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ORIGINAL ARTICLE

Immunomodulatory Effect of Lentinan on Aberrant T Subsets and Cytokines Profile in Non-small Cell Lung Cancer Patients

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Abstract

As a purified active component from traditional Chinese medicine, lentinan administration can be applied as beneficial chemo-immunotherapy for anti-tumor. In this study, the immunomodulatory effects of lentinan on aberrant T subsets and cytokines profile were evaluated for non-small cell lung cancer (NSCLC). Of all NSCLC patients treated with NP chemotherapeutic protocol (combination of vinorelbin and cisplatin), 73 cases were recruited in this retrospective cohort trial study, of which 38 cases received additional lentinan. The changes of aberrant T subsets and cytokines profile were compared between two groups (chemotherapy in combination with lentinan vs. conserved single chemotherapy) by flow cytometry and molecular biology. Higher subset ratio of $CD3^+CD8^+$ cytotoxic T cells was confirmed in the peripheral blood of NSCLC patients. Chemo-immunotherapy of lentinan resulted in a significant increase of CD3 + CD56+ NKT cells (15.7 ± 3.1%), compared with 8.6 ± 1.4% of NKT cells in single chemotherapy group, and up-regulated $CD3^+CD8^+$ and $CD3^+CD4^+$ subsets as well, but caused the decrease of $CD4^+CD25^+$ Tregs induction, accompanied by significant alleviation of IL-10 and TGF-β1, and elevation of IFN-γ, TNF-α, and IL-12 ($P < 0.05$). It could be confirmed that lentinan could not only enhance the cellular immunity and promote the beneficial of anti-tumor by associated immunotherapy, but also had the ability to inhibit the expansion of immune suppressive Tregs in the NSCLC patients, in whom there was a raised Tregs induction compared to health control. Lentinan-based chemo-immunotherapy is a promising strategy for anti-tumor via enhancing the proliferation of cytotoxic T cells, followed by the elevation of inflammatory chemokines/cytokines. Meanwhile, the percentage of CD4+ CD25+ Tregs is down-regulated, leading to a shift in the inflammatory status from Th2 to Th1 in NSCLC patients treated with lentinan.

Keywords Non-small cell lung cancer · Chemo-immunotherapy · Lentinan · T cell subsets

Introduction

Plenty of studies have indicated that the chemotherapeutical drugs are cytotoxic to normal lung cells, which means chemotherapy can offer negative impact to patients' quality of life (QOL) and even their survival period in a certain degree, regardless of their superior effects on anti-tumor [1]. The severe adverse reactions caused by drug toxicity may limit the choice of chemotherapy scheme and its successful implementation, which would lead to negative treatment efficiency. Therefore,

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the prognosis of single chemotherapy with potential risk is generally unsatisfactory.

In the past decades, accumulating clinical researches have reported that traditional Chinese medicine and their purified active components can be applied as beneficial chemo-immunotherapy for anti-cancer, as they may target to modulate immunity, reduce side events, decrease the metastatic possibility and improve the QOL of advanced tumor patients [2]. As a neutral polysaccharide purified from *Lentinula edodes* mycelia extract (LEME), lentinan has been officially approved as an immunomodulatory injection for anti-tumor in Japan first [3]. It was reported that orally LEME in combination with postoperative adjuvant chemotherapy could improve the QOL of patients suffering from advanced breast cancer or gastrointestinal cancer [4, 5]. The antitumor activity of lentinan is mediated by the augmented activities of NK subsets, cytotoxic lymphocytes, macrophages and so on [6].

Although parts of clinical trials proved its application on solid tumor, little is known about the regulatory functions of lentinan on cytotoxic T cells and proinflammatory chemokine/cytokine expression in non-small cell lung cancer (NSCLC) patients. As a retrospective cohort trial, this study is to examine the immunomodulatory effect of lentinan on aberrant T-lymphocytes and cytokine profiles (such as cytokines interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), etc.), as the assistance of chemotherapy in NSCLC patients.

