Increased serum TRAIL and DR5 levels correlated with lung function and inflammation in stable COPD patients

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Background: Chronic obstructive pulmonary disease (COPD) is associated with abnormal systemic inflammation, and apoptosis is one of the pathogenic mechanisms of COPD. Several studies have suggested that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptors were not only involved in diseases associated with apoptosis but also in inflammatory diseases. However, limited data about the possible relationship between COPD and TRAIL/TRAIL-receptors are available.

Objective: To evaluate the potential relationship between TRAIL/TRAIL-receptors and COPD.

Methods: Serum levels of TRAIL, decoy receptor 5 (DR5), C-reactive protein, and tumor necrosis factor-α were analyzed using multiplex enzyme-linked immunosorbent assay kits. Then, serum levels of TRAIL and DR5 in 57 COPD patients with 35 healthy controls were compared and correlated with lung function and systemic inflammation.

Results: Mean levels of serum TRAIL and DR5 were significantly higher in COPD patients than those in controls (50.17±17.70 versus 42.09±15.49 pg/mL, P=0.029; 48.15±22.88 versus 38.94±10.95 pg/mL, P=0.032, respectively). Serum levels of TRAIL and DR5 correlated inversely with forced expiratory volume in 1 second % predicted, an index of lung function in COPD (r=−0.354, P=0.007 for TRAIL; r=−0.394, P=0.002 for DR5) in all participants (r=−0.291, P=0.005 for TRAIL; r=−0.315, P=0.002 for DR5), while DR5 correlated positively with C-reactive protein (r=0.240, P=0.021 for total subjects) and TRAIL correlated positively with tumor necrosis factor-α (r=0.371, P=0.005 for COPD; r=0.349, P=0.001 for total subjects).

Conclusion: Our results suggested that circulating TRAIL and DR5 increased in COPD patients and were associated with lung function and systemic inflammation in COPD. Future studies are needed to verify whether and how TRAIL and its receptors play roles in COPD.

Keywords: chronic obstructive pulmonary disease, tumor necrosis factor-related apoptosis-inducing ligand, decoy receptor 5, apoptosis, inflammation

Introduction

Chronic obstructive pulmonary disease (COPD), a kind of chronic airway inflammatory disease, has been considered as the third most life-threatening disease, with high morbidity and mortality.¹ The pathogenesis and pathology of COPD are complicated, but abnormal airway inflammation is the most important hypothesis, combined with imbalance between protease/antiprotease system, oxidative stress, and immunological mechanisms.² In addition, it has been treated not only as a respiratory disease, but also as a systemic process, which could cause target organ injury, leading to various comorbidities of COPD patients.³⁴ Over the past few years, a growing number of studies have reported that the apoptotic alveolar epithelial and endothelial cells were significantly increased in the lungs of COPD patients, which suggested that apoptosis
might be involved in the pathogenesis of COPD. However, the functional relationship between apoptosis and systemic inflammation in COPD pathogenesis remains unclear.

Tumor necrosis factor (TNF) plays an important role in the pathogenesis of COPD. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a member of the TNF family and mainly expressed by CD11c+ dendritic cells, airway epithelial cells, human peripheral blood T-lymphocytes, natural killer cells, eosinophils, neutrophils, and monocytes can be activated by cytokine or antigen exposure. Decoy receptor 5 (DR5) is one of the most important receptors of TRAIL, the cytoplasmic death domains of which can activate nuclear factor kappa B cells (NF-κB), inducing apoptotic cell death. Moreover, DR5 has been confirmed to be the highest affinity receptor for TRAIL, and it plays a vital role in TRAIL-induced apoptosis.

