

Detection and significance of TregFoxP3⁺ and Th17 cells in peripheral blood of non-small cell lung cancer patients

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Abstract

Introduction: The aim of this study was to explore the relationships between TregFoxP3⁺ cells and Th17 cells and occurrence of lung cancer.

Material and methods: The proportions of TregFoxP3⁺ and Th17 cells, the expression of FoxP3 and ROR γ t mRNA, and the levels of related cell factors such as transforming growth factor- β (TGF- β), interleukin IL-17 (IL-17) and IL-23 were determined respectively by flow cytometry analysis, real-time-polymerase chain reaction (PCR), and ELISA in peripheral blood of 18 healthy people and 26 patients with non-small cell lung cancer (NSCLC).

Results: The levels of TregFoxP3⁺ and Th17, expression of FoxP3 and ROR γ t mRNA, and ratios of TregFoxP3⁺/Th17 and FoxP3/ROR γ t in peripheral blood with NSCLC were higher than those in healthy controls ($p < 0.05$). The proportion of Th17 cells from NSCLC patients was positively correlated with that of TregFoxP3⁺ ($r = 0.81$, $p < 0.05$). The receiver-operating characteristic (ROC) curve demonstrates that the increased level of TregFoxP3⁺/Th17 in the peripheral blood may be a useful indicator in early diagnosis of non-small cell lung carcinoma. The TregFoxP3⁺/Th17 and FoxP3/ROR γ t levels for patients in stage IV were higher than those of patients in stages I, II, and III ($p < 0.05$). The levels of TGF- β , IL-17, and IL-23 were higher in NSCLC patients than those in healthy controls.

Conclusions: The results suggest that ratios of Treg/Th17 correlate with the stage of NSCLC.

Key words: carcinoma, non-small cell lung, TregFoxP3⁺, ROR γ t, Th17.

Introduction

Non-small cell lung cancer (NSCLC) accounts for 80–85% of lung cancer cases. However, surgery, radiotherapy and chemotherapy treatment are not satisfactory because the immunity is inhibited to some extent in patients with NSCLC. The inhibition degree is closely related to tumor progression and prognosis. Non-small cell lung cancer patients often have immunosuppression, which leads to Th1/Th2 imbalance and Th2 drift, affecting anti-tumor immunity [1–3]. Regulatory T cells may contribute to this imbalance and may redirect immunity towards Th2. The cell differentiation pathways and immune effector mechanisms of Treg and Th17 are similar to Th1 and Th2. Therefore, it is important to explore whether there is Treg and Th17 imbalance and its regulatory mechanism of tumor immunity in NSCLC.

CD4⁺ T cells play a key role in immune regulation, including Th1, Th2, Th17 and Treg cells. Treg expresses a variety of surface molecules. Although FoxP3 is not a superficial molecule, FoxP3 is widely recognized as a specific marker of Treg cells [4, 5]. TregFoxP3 inhibits effector T cell proliferation, proinflammatory cytokine production and antibody secretion. It is of great importance in the prevention of autoimmune responses and maintenance of immune balance. Natural Treg cell (nTreg) mediates peripheral tolerance and induces Treg (iTreg) to inhibit inflammation. It is reported that high expression of nTreg in peripheral blood may be associated with anti-tumor immunity block. The iTreg increase is of anti-inflammatory effect. Both iTreg and nTreg contribute to the tumor tolerance [6]. Th17 regulates expression and secretion of effect factor interleukin-17 (IL-17), and is related to occurrence and development of inflammatory and autoimmune diseases. Further, it is shown that IL-6 promotes naive T cells to express high IL-17, and mediates Treg into Th17. TregFoxP3 promotes differentiation of Th17 cell through the secretion of transforming growth factor-β (TGF-β). It is shown that the proportion of TregFoxP3 and Th17 in CD4⁺ T cells increases in patients with tumors. Though they both increase, it is found in mouse models that TregFoxP3 increases more significantly than Th17 [7].

It was shown recently that TregFoxP3⁺ maintains immune tolerance in cancer patients. Th17 is a newly found cell group that participates in autoimmune diseases, cancer, inflammation, and transplant rejection [8]. In this study, the proportion of TregFoxP3⁺ and Th17, mRNA expression and related cell factor levels of FoxP3 and RORγt in peripheral blood of NSCLC patients were detected, in order to explore their regulatory effects in tumor development and immune response.

