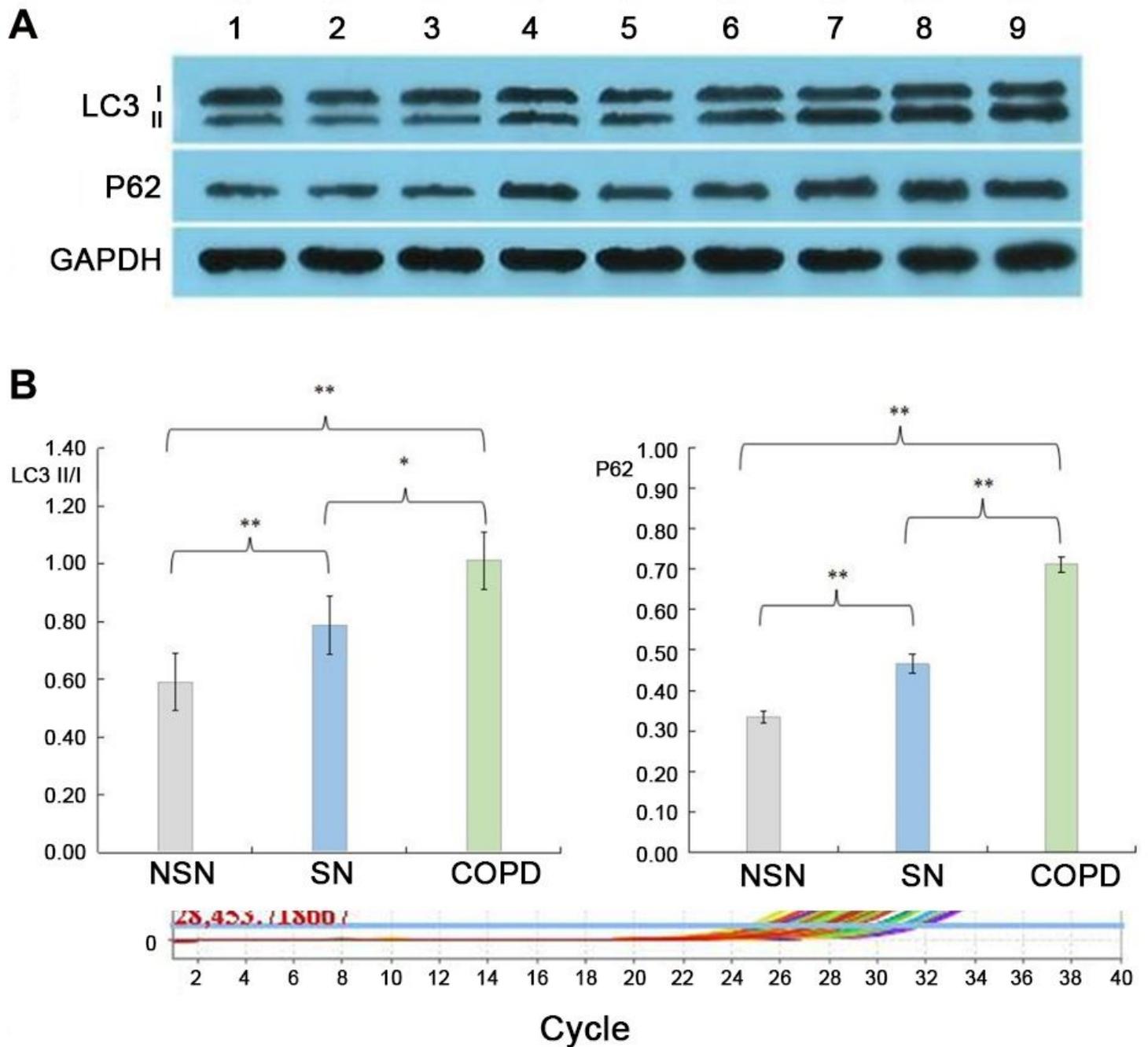


Figure 6

Amplification curves of the LC3 and P62 genes



**Figure 7**

(A) Representative western blots images: bands 1, 2, and 3 represent the non-smoking group with normal lung function; bands 4, 5, and 6 represent the smoking group with normal lung function; while bands 7, 8, and 9 represent the COPD group. (B) Quantitation of immunoreactive bands

## Supplementary Files

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- [Westernblotoriginalphoto.jpg](#)