Materials and Methods

Patients

Of all NSCLC patients treated with NP chemotherapeutic protocol, 73 cases were recruited in this retrospective cohort study from June 2010 to December 2015. All patients were screened according to the inclusion criteria and signed agreement to accept this treatment strategy. Inclusion criteria was showed as follows: (1) TNM I~III according to the classification of Union for International Cancer Control (UICC, 2010); (2) Maximum lesion diameter ≥ 10 mm detected by common CT or MRI; (3) Karnofsky grade ≥ 60; (4) Expected survival time ≥ 3 months; (5) No function-anomaly or bad damage of the main organs. Besides, patients with bone marrow dysfunction (WBC < 4000/mm^3, PLT < 10,000/mm^3, Hb < 8 g/dL), Eastern Cooperative Oncology Group (ECOG) score ≥ 4, and patients with multiple cancer lesions or metastatic tumors had been excluded. All patients were treated with NP chemotherapeutic protocol, of which 38 cases received the additional administration of lentinan injection (Fuzhou Jinling Pharmaceutical Co.) intramuscularly. Besides, 25 cases of healthy volunteers were included as control. The study protocol was approved by the Ethics Committee of local Hospital, and the informed consent was obtained from all patients and control subjects.

Therapies

All patients received NP first-line chemotherapy: 25 mg/m^2 of vinorelbin (i.v. drip) on day 1 and 8; 30 mg/m^2 of hydrated cisplatin (DDP) on first 3 days. Every 4 weeks was a cycle and three cycles finished one treatment course. Each patient was observed after one course at least. For chemo-immunotherapy group ($n = 38$), patients were additionally treated with 4 mg of lentinan per intramuscular injection, once daily. The immunotherapy was administrated and lasted till the end of treatment course (12 weeks as a course). The patients in conserved chemotherapy group ($n = 35$) only received single NP chemotherapy. All patients in two groups received support care and symptomatic therapies, such as antiemetic, fluid infusion, bone marrow suppression and so on.

Collection of Blood Samples

For the baseline of characteristics of all NSCLC patients, peripheral blood samples were collected before the initiation of single chemotherapy or chemo-immunotherapy. And the blood samples of healthy controls were collected from 25 cases of volunteers, when they came for health check at the same period of time. Peripheral blood samples of all NSCLC patients were obtained at the first follow-up within 1 week after the treatment course of chemo-immunotherapy or single chemotherapy completed. Each sample would be tested triplicate and shown in the form of mean value.

Preparation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated by Ficoll-Hypaque gradient centrifugation from venous blood. PBMCs were suspended at 4×10^6 cells/ml in RPMI640 medium supplemented with 10% fetal calf serum, 5 mM Hepes and antibiotics. The suspension was cultured for 30 min in tissue culture dishes to remove adherent cells.

Flow Cytometry

All samples from both healthy controls and NSCLC patients were stained with antibodies as follows: CD3-FITC/CD4-PE/CD8-PC5, CD3-FITC/CD56-PE, CD4-PE/CD25-FITC/FOXP3–PC5, and appropriate isotype controls (Bio-Rad Co. LLC, Hercules, CA, USA). All steps were completed according to the product instructions.

ELISA Assay

The levels of the cytokines IFN-γ, interleukin 10 (IL-10), IL-12, TNF-α, and Transforming growth factor beta 1 (TGF-β1) in the cultured PBMCs were determined by ELISA kit (Sigma-Aldrich Co. LLC., St. Louis, USA).

Follow-Up

All patients were followed-up after treatment until December 2015 or death. The end points in this study were progression-free survival (PFS), estimation of the objective response rate (RR), and evaluation of adverse events. PFS was defined from the first day of treatment to clinical/radiological determination of progression. The objective RR was defined as the rate of complete response (CR) plus partial response (PR) with the duration of more than 4 weeks.

Statistical Analyses

All data are exhibited in the form of the mean ± SEM. Statistical significance between two groups on patient demographics was evaluated by unpaired and two-tailed *t*-tests when a statistically significant drug effect was observed in GraphPad Prism 5. The data of response rate were analyzed using the chi-square (χ^2). The survival curves were evaluated by using Kaplan-Meier product-limit method, and the hazard ratio (HR) and their corresponding 95% confidence intervals (CI) were calculated accordingly. The criterion *P* value for statistical significance was 0.05.

Results and Discussion

Baseline Character

The information between two groups was processed and analyzed with no significance on gender, age and course of disease ($P > 0.05$). The average age of all NSCLC patients in this study was 53 yr. (age range: 27 to 75 yr). The percentages of male gender between chemo-immunotherapy group and conservative chemotherapy group were 42.1%(16/38) vs. 40.0%(14/35). The mean time since diagnosis was 4.4 (2–11) months.