Material and methods

Samples

Twenty-six NSCLC patients (diagnosed in the same hospital and confirmed by pathological examination) from January 2011 to December 2011 were enrolled in this study. Peripheral blood was taken before surgery with heparin anticoagulant. According to the TNM classification for NSCLC (American Joint Cancer Committee, AJCC), patients were divided into two groups. Patients with stage I, II and III NSCLC were the early-mid group. And those with stage IV NSCLC were the advanced group. Fifteen patients had squamous carcinoma and 11 patients had adenocarcinoma, according to the WHO classification criteria. None of the enrolled patients had received radiotherapy or other immunotherapy previously and they did not have any immune-related disease. For the healthy control group, 18 healthy individuals were selected randomly from the Department of Physical Examination in Qianfoshan Hospital, Shandong Province. All the healthy individuals did not receive immunotherapy and did not have autoimmune disease history. The characteristics of the patients are summarized in Table I. Conduct of clinical trials for this study was approved by the Medical Ethical Committee of Qianfoshan Hospital. All participants signed the informed consent.

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Antibodies, reagents and equipments

Anti-Human-CD4-FITC, anti-Human-CD25-PE, anti-Human-IL-17A-APC, anti-Human-FoxP3-APC and anti-Mouse-IgG1-APC were purchased from eBioscience (San Diego, CA, USA).

Phorbol-12-myristate-13-acetate (PMA), ionomycin (Ion) and monensin were purchased from Sigma (St. Louis, MO, USA). The Foxp3 Staining Buffer Set and Fixation/Permeabilization were also obtained from eBioscience. RPMI-1640 cell culture medium was purchased from Hyclone (Utah, USA). Trizol, PrimeScript RT reagent Kit with gDNA and SYBR Premix Ex Taq (Tli RNaseH Plus) were purchased from TaKaRa, Japan. Anti-human TGF-β, IL-17, IL-23 ELISA kit was purchased from Hangzhou Unitech Biological Technology Co., Ltd.

The Beckman Coulter Epics XL-4-type flow cytometer was purchased from Beckman, High Wycombe, USA. The ABI PRISM 7300 Real-Time PCR System was purchased from Applied Biosystems Inc, California, USA. The SPECTRA MAX190 microplate reader was purchased from MD, Northern California, USA.

Isolation of peripheral blood mononuclear cells

Peripheral blood (4 ml) with heparin was diluted by adding an equal amount of PBS. Then the

Table I. Clinical data of the healthy control group and the NSCLC group

Parameter	Healthy control group (n = 18)	NSCLC group (n = 26)
Age ($\bar{x} \pm s$)	54.2 ± 5.4	56.4 ± 4.7
Gender (male/female)	12/6	17/9
Histological classification:		
Squamous carcinoma	–	15
Adenocarcinoma	–	11
TNM stage:		
I–II	–	6
III	–	12
IV	–	8

diluted peripheral blood was added into the liquid surface of the lymphocyte separation buffer slowly along the wall of the centrifuge tube, followed by centrifugation at 2000 rpm for 20 min until the cells were layered. The middle layer was carefully transferred to a 15 ml centrifuge tube, with addition of 5-fold volume of PBS buffer. After mixing, centrifugation at 1500 rpm for 10 min was performed. The supernatant was discarded and the precipitation was peripheral blood mononuclear cells (PBMCs). The cell number was adjusted to a concentration of 2×10^6 /ml.

Th17 cell labeling

Peripheral blood mononuclear cells (1 ml) were seeded onto a 24-well cell culture plate. Then cells were cultured in 1640 medium supplemented with 10% fetal bovine serum at 37°C with 5% CO₂. Stimulus reagents PMA (50 ng/ml), ionomycin (1 µg/ml) and monensin (2 µg/ml) were also added. Cells were cultured in the stimulus medium for 5 h before they were collected by centrifugation. Then cells were resuspended in 100 µl PBS buffer and incubated with FITC-labeled anti-human CD4 antibody in the dark for 30 min. After fixation and permeabilization, APC-labeled anti-human IL-17A and mouse IgG1 isotype-APC antibodies were added and incubated for 30 min. After washing, cells were resuspended in PBS before cytometric analysis.

TregFoxp3⁺ cell labeling

Peripheral blood mononuclear cells (1 ml) were centrifuged, resuspended in 100 µl PBS buffer and incubated with FITC-labeled anti-human CD4 and PE-labeled anti-human CD25 antibodies at room temperature in the dark for 30 min. After fixation and permeabilization, APC-labeled anti-FoxP3 and mouse IgG1 isotype-APC antibodies were added and incubated at room temperature in the dark for 30 min. After washing, cells were resuspended in PBS before cytometric analysis.