Previous studies mainly focused on the function of TRAIL in tumors, since it could activate the extrinsic apoptotic pathway in immortalized cell lines and primary tumor cells. In recent years, some studies have found that TRAIL and its receptors played important roles in certain inflammatory responses. Furthermore, the underlying roles of the TRAIL/TRAIL-receptor system in the conditions of chronic pulmonary diseases, such as asthma, lung fibrosis, and pulmonary hypertension, have also been investigated. It has been shown that the TRAIL/TRAIL-receptors system was one of various mechanisms implicated in alveolar apoptosis of emphysematous lung. Morissette et al previously reported that DR5 was upregulated in the lung tissues of patients with emphysema when compared with healthy controls (smokers and nonsmokers). They also discovered that cigarette smoking and oxidative stress could sensitize alveolar epithelial cell to TRAIL-mediated apoptosis, and TRAIL-mediated apoptosis was abnormally increased in the lung of patients with emphysema. Despite these findings, studies concerning the association between TRAIL/TRAIL-receptors system and COPD are still limited. Therefore, the main objectives of this study were to measure the circulating TRAIL and DR5 in stable COPD patients and analyze their correlations with pulmonary function and systemic inflammatory markers of COPD.

Methods
Subjects
Between January 2013 and October 2014, 57 patients with COPD were recruited from the outpatient department of West China Hospital. They all underwent a chest X-ray and a standard pulmonary function test using a Spirotel® spirometer purchased from Mir Medical International Research Srl (Rome, Italy). COPD was diagnosed and classified for this research in accordance with Global Initiative for Chronic Obstructive Lung Disease criteria. Patients with COPD had to have a forced expiratory volume in 1 second to forced vital capacity ratio (FEV1/FVC) less than 70%, and a less than 12% increase in FEV1 after inspiring bronchodilator, and they should not have had an exacerbation within the last 3 months prior to the study. None of the patients had ever received inhaled corticosteroids or antibiotics. They were divided into nonsmokers with COPD and smokers with COPD.

Statistical analysis
Data were expressed as mean ± standard deviation. After normal distribution of these data was been confirmed, differences between groups were statistically analyzed using an unpaired Student’s t-test and, for multiple comparisons, one-way analysis of variance was used. The nonnormal data were transformed to be normally distributed through log transformation. Correlations between variables were analyzed using Pearson’s correlation analysis. The indication of statistically significant difference was P<0.05. Data were analyzed using SPSS 19.0 for Windows (IBM, Chicago,
Results
Clinical characteristics of subjects
Demographic and clinical characteristics (including smoking pack-years, body mass index [BMI], and serum levels of TRAIL and DR5) of patients with COPD and healthy controls are summarized in Tables 1 and 2. The age, sex, BMI, blood oxygen saturation, proportion of smoking, and smoking pack-years of both groups were similar. There were significant reductions in FEV₁, FEV₁ % predicted, and FEV₁/FVC in the COPD group compared with those in the control group, suggesting that the COPD group had airflow limitation. In multigroup analysis, age, BMI, blood oxygen saturation of all the groups were similar. Even though the sex composition and smoking pack-years were different between COPD smokers with COPD nonsmokers and healthy nonsmokers, they were similar between smoking COPD group and healthy smokers.

Serum levels of TRAIL and DR5 increase in COPD patients
The circulating TRAIL levels in COPD patients were significantly upregulated in comparison with healthy volunteers (50.17±17.70 versus 42.09±15.49 pg/mL, \(P = 0.029\), Figure 1A). Similar result was found in DR5 (48.15±22.88 versus 38.94±10.95 pg/mL, \(P = 0.032\), Figure 1B). In addition, in multigroup analysis, we found that TRAIL and DR5 expressions were higher in smokers with COPD than those in nonsmokers with COPD (55.68±16.44 versus 45.55±17.65 pg/mL, \(P = 0.024\); 55.06±25.26 versus 42.35±19.22 pg/mL, \(P = 0.008\), respectively), healthy smokers (41.03±21.00 pg/mL, \(P = 0.013\); 39.38±10.64 pg/mL, \(P = 0.021\), respectively), and healthy nonsmokers (42.65±12.22 pg/mL, \(P = 0.007\); 38.71±11.34 pg/mL, \(P = 0.002\), respectively). However, there was no difference observed among the healthy nonsmokers, healthy smokers, and nonsmokers with COPD (Figure 2A and B).