The Aberrant T-Lymphocytes and Cytokine Profile in NSCLC

Whether lymphocyte profile would alter in the systemic circulation of NSCLC patients was estimated as well (Fig. 1a). Compared with the health control ($n = 25$), the percentage of $CD3^+CD8^+$ cells (control vs. NSCLC: 21.4 ± 1.4% vs. 24.7

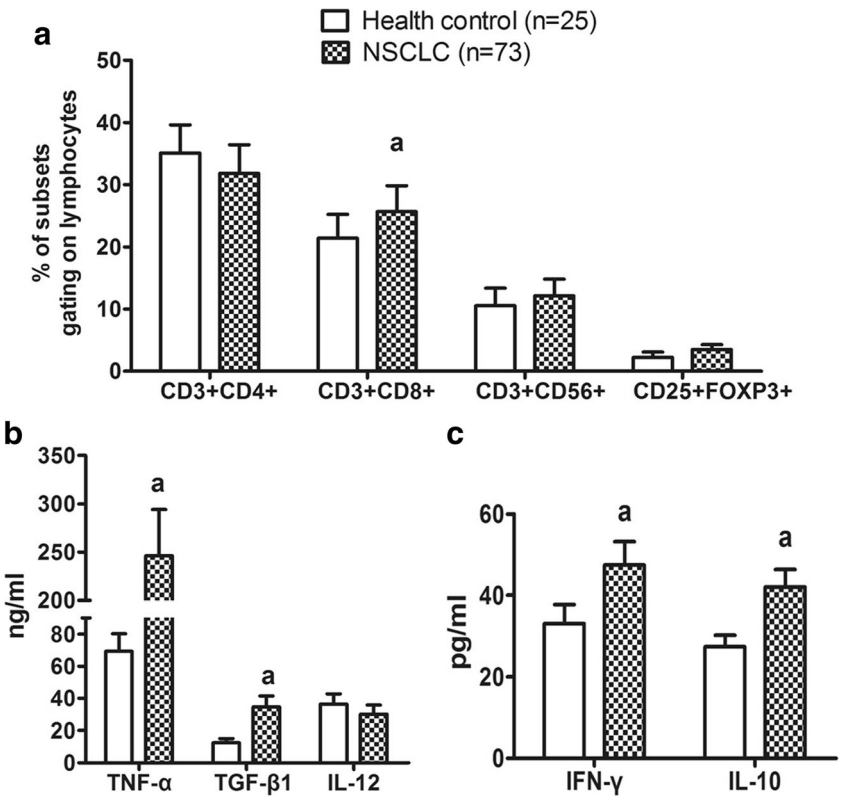

Fig. 1 The baseline of altered T cell subsets and cytokine profiles in NSCLC patients. Data are shown as means ± SEM. [a]$P < 0.05$ compared with healthy control group (analyzed by unpaired and two-tailed Student's *t* test)

± 1.5%, $P = 0.047$) were distinctly elevated in the PBMCs of NSCLC patients. Additionally, the percentages of $CD4^+CD25^+$ regulatory T cells (Tregs), $CD3^+CD4^+$, $CD3^+CD56^+$ were also learnt in the NSCLC patients compared with health control with no significant difference.

The differences of cytokine profile between NSCLC and health control were also assessed and exhibited in Fig. 1b. The remarkable increases of production of inflammatory cytokines, including IL-10, TGF-β1, IFN-γ and TNF-α in NSCLC patients were observed compared with those in health control. In contrast, production of pre-inflammatory cytokine IL-12 was significantly reduced in the NSCLC patients.

Immunomodulatory Effects of Lentinan

The regulatory effect of lentinan on T cell subtypes was investigated as well. In the present study, chemo-immunotherapy of lentinan resulted in a significant increase of $CD3^+CD56^+$ NKT cells (15.7 ± 3.1%), compared with 8.6 ± 1.4% of NKT cells in single chemotherapy group, and up-regulated $CD3^+CD8^+$ and $CD3^+CD4^+$ subsets as well, but caused the decrease of $CD4^+CD25^+$ Tregs induction, accompanied by significant alleviation of IL-10 and TGF-β1, and elevation of IFN-γ, TNF-α, and IL-12 (Figs. 2 and 3, $P < 0.05$). It could be confirmed that lentinan could not only enhance the cellular immunity and promote the beneficial of anti-tumor by associated immunotherapy, but also had the ability to inhibit the expansion of immune suppressive Tregs in the NSCLC patients, in whom there was a raised Tregs induction compared to health control (Fig. 1a and 2e).