Flow cytometric analysis of Th17 cells and TregFoxp3⁺ cells

After proper labeling, peripheral blood Treg-FoxP3⁺ cells were detected by CD4⁺ CD25⁺ gating

and Th17 cells by IL-17A⁺ gating. The proportion of CD4⁺ CD25⁺ FoxP3⁺ cell subsets in CD4⁺ cell subsets and the percentage of IL-17A⁺ cell subsets in CD4⁺ cell subsets were analyzed respectively.

Quantitative real-time-polymerase chain reaction

Peripheral blood mononuclear cells were isolated as described above. Total RNA was extracted by Trizol. Then the RNA was reversed transcribed to form cDNA. The PCR procedures for the reverse transcription were 42°C for 2 min, 37°C for 15 min, 85°C for 5 s. Quantitative real-time RT-PCR was conducted using an ABI PRISM 7500 Real-Time PCR System. The PCR conditions were initial template denaturation at 95°C for 30 s, then 40 cycles of 95°C for 5 s, 60°C for 31 s, 72°C for 90 s, and a final extension at 72°C for 5 min. β-Actin was used as an internal control. Sequences of primer pairs used in this experiment were summarized in Table II.

Serum TGF-β, IL-17, IL-23 level detection by ELISA

Experiments were performed according to the instructions of the anti-human TGF-β, IL-17 and IL-23 ELISA kit. Briefly, 50 µl of sample dilution buffer, 50 µl of standard sample, 50 µl of sample and 50 µl of antibodies were added to each well and incubated for 3 h. After washing, 100 µl of peroxidase were added and incubated for 45 min, followed by another washing. Chromogenic substrate was added and then the terminated buffer was added. The OD value at 450 nm and 570 nm was measured and the concentration of the test sample was calculated accordingly.

Statistical analysis

SPSS 18.0 software, two samples independent *t*-test, nonparametric rank sum test and linear correlation analysis were used for statistical analysis. The results were expressed as $\bar{x} \pm s$. A value of *p* of less than 0.05 was considered statistically significant.

Table II. Primer sequences for real-time quantitative PCR

Primer name	Primer sequences	Product length [bp]
FoxP3	Forward: 5'-CTGGCAAATGGTGTCTGCAAGT-3' Reverse: 5'-CTGCCCTTCTCATCCAGAAGATG-3'	107
RORγt	Forward: 5'-CTGCAAGACTCATCGCAAAG-3' Reverse: 5'-TTTCCACATGCTGGCTACACA-3'	83
β-Actin	Forward: 5'-TGACGTGGACATCCGCAAAG-3' Reverse: 5'-CTGGAAGGTGGACAGCGAGG -3'	205

Results

The level of TregFoxP3⁺ cells and Th17 cells and the ratio of TregFoxP3⁺/Th17 are elevated in the peripheral blood of NSCLC patients

To find out whether there is an imbalanced level of TregFoxP3⁺ cells and Th17 cells in NSCLC patients, we first analyzed the levels of TregFoxP3⁺ cells and Th17 cells and their ratio in peripheral blood of NSCLC patients compared with healthy controls. The cells were analyzed by flow cytometry and the re-

sults are shown in Figure 1 and Figure 2. The proportion of TregFoxP3⁺ cells in CD4⁺ cells in the healthy control group and NSCLC group were 3.38% (Figure 1 A) and 10.02% (Figure 1 B) respectively. The percentage of Th17 cells in CD4⁺ cells in the healthy control group and NSCLC group were 2.13% (Figure 2 A) and 5.07% respectively (Figure 2 B). Statistically the proportions of TregFoxP3⁺ and Th17 cells in the peripheral blood of the 26 NSCLC patients were significantly higher than those in the peripheral blood of the healthy control group ($p < 0.05$). Also the ratio