TRAIL and DR5 are correlated with lung function, TNF-\(\alpha\), and CRP
We analyzed the correlations of TRAIL and DR5 with FEV₁ % predicted, FEV₁/FVC, CRP, and TNF-\(\alpha\) in COPD patients and in all subjects, and found that TRAIL was inversely correlated with FEV₁ % predicted (\(r = -0.354, P = 0.007\), Figure 3A) and FEV₁/FVC (\(r = -0.32, P = 0.013\), Figure 3B) in COPD. Similar results were also found in all participants (\(r = -0.291, P = 0.005\); \(r = -0.321, P = 0.002\), respectively, Figure 3A and B). We also observed that DR5 had a negative correlation with FEV₁ % predicted (\(r = -0.394, P = 0.002\) for COPD; \(r = -0.315, P = 0.002\) for total subjects, Figure 3C) and FEV₁/FVC (\(r = -0.316, P = 0.017\) for COPD; \(r = -0.358, P = 0.000\) for subjects, Figure 3D). More importantly, we still found a positive relationship between TRAIL and TNF-\(\alpha\) in COPD and in all subjects (\(r = 0.371, P = 0.005\); \(r = 0.349, P = 0.001\), respectively; Figure 4A) and a positive correlation between DR5 and CRP in subjects (\(r = 0.240, P = 0.021\), Figure 4B). All these correlations are shown in the Tables 3 and 4.

Discussion
In this study, we explored the serum levels of TRAIL and DR5 in 57 COPD cases and 35 controls using multiplex enzyme-linked immunosorbent assay (Luminex platform). We found that serum TRAIL and DR5 levels were significantly higher in patients with COPD than those in healthy controls. Moreover, in COPD patients, circulating TRAIL and DR5 had relationships with smoking history. Further analyses indicated that both TRAIL and DR5 concentrations correlated negatively with lung function, while TRAIL and DR5 correlated positively with TNF-\(\alpha\) and CRP, respectively. To the best of our knowledge, this is the first study to assess the association between TRAIL/DR5 and COPD.

It has been proven that circulating TRAIL and DR5 play important roles in many diseases, such as cancer,29-31
asthma,\textsuperscript{32} allergic rhinoconjunctivitis,\textsuperscript{33} scleroderma,\textsuperscript{34} and chronic hepatitis B.\textsuperscript{35} Recent studies have also connected TRAIL and its receptors to COPD. It has been reported that alveolar epithelial cell was sensitive to TRAIL-mediated apoptosis due to the presence of cigarette smoke extract and \textsubscript{2}O\textsubscript{3}, while recombinant TRAIL could activate caspase-3 and caspase-8 of emphysematous lung but decrease those apoptosis-related proteins in the nonemphysematous lung,\textsuperscript{28} which might be a new mechanism for persistent alveolar destruction after smoking cessation. Moreover, TRAIL could induce the proliferation of smooth muscle cells through activating NF-\kappaB and inducing insulin-like growth factor-1 receptor, which may play a role in vascular remodeling in COPD.\textsuperscript{36,37} Besides, it could also activate proinflammatory signaling pathways, like the MAPK and phosphoinositoide 3-kinase pathways, which are classic signal pathways in COPD.\textsuperscript{38–40} Similarly, a previous investigation has shown that DR5 levels were higher in the lung of subjects with emphysema than normal smokers and nonsmokers, regardless of smoking cessation, and that oxidative stress increased DR5 expression in A549 cells.\textsuperscript{27} DR5 expression in alveolar epithelial cells was upregulated in idiopathic pulmonary fibrosis with overexpression of proapoptotic marker p53.\textsuperscript{24} All of the accumulated evidences suggested that TRAIL and DR5 are also expressed in the cells of lung tissue and had strong relationships with chronic pulmonary inflammatory disease, including COPD.

Therefore, our study took a further step toward exploring the involvements of TRAIL and DR5 in COPD. In this study, we found higher serum TRAIL and DR5 concentrations in COPD patients compared with those in healthy subjects, which was in accordance with previously published results.