RR & PFS

In chemo-immunotherapy of lentinan, three patients (3/38, 7.9%) achieved a CR and 14 cases (14/38, 36.8%) achieved a PR, for an overall objective RR of 44.7%. Another 28.9%(11/38) of patients had stable disease (SD) and 26.3%(10/38) of patients had progressive disease (PD). In conservative chemotherapy group, one patient (1/35, 2.9%) achieved a CR and 11 cases (11/35, 31.4%) achieved a PR, for an overall objective RR of 34.3%, while another 22.9%(8/35) of patients had SD and 42.9%(15/35) of patients had PD. The objective RR between two groups was similar (44.7% vs 34.3%; $\chi^2 = 0.67$, $P = 0.50$).

The Kaplan–Meier curves for progression-free survival (PFS) were shown in Fig. 4. Lentinan-based regimens prolonged PFS comparing to conservative regimens in certain degree of trend (median 15.0 vs 11.0 months, HR = 1.65, 95% CI: 0.93–2.90, $P = 0.09$).

Commonly, overall survival (OS) was defined as the period of time from the first day of treatment to death due to any causes. The factors affecting the OS are complicated, such as the patient's own factors, medical factors, and so on. Considering data on treatment administered after progression were not collected in this study, an exploratory analysis of lentinan treatment on OS was precluded. Despite there was no data on overall survival, the addition of lentinan prolonged PFS in this study. As a clinical study endpoint, PFS is a good indicator and can be influenced by fewer factors. Therefore, the results of PFS are closely related to this study, and the magnitude of this benefit is clinically meaningful.

Discussion and Conclusion

Pharmacological studies showed that lentinan, the main polysaccharide of Lentinus edodes, could activate T cells, strengthen the macrophage phagocytosis, and enhance the body's immune function [7]. It also can adjust the body cell metabolism [8], inhibit side effects of radiotherapy and markedly prolonged survival at five-years follow-up in a variety of cancers, such as gastric cancer, breast cancer, esophageal cancer, nasopharynx cancer, NSCLC and colon-rectum carcinoma [5, 9, 10].

In the previous study, it was reported that lentinan could break out Th1/ Th2 balance and skew to Th1, regulated by the altered balance between reductive macrophages and oxidative macrophages through the distinctive induction of IL-12 and NO [11]. The development of Th1 cells requires IL-12 and is impaired by PGE2 and IL-10, which was mainly expressed by Th2 cells [12, 13]. It was well known that the elevation of IL-10 and TGF-β expression from Th2 cells would down-regulate the activities of antigen-presenting cells and Th1 cells, which resulted into the immunosuppression and related immune escape of tumors. The formation of Th1 further stimulated the enhancement of IL-12, intracellular glutathione content, proliferations of CD4+ and CD8+ T cells, NK cells, macrophage and associated secretion of cytokines (i.e. IFN-γ, TNF-α, etc.) [14, 15]. To the efficient immunotherapy against tumors, it may be one of the critical elements to induce a reductive form of macrophages in tumor stromal tissues to maintain Th1 response [16]. Most of current studies on biological mechanism of lentinan were based on in vitro cell cultures. In this clinical study, the consistent biological mechanism of lentinan in both cellular levels of CD4+/CD8+ T cells, NKT cells, and related cytokines releasing was evaluated and proved in NSCLC patients. As a kind of special T cell subset, NKT cells can express the receptors of both T cell receptor and NK cell receptor, and have the NK cells/CD8+ T cells-like cytotoxic activity after activation. Its activation is commonly accompanied with the activations of T cells, B cells and NK cells and play significant roles in immunomodulation for anti-tumor as well [17]. Therefore, it can be assumed that NKT cells may also play important role in the anti-tumor effect of lentinan.