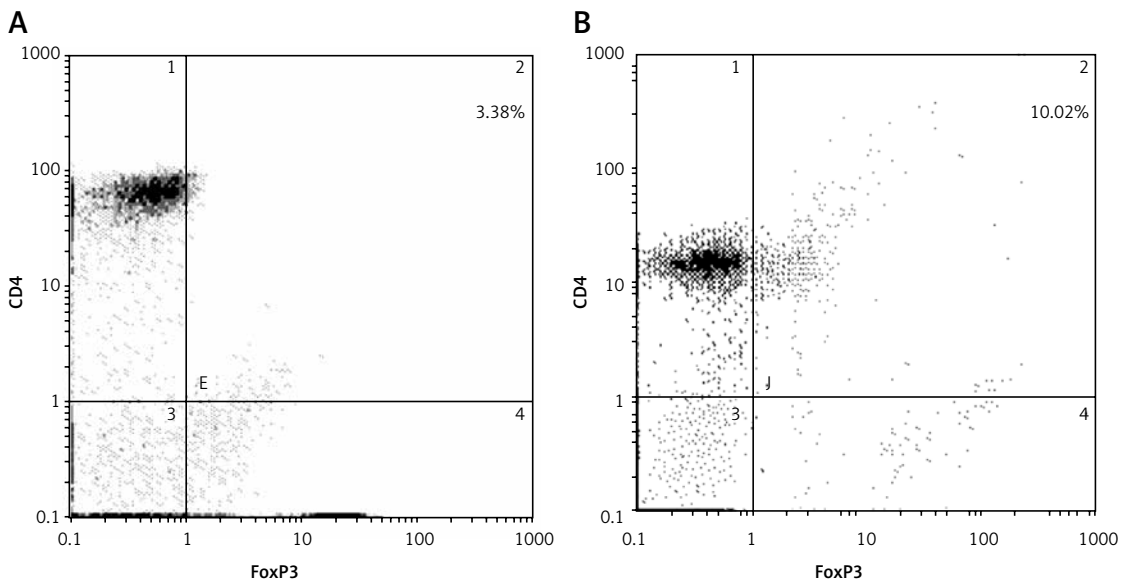


Figure 1. Proportions of TregFoxP3⁺ in the healthy control group (A) and the NSCLC group (B). Isolated PBMCs were labeled by anti-CD4, anti-CD25 and anti-FoxP3 antibodies before cytometric analysis. Representative flow cytometric results are shown. The cells positive for CD4 and FoxP3 were gated (upper right quadrant). Experiments were conducted three times. A p value of less than 0.05 was considered statistically significant

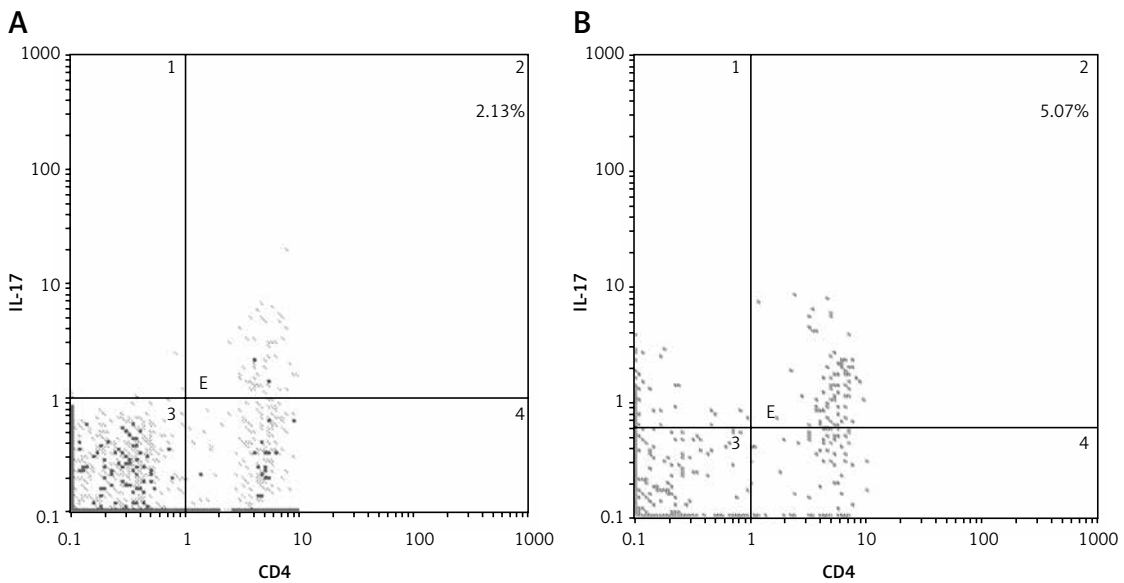


Figure 2. Proportions of Th17 in the healthy control group (A) and the NSCLC group (B). Isolated PBMCs were labeled by anti-CD4 and anti-Th17A antibodies before cytometric analysis. Representative flow cytometric results are shown. The cells positive for CD4 and Th17A were gated (upper right quadrant). Experiments were conducted three times. A p value of less than 0.05 was considered statistically significant