**Table 2** Characteristics of multigroups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy nonsmokers</th>
<th>Healthy smokers</th>
<th>Nonsmokers with COPD</th>
<th>Smokers with COPD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>9/14</td>
<td>12/0</td>
<td>13/18</td>
<td>26/0</td>
<td>0.000*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.74±11.13</td>
<td>59.00±13.98</td>
<td>61.61±12.13</td>
<td>62.35±10.46</td>
<td>0.324</td>
</tr>
<tr>
<td>Pack-years (in smoker)</td>
<td>0</td>
<td>22.52±11.57</td>
<td>0</td>
<td>24.78±14.08</td>
<td>0.000*</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (L)</td>
<td>2.43±0.72</td>
<td>3.23±0.59</td>
<td>1.88±0.58</td>
<td>2.09±0.55</td>
<td>0.000</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.06±0.90</td>
<td>4.13±0.71</td>
<td>3.04±0.77</td>
<td>3.61±0.78</td>
<td>0.000</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC</td>
<td>110.83±18.72</td>
<td>107.66±12.64</td>
<td>85.03±22.07</td>
<td>77.65±16.87</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>24.45±3.59</td>
<td>23.75±3.70</td>
<td>23.66±3.70</td>
<td>23.18±3.14</td>
<td>0.662</td>
</tr>
<tr>
<td>SO\textsubscript{2} (%)</td>
<td>96.57±1.56</td>
<td>96.58±1.78</td>
<td>96.36±1.77</td>
<td>96.79±1.68</td>
<td>0.836</td>
</tr>
<tr>
<td>TRAIL (pg/mL)</td>
<td>42.65±12.22</td>
<td>41.03±21.00</td>
<td>45.55±17.65</td>
<td>55.68±16.44</td>
<td>0.019</td>
</tr>
<tr>
<td>DR5 (pg/mL)</td>
<td>38.71±11.34</td>
<td>39.38±10.64</td>
<td>42.35±19.22</td>
<td>55.06±25.26</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Notes: Data are presented as mean ± SD. *There is no difference between healthy smokers and smokers with COPD. †The data were log transformed in the statistical analyses.

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; DR5, decoy receptor 5; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.
Increased serum TRAIL and Dr5 levels in COPD

Figure 2 Multigroup analysis: TRAIL and Dr5 in the plasma from the healthy nonsmokers group, healthy smokers group, COPD nonsmokers group, and COPD smokers group.

Notes: (A) TRAIL was highly expressed in the smoking COPD compared with nonsmokers with COPD, healthy smokers, and healthy nonsmokers (P=0.024, P=0.013, and P=0.007, respectively); (B) a similar tendency was in Dr5 (P=0.008, P=0.021, and P=0.002, respectively). There was no difference between the healthy nonsmokers and smokers. DR5 was log transformed in the statistical analyses. The levels of TRAIL and DR5 are presented as mean (error bar) and compared by one-way analysis of variance (*P<0.05).

Abbreviations: COPD, chronic obstructive pulmonary disease; DR5, decoy receptor 5; NS, nonsmokers; S, smokers; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Figure 3 TRAIL and Dr5 were correlated with pulmonary function.

Notes: TRAIL in the serum was inversely correlated with (A) FEV1 % predicted (r=−0.354, P=0.007 for COPD; r=−0.291, P=0.005 for all participants) and (B) FEV1/FVC (r=−0.32, P=0.013 for COPD; r=−0.321, P=0.002 for all participants). Dr5 was inversely correlated with (C) FEV1 % predicted (r=−0.394, P=0.002 for COPD; r=−0.315, P=0.002 for total subjects) and (D) FEV1/FVC (r=−0.316, P=0.017 for COPD; r=−0.358, P=0.000 for total subjects). DR5 was log transformed in the statistical analyses. The orange solid line denotes the line of best fit in COPD, the blue line represents the line of best fit in all subjects, and Pearson’s correlation coefficient is presented as an r-value.

Abbreviations: COPD, chronic obstructive pulmonary disease; DR5, decoy receptor 5; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.
Meanwhile, in the analysis of stratification, both TRAIL and DR5 levels were higher in smoking COPD than those in nonsmoking COPD patients, healthy smokers, and healthy nonsmokers, indicating that circulating TRAIL/DR5 levels may have relations with smoking history and that different mechanisms may exist between smokers COPD and nonsmokers COPD, although the exact reason remains unclear. The differences between COPD smokers and COPD nonsmokers or healthy nonsmokers might attribute to the differences in either the sex or pack-years. However, there were no differences between smoking COPD group and healthy smokers regarding the sex composition and smoking pack-years. It can be deduced that TRAIL and DR5 may only contribute to the COPD induced by smoking cigarette, as cigarette smoking is responsible for the apoptosis of alveolar structural cells. More interestingly, the apoptosis progress would not stop after smoking cessation, indicating that other mechanisms perpetuate apoptosis in COPD. Whether the TRAIL/TRAIL-receptors play any role in these mechanisms remains an open question and deserves further research.