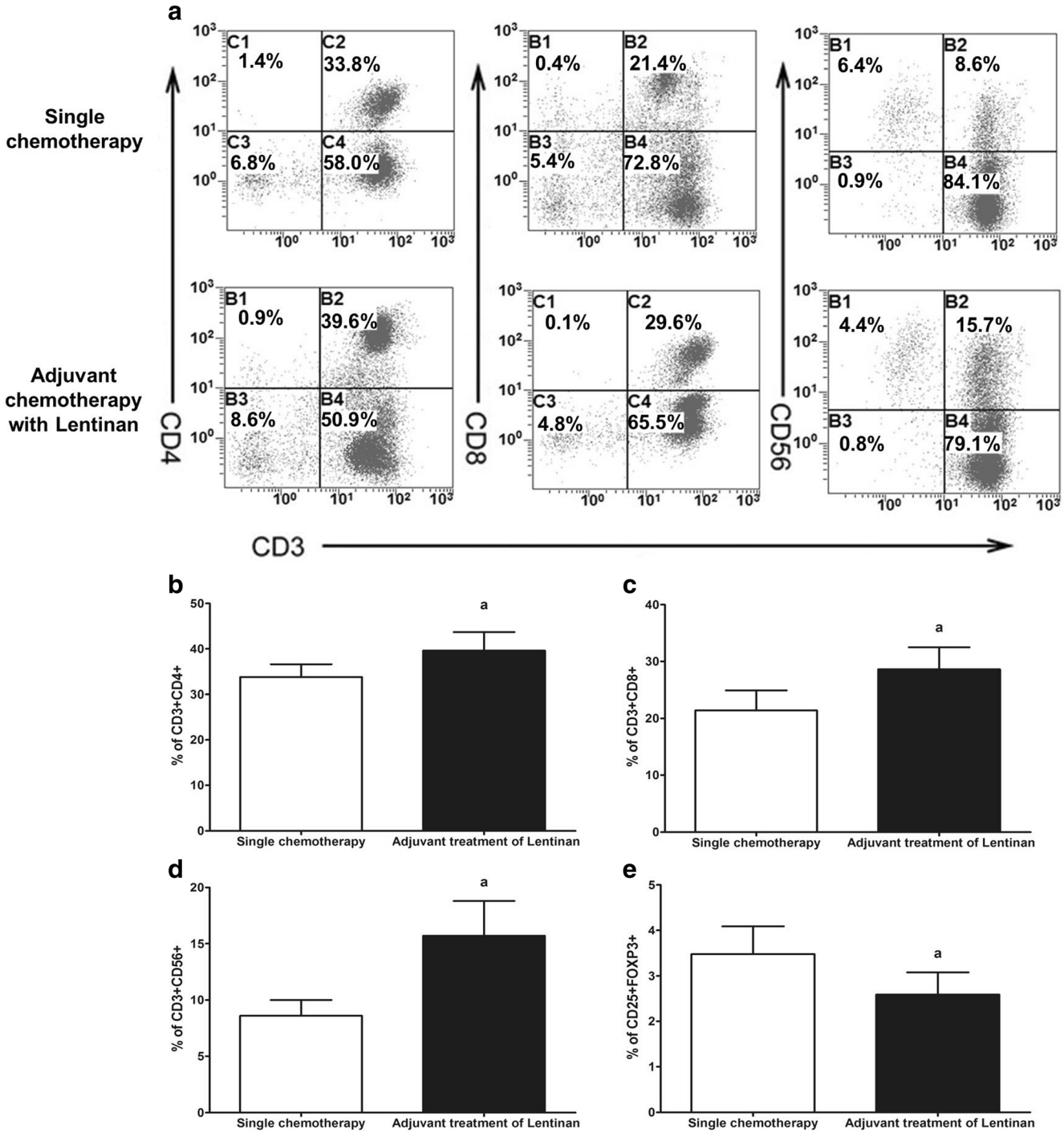

Fig. 2 Immunomodulatory effects of lentinan on aberrant T subsets. Data are shown as means ± SEM. [a]$P < 0.05$ compared with single chemotherapy group (analyzed by unpaired and two-tailed Student's *t* test)