Table III. Proportions of TregFoxP3⁺ and Th17 ($\bar{x} \pm s$, %) and the TregFoxP3⁺/Th17 ratio of the healthy control group and the NSCLC group

Variable	Healthy control group (n = 18)	NSCLC group (n = 26)	Value of p
TregFoxP3 ⁺	3.65 ±0.97	7.02 ±2.41	< 0.05
Th17	2.21 ±0.64	3.61 ±0.82	< 0.05
TregFoxP3 ⁺ /Th17	1.54 ±0.45	1.92 ±0.51	< 0.05

Table IV. Level of FoxP3 and RORγt mRNA ($\bar{x} \pm s$, %) and FoxP3/RORγt ratio of the healthy control group and the NSCLC group

Variable	Healthy control group (n = 18)	NSCLC group (n = 26)	Value of p
FoxP3	0.68 ±0.16	4.31 ±0.71	< 0.05
RORγt	0.43 ±0.14	1.38 ±0.56	< 0.05
FoxP3/RORγt	0.51 ±0.12	2.37 ±0.48	< 0.05

of TregFoxP3⁺/Th17 of the NSCLC patients was higher than that of the healthy control group. The difference was statistically significant ($p < 0.05$) (Table III). These data indicate that in the peripheral blood of patients with NSCLC, the TregFoxp3 + Th17 cells account for a higher proportion of mononuclear cells.

The expression level of FoxP3 mRNA and RORγt mRNA and the ratio of FoxP3/RORγt are elevated in the peripheral blood of NSCLC patients

FoxP3 and RORγt are lineage-defining transcription factors for Treg and Th17 respectively. To further verify the higher level of Treg and Th17 in the peripheral blood of patients with NSCLC at the gene

level, we next checked the expression of FoxP3 mRNA and RORγt mRNA. As revealed by real-time quantitative PCR results (Table IV), in the PBMCs of NSCLC patients, the FoxP3 and RORγt mRNA expression levels were 4.31 ±0.71% and 1.38 ±0.56% respectively, while in the healthy control group, the FoxP3 and RORγt mRNA expression levels were 0.68 ±0.16% and 0.43 ±0.14% respectively. Statistically the FoxP3 and RORγt mRNA expression levels in the PBMCs of NSCLC patients were significantly higher than those in the healthy control group ($p < 0.05$). Similarly, the FoxP3/RORγt ratio in the NSCLC group (2.37 ±0.48%) was higher than in the healthy control group (0.51 ±0.12%). There was a significant difference between the two groups ($p < 0.05$). These results are consistent with the cytometric results and further confirm the higher proportion of TregFoxp3+ Th17 cells in the peripheral blood of patients with NSCLC.

TregFoxP3⁺ cells and Th17 cells are positively linearly correlated in the peripheral blood of NSCLC patients

To address the issue of whether TregFoxP3⁺ cells and Th17 cells are correlated, the linear correlation method was used. As shown in Figure 3, there was a strong positive linear correlation between the TregFoxP3⁺ cells and Th17 cells ($r = 0.81$, $r^2 = 0.674$, $p < 0.05$). These data reveal that a TregFoxP3⁺/Th17 imbalance exists in NSCLC patients.

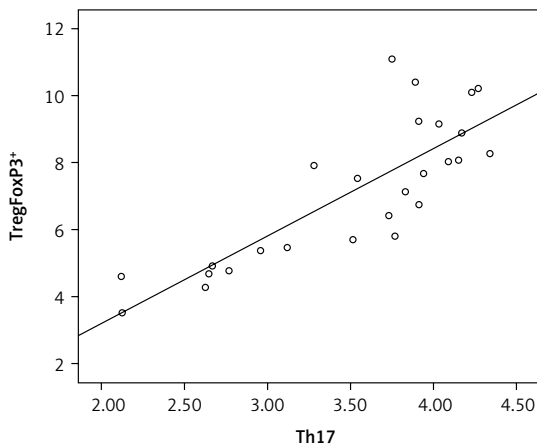


Figure 3. TregFoxP3⁺ and Th17 correlation analysis in NSCLC patients. The linear correlation method was used. The sign and the absolute value of a correlation coefficient describe the direction and the magnitude of the relationship between two variables. The value of a correlation coefficient ranges between -1 and 1. The greater the absolute value of a correlation coefficient, the stronger the linear relationship. In our study the correlation coefficient (r) between TregFoxP3⁺ and Th17 was 0.81