More importantly, we observed that either TRAIL or DR5 had a negative relationship with pulmonary function (FEV1,% predicted) in COPD patients, suggesting that TRAIL and DR5 may be related to the severity of airway obstruction. Last but not least, we found that serum CRP and TNF-α levels in COPD patients were elevated relative to controls, and TRAIL and DR5 correlated positively with TNF-α and CRP, respectively. It is well established that circulating TNF-α and CRP levels were elevated in COPD, thus considered as appropriate biomarkers of systemic inflammation in COPD.41–43 and were associated with the presence of airflow obstruction.44,45 Therefore, our results suggested that TRAIL and DR5 might function as biomarkers of the severity of systemic inflammation in COPD. However, the underlying mechanisms could not be clarified in the present study. It is well known that TRAIL and its receptors are apoptosis-related proteins. As mentioned earlier, several disease mechanisms contribute to the development of COPD such as inflammation, proteinase/antiproteinase imbalance, oxidative stress, etc. There are a series of complicated interactions between apoptosis and

Table 3 Correlations between TRAIL, lung function, and TNF-α

<table>
<thead>
<tr>
<th></th>
<th>TRAIL (pg/mL) (COPD)</th>
<th>TRAIL (pg/mL) (total subjects)</th>
</tr>
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<tbody>
<tr>
<td>FEV1,% predicted</td>
<td>$r=−0.354, P=0.007$</td>
<td>$r=−0.291, P=0.005$</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>$r=−0.32, P=0.013$</td>
<td>$r=−0.321, P=0.002$</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>$r=0.371, P=0.005$</td>
<td>$r=0.349, P=0.001$</td>
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Notes: TNF-α was log transformed in the statistical analyses. Correlations between variables were analyzed by Pearson’s correlation coefficient.

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TNF-α, tumor necrosis factor-α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Table 4 Correlations between DR5, lung function, and CRP

<table>
<thead>
<tr>
<th></th>
<th>DR5 (pg/mL) (COPD)</th>
<th>DR5 (pg/mL) (total subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1,% predicted</td>
<td>$r=−0.394, P=0.002$</td>
<td>$r=−0.315, P=0.002$</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>$r=−0.316, P=0.017$</td>
<td>$r=−0.358, P=0.000$</td>
</tr>
<tr>
<td>CRP (ng/mL)</td>
<td>$r=0.158, P=0.24$</td>
<td>$r=0.240, P=0.021$</td>
</tr>
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</table>

Notes: DR5 and CRP were log transformed in the statistical analyses. Correlations between variables were analyzed by Pearson’s correlation coefficient.

Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DR5, decoy receptor 5; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TNF-α, tumor necrosis factor-α.
all other pathways.\textsuperscript{7} Our findings might be further evidence of the relationship between apoptosis and inflammation in COPD. Nevertheless, further experiments should be performed in order to clarify how TRAIL and DR5 are induced in COPD and which signaling pathways they involve.

Our research also had some limitations. First, we recruited relatively small number of samples, which might lead to an inexact assessment of serum TRAIL and DR5 concentration. Second, most of our patients had relatively mild COPD, so this result might not be applicable for patients with more severe airway obstruction. Furthermore, we measured the serum levels of TRAIL and DR5 only once and chose the stable COPD patients, so it was unlikely to assess their dynamic levels during the progression of COPD and their expressions in the acute exacerbation of COPD. Finally, only two systemic inflammatory indicators (CRP and TNF-\textgreek{\alpha}) were analyzed, thus we could not gain more detailed insights into whether and how TRAIL/DR5 contribute to the disease.

In conclusion, this is the first study to research the correlations of serum TRAIL and DR5 with lung function and systemic inflammation in COPD. The results indicate that circulating TRAIL and DR5 may serve as blood-based markers for COPD severity and systemic inflammation and further confirm the roles of TRAIL and its receptors in COPD. However, more fundamental researches to demonstrate how TRAIL/TRAIL-receptors contribute to the pathogenesis of COPD should be made.

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Author contributions
All authors contributed toward data collection, statistical analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

References