In this study, the solid enhancement of $CD3^+CD8^+$ cytotoxic T cells, Tregs and $CD3^+CD56^+$ NKT-like cells were confirmed in the peripheral blood of NSCLC patients, followed with a reduced level of $CD3^+CD4^+$ T cells. Considering the profile of lymphocyte subtypes might be correlated to the pathological stage and specific situations (i.e. gender, age, etc.) in NSCLC, it was reasonable to investigate the differences between NSCLC patients and healthy controls [18]. It was reported that both the percentages of $CD8^+$ T cells and Tregs were extremely high in tumor tissues of NSCLC patients, showing immunosuppressive effects and inhibiting T lymphocyte proliferation and cytotoxicity by secreting IL-10, TGF-β and so on [19, 20]. In other clinical studies, a significant increase in $CD8^+$ cells, but not $CD4^+$ was observed in

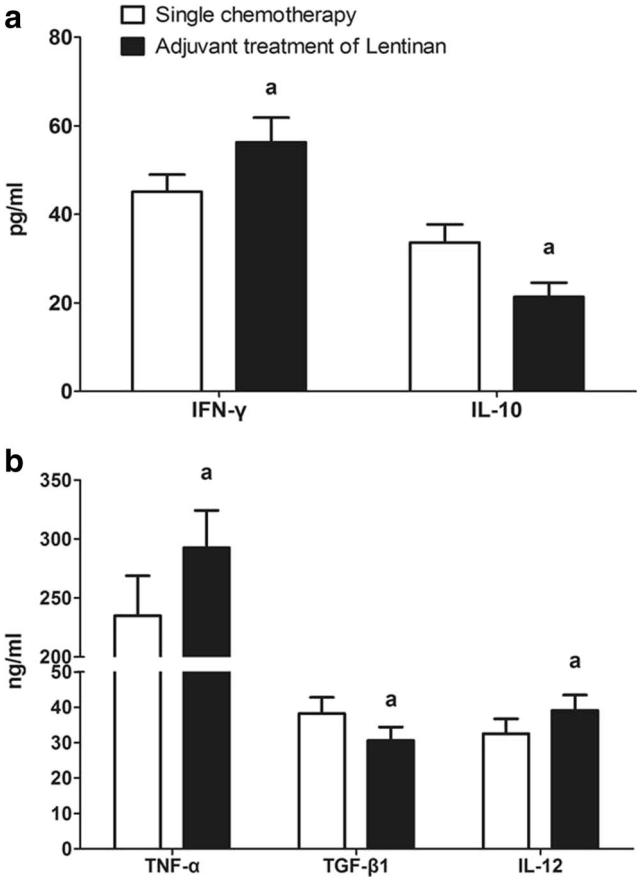

Fig. 3 Immunomodulatory effects of lentinan on cytokine/chemokine profiles. Data are shown as means ± SEM. $^{a}P < 0.05$ compared with single chemotherapy group (analyzed by unpaired and two-tailed Student's *t* test)

NSCLC patients with respect to healthy subjects [21, 22], which indicated that our findings were not totally in accordance with their studies. There was no remarkable difference between NSCLC and healthy controls in their report.

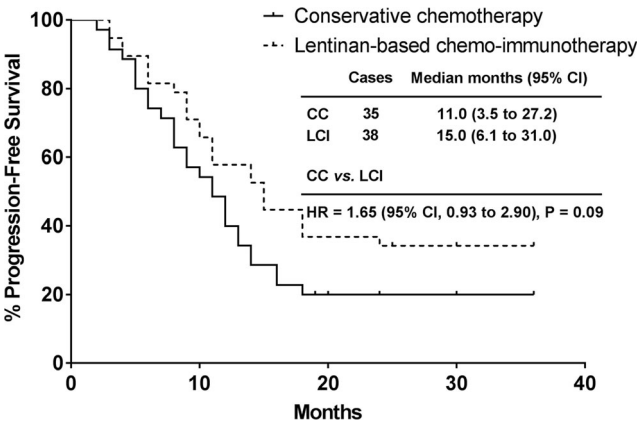

Fig. 4 Progression-free survival for NSCLC patients administrated with conservative chemotherapy (CC) or lentinan-based chemo-immunotherapy (LCI). HR, hazard ratio

Differently, the distinctly decrease of $CD4^+$ in our result could be recognized as the primary damage of immune system in these NSCLC patients. As a heterogeneous subset of T lymphocytes, NKT cells play important functions in both innate and adaptive immune system through their own cytotoxicity and mediating pro-inflammatory productions, which exhibited their potential activities in anticancer and inhibition of tumor diffusion [23].