The performance of TregFoxP3⁺/Th17 ratio as a diagnostic indicator of NSCLC by ROC curve

A receiver operating characteristic (ROC) curve analysis is a useful method for diagnostic decision making. To evaluate the performance of TregFoxP3⁺/Th17 ratio as an NSCLC diagnostic indicator and to assess its clinical significance,

we examined the TregFoxP3⁺/Th17 ratio of 26 NSCLC patients and 18 healthy people by ROC curve analysis. As shown in Figure 4, the area under the ROC curve (AUC) was 0.809 ($p = 0.001$). The best diagnostic critical value was 1.695. The sensitivity and specificity were 0.846 and 0.667 respectively. This indicates that the TregFoxP3⁺/Th17 ratio may be a useful indicator of early diagnosis of NSCLC.

TregFoxP3⁺/Th17 ratio was different among NSCLC patients with different TNM staging

To check whether the TregFoxP3⁺/Th17 ratio is imbalanced in NSCLC patients, we compared the TregFoxP3⁺/Th17 ratio in different TNM staging NSCLC patients. The non-parametric rank sum test was used. In the 26 NSCLC patients, 6 cases were at stage I-II, 12 cases were at stage III and 8 cases were at stage IV. The results showed that the TregFoxP3⁺/Th17 ratio in stage IV NSCLC patients was significantly higher than that in stage I-II and stage III NSCLC patients ($p < 0.05$) (Figure 5).

FoxP3/ROR γ t ratio was different among NSCLC patients with different TNM staging

Furthermore, to check whether the FoxP3/ROR γ t ratio is also imbalanced in NSCLC patients, we next compared the FoxP3/ROR γ t ratio in NSCLC patients with different TNM staging. We used the non-parametric rank sum test. In the 26 NSCLC patients, 6 cases were at stage I-II, 12 cases were at stage III and 8 cases were at stage IV. As shown in Figure 6, the FoxP3/ROR γ t ratio in stage IV NSCLC patients was significantly higher than that in stage I-II and stage III NSCLC patients ($p < 0.05$). This result was similar to that of the

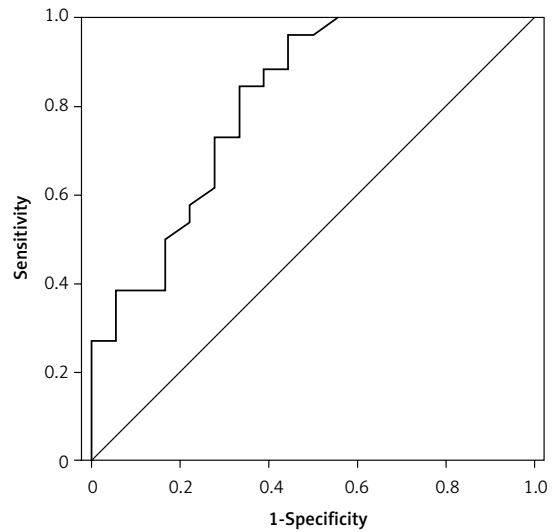


Figure 4. Receiver-operating characteristic (ROC) curve analysis to assess the performance of TregFoxP3⁺/Th17 in the diagnosis of non-small cell lung carcinoma. The TregFoxP3⁺/Th17 ratio was used as an indicator variable. The ROC curve was constructed by plotting the true positive rate (sensitivity) and false positive rate (specificity) associated with each unique value of TregFoxP3⁺/Th17 ratio. An indicator variable with high discriminatory ability will have a curve with an AUC near 1, and an indicator variable with low discriminatory ability will have an AUC near 0.5. In this study, the AUC was 0.809

TregFoxP3⁺/Th17 ratio, suggesting that the imbalance in stage IV NSCLC patients was more significant.