The recent approved immunotherapy strategies in clinical trials, such as monoclonal antibodies for specific tumor cells, oncolytic viruses, immune checkpoint blockade (anti-PD-1 or anti-PD-L1) and adoptive T cell therapy (CAR T cell) have clearly established as an important modality for anti-cancer besides the traditional approaches of surgery, radiotherapy, and chemotherapy or targeted therapy. However, to date immunotherapy has been shown to induce durable clinical benefit in only a fraction of the patients [24]. The use of combination strategies is likely to increase the number of patients that might benefit from immunotherapy. In this study, even though that the chemo-immunotherapy of lentinan did not result in relatively higher RR than single chemotherapy, the NSCLC patients still benefited from this combination immunotherapy with higher PFS in a certain degree of trend. Besides, various malignancies can lead to lymphocyte infiltrates, which can make tumor cells escaped from the immune response for the stimulation and overexpression of inhibitory receptors on T cells, and may undermine the immune system directly [25, 26]. Therefore, the immune function in tumor patients is impaired commonly more or less. The addition of lentinan can be defined as immune agonists for effector T cells, and may contribute to the combination with the strategy of immune checkpoint blockade. Although lentinan may not increase the immune response rate of other immunotherapies, it still has opportunity to prolong the survival benefits of PFS or even OS in NSCLC patients.

The addition of lentinan decreased the immune suppressive Tregs concomitant with promoted $CD3^+CD4^+$ subsets, $CD3^+CD8^+$ cytotoxic T cells and $CD3^+CD56^+$ NKT-like cells. Besides, lentinan distinctly suppressed anti-inflammatory IL-10 and TGF-β1 and elevated the expressions of pro-inflammatory chemokines/cytokines (IFN-γ and TNF-α) and pre-inflammatory IL-12 as well. The higher frequency of cytotoxic NKT cells, followed with Tregs down-regulation is related to the hyper-induction of IFN-γ and TNF-α in NSCLC. Lentinan-based chemo-immunotherapy is a promising strategy for anti-tumor via enhancing the proliferation of cytotoxic T cells ($CD3^+CD8^+$) and $CD3^+CD56^+$ NKT cells, followed by the elevation of pro-inflammatory chemokines/cytokines (IFN-γ, and TNF-α) and pre-inflammatory IL-12. Meanwhile, the percentage of $CD4^+CD25^+$ Tregs is down-regulated, leading to a shift in the inflammatory status from Th2 to Th1 in NSCLC patients treated with lentinan.

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Author's Contributions All authors of XEW, YHW, QZ and GMX were involved in the study concept, statistical analysis and preparation of the manuscripts. The co-authors of MP, JZ, MC and LJM were mainly focused on data collection. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest All authors declare that they have no conflict of interests.