ELISA detection of expression levels of TGF- β , IL-17 and IL-23 in the serum of patients with NSCLC

Transforming growth factor- β expression is relevant with TregFoxP3⁺ cells. Interleukin-17 and IL-23 expression is associated with Th17

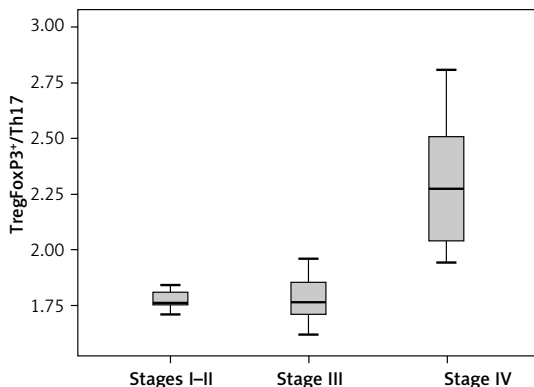


Figure 5. TregFoxP3⁺/Th17 among different TNM stages in NSCLC patients. The non-parametric rank sum test was conducted. According to TNM staging, 6 patients in stage I-II, 12 patients in stage III and 8 patients in stage IV were enrolled in this analysis. Experiments were performed three times. A p value of less than 0.05 was considered statistically significant

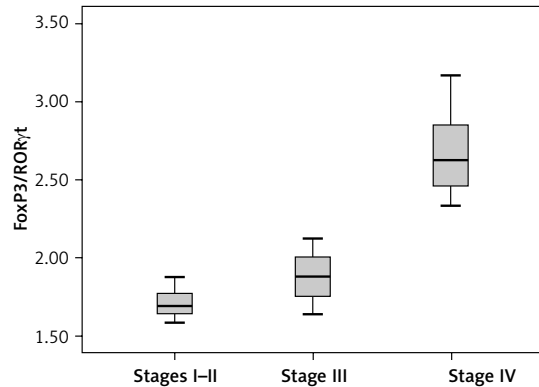


Figure 6. FoxP3/ROR γ t among different TNM stages in NSCLC patients. The non-parametric rank sum test was conducted. According to TNM staging, 6 patients in stage I-II, 12 patients in stage III and 8 patients in stage IV were enrolled in this analysis. Experiments were performed three times. A p value of less than 0.05 was considered statistically significant

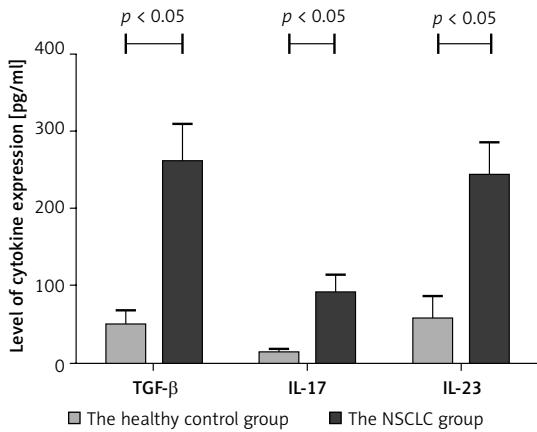


Figure 7. The level of TGF-β, IL-17 and IL-23 in the serum of the healthy control group and the NSCLC group ($\bar{x} \pm s$, pg/ml). ELISA assay was performed to detect the expression levels of TGF-β, IL-17 and IL-23. Experiments were performed three times and the data were expressed as $\bar{x} \pm s$. A *p* value of less than 0.05 was considered statistically significant

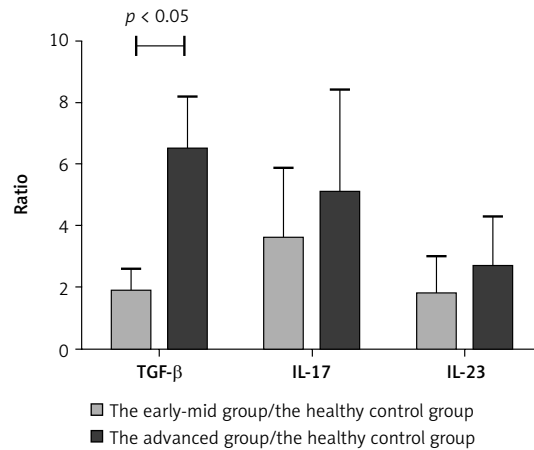


Figure 8. The ratio of TGF-β, IL-17 and IL-23 in the early-mid group/healthy control group and the advanced stage group/healthy group. The relative level of TGF-β, IL-17 and IL-23 was compared between the early-mid group and the healthy control group; and between the advanced stage group and the healthy group. Experiments were performed three times. A *p* value of less than 0.05 was considered statistically significant

cells secrete IL-17 and their expansion is regulated by IL-23. TregFoxP3⁺/Th17 imbalance in NSCLC patients may be related to the regulation of both cytokines. Therefore we detected the expression levels of TGF-β, IL-17 and IL-23 in the serum of patients with NSCLC by ELISA. In the serum of NSCLC patients, expression levels of TGF-β, IL-17 and IL-23 were higher than those in the healthy control group (Figure 7). There was a significant difference (*p* < 0.05). Also we compared the expression levels of TGF-β, IL-17 and IL-23 in the early-mid group, the advanced group and the healthy control group. As shown in Figure 8, the TGF-β level in the early-mid group and the advanced group was significantly higher than the healthy control group (*p* < 0.05), while the difference in the IL-17 and IL-23 level was not significant among the three groups.

Discussion

In vitro studies have shown that Treg cells can induce immune tolerance against tumor-specific antigens and affect the activation and proliferation of CD4⁺ T cells through the secretion of inhibitory cytokines IL-10 and TGF-β [9]. Transforming growth factor-β is mainly involved in the induction, maintenance and function of TregFoxP3 cells [10, 11]. Also TGF-β plays an important role in immunosuppression through inhibition of killer T cell maturation and macrophage activation [5, 12, 13].

Initially, IL-23 was considered to be required for the induction and differentiation of Th17 cells. Langrish *et al.* found that in *p19* gene defective mice (*p19*^{-/-} mice), in which the expression of IL-23 was deficient, Th17 cells could be generated normally [14]. However, the survival of Th17 cells was seriously affected. On the other hand, further studies found that IL-23 can not only promote the func-

tion of Th17 cells but also can recruit Th17 cells to sites of inflammation [15, 16]. Furthermore, *in vivo* data showed that IL-23 participated in the Th17 cell mediated immune response against bacterial infections and in Th17 cell mediated chronic inflammation in disease [17, 18].

Our results showed that, compared with the healthy control group, the proportion of TregFoxP3⁺ cells and Th17 cells, the expression levels of FoxP3 mRNA and RORγt mRNA, the TregFoxP3⁺/Th17 ratio and the FoxP3/RORγt ratio were significantly higher in the peripheral blood of the NSCLC patients. These data suggest that TregFoxP3⁺ cells and Th17 cells account for a higher proportion in the peripheral blood mononuclear cells of patients with NSCLC. TregFoxP3⁺ cells were positively linearly correlated with Th17 cells. The ROC curves showed that the TregFoxP3⁺/Th17 ratio may be a useful indicator for early diagnosis of NSCLC. So far there are no reports indicating the TregFoxP3⁺/Th17 ratio as an early diagnosis indicator. However, in our study the AUC in the ROC curve was 80.5%, making the TregFoxP3⁺/Th17 ratio valuable for diagnosis. The NSCLC patients in stage IV had a significantly higher ratio of TregFoxP3⁺/Th17 and FoxP3/RORγt than patients in stage I-II and stage III. This suggests that the ratios of Treg/Th17 in NSCLC patients correlate with TNM staging of NSCLC. With the progression of NSCLC, the induction of Treg cells is strongly promoted while the induction of Th17 cells is relatively weak. The expression of cytokines was also elevated in NSCLC patients. Compared with the healthy control group, the expression levels of TGF-β, IL-17 and IL-23 in the serum of NSCLC patients were

significantly higher. The Treg cell related TGF- β level in the early-mid group and the advanced group was significantly higher than the healthy control group, while there was no significant difference in the expression levels of the Th17 cell related cytokine IL-17 and IL-23 among the three groups. These results indicate that TGF- β participates in the positive feedback regulation during Treg differentiation and plays an important role in immunosuppression. Also the weak induction of Th17 cells in advanced lung cancer may be caused by the limited expression of IL-17 and IL-23. In fact, in advanced lung cancer, tumor cells secreted a large number of cytokines, forming the immune tolerance environment to favor the growth of cancer cells. It is possible that these cytokines suppressed the expression of inflammatory cytokines such as IL-17, IL-23 and IL-6, resulting in the inhibited expression of Th17 cells in advanced NSCLC [19].

Taken together, we speculate that TregFoxP3⁺ can change the micro-environment of the immune response, leading to aggravated immunosuppression and further promoting the proliferation and metastasis of tumor cells. On the other hand, in advanced NSCLC, strong expression of TregFoxP3⁺ cells competitively inhibited the differentiation of Th17 cells [20–22], weakening the inflammatory response that inhibits tumor growth and at the same time promoting the immune tolerance environment suifor tumor growth. In summary, to a certain extent, the expression levels of Treg-FoxP3⁺ cells and Th17 cells reflect the invasion and the metastasis of lung cancer and its ability to escape immune surveillance. Further studies on this will be beneficial and valuable for the early diagnosis and prognosis of NSCLC.

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