References

1. Tolcher AW, Patnaik A, Papadopoulos KP, Rasco DW, Becerra CR, Allred AJ, Orford K, Aktan G, Ferron-Brady G, Ibrahim N, Gauvin J, Motwani M, Cornfeld M (2015) Phase I study of the MEK inhibitor trametinib in combination with the AKT inhibitor afuresertib in patients with solid tumors and multiple myeloma. Cancer Chemother Pharmacol 75:183–189
2. Guo Q, Li J, Lin H (2015) Effect and molecular mechanisms of traditional Chinese medicine on regulating tumor immunosuppressive microenvironment. Biomed Res Int 2015:261620
3. Tanaka K, Ishikawa S, Matsui Y, Tamesada M, Harashima N, Harada M (2011) Oral ingestion of Lentinula edodes mycelia extract inhibits B16 melanoma growth via mitigation of regulatory T cell-mediated immunosuppression. Cancer Sci 102:516–521
4. Nagashima Y, Sanpei N, Yamamoto S, Yoshino S, Tangoku A, Oka M (2005) Evaluation of host immunity and side effects in breast cancer patients treated with adjuvant chemotherapy (FEC therapy). Gan To Kagaku Ryoho 32:1550–1552
5. Okuno K, Uno K (2011) Efficacy of orally administered Lentinula edodes mycelia extract for advanced gastrointestinal cancer patients undergoing cancer chemotherapy: a pilot study. Asian Pac J Cancer Prev 12:1671–1674
6. Nakano H, Namatame K, Nemoto H, Motohashi H, Nishiyama K, Kumada K (1999) A multi-institutional prospective study of lentinan in advanced gastric cancer patients with unresectable and recurrent diseases: effect on prolongation of survival and improvement of quality of life. Kanagawa Lentinan Research Group. Hepato-Gastroenterology 46:2662–2668
7. Wang W, Dai X, Ouyang X (2006) Efficacy of lentinan combined with chemotherapy in advanced non-small cell lung cancer. Zhongguo Fei Ai Za Zhi 9:78–81
8. Piao BK, Wang YX, Xie GR, Mansmann U, Matthes H, Beuth J, Lin HS (2004) Impact of complementary mistletoe extract treatment on quality of life in breast, ovarian and non-small cell lung cancer patients. A prospective randomized controlled clinical trial. Anticancer Res 24:303–309
9. Kidd PM (2000) The use of mushroom glucans and proteoglycans in cancer treatment. Altern Med Rev 5:4–27
10. Yamaguchi Y, Miyahara E, Hihara J (2011) Efficacy and safety of orally administered Lentinula edodes mycelia extract for patients undergoing cancer chemotherapy: a pilot study. Am J Chin Med 39: 451–459
11. Murata Y, Shimamura T, Tagami T, Takatsuki F, Hamuro J (2002) The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. Int Immunopharmacol 2:673–689
12. Chehimi J, Trinchieri G (1994) Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. J Clin Immunol 14: 149–161
13. Hsieh CS, Heimberger AB, Gold JS, O'Garra A, Murphy KM (1992) Differential regulation of T helper phenotype development by interleukins 4 and 10 in an alpha beta T-cell-receptor transgenic system. Proc Natl Acad Sci U S A 89:6065–6069
14. Murata Y, Shimamura T, Hamuro J (2002) The polarization of T(h)1/T(h)2 balance is dependent on the intracellular thiol redox status of macrophages due to the distinctive cytokine production. Int Immunol 14:201–212
15. Karpus WJ, Swanborg RH (1989) CD4+ suppressor cells differentially affect the production of IFN-gamma by effector cells of experimental autoimmune encephalomyelitis. J Immunol 143:3492–3497
16. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G (1993) Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 178:1041–1048
17. Suzuki M, Kikuchi T, Takatsuki F, Hamuro J (1994) Curative effects of combination therapy with lentinan and interleukin-2 against established murine tumors, and the role of CD8-positive T cells. Cancer Immunol Immunother 38:1–8
18. Chen C, Chen D, Zhang Y, Chen Z, Zhu W, Zhang B, Wang Z, Le H (2014) Changes of CD4+CD25+FOXP3+ and CD8+CD28- regulatory T cells in non-small cell lung cancer patients undergoing surgery. Int Immunopharmacol 18:255–261
19. Filaci G, Fenoglio D, Fravega M, Ansaldo G, Borgonovo G, Traverso P (2007) CD8+ CD28- T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. J Immunol 179:4323–4334
20. Tsukishiro T, Donnenberg AD, Whiteside TL (2003) Rapid turnover of the CD8(+)CD28(−) T-cell subset of effector cells in the circulation of patients with head and neck cancer. Cancer Immunol Immunother 52:599–607
21. Karagöz B, Bilgi O, Gümüs M, Erikçi AA, Sayan O, Türken O, Kandemir EG, Oztürk A, Yaylaci M (2010) CD8+CD28- cells and CD4+CD25+ regulatory T cells in the peripheral blood of advanced stage lung cancer patients. Med Oncol 27:29–33
22. Meloni F, Morosini M, Solari N, Passadore I, Nascimbene C, Novo M (2006) Foxp3 expressing CD4+CD25+ and CD8+CD28- T regulatory cells in the peripheral blood of patients with lung cancer and pleural mesothelioma. Hum Immunol 67:1–12
23. Giroux M, Denis F (2005) CD1d-unrestricted human NKT cells release chemokines upon Fas engagement. Blood 105:703–710
24. Sathyanarayanan V, Neelapu SS (2015) Cancer immunotherapy: strategies for personalization and combinatorial approaches. Mol Oncol 9:2043–2053
25. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ (2009) Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 10:29–37
26. Wherry EJ (2011) T cell exhaustion. Nat Immunol 12:492–499