

ARTICLE

Chinese Herbal Prescription QYSL Prevents Progression of Lung Cancer by Targeting Tumor Microenvironment

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ABSTRACT

Objectives: Lung cancer is a common and malignant tumor in adults and ranks first in the incidence and mortality of the top five malignant tumors in China. Our previous studies have shown that QYSL prescription can balance lung cancer mice Th1/Th2 and inhibit tumor cell immune escape. Here, we examined the effects of QYSL on lung cancer associated macrophage and the potential associated mechanism. **Methods:** C57BL/6 mice were injected with Lewis lung cancer cells and treated with QYSL. FACS, RT-PCR, and western blot were used to examine the effect of QYSL on tumor immune microenvironment. **Results:** We found QYSL inhibited tumor growth in mice with lung cancer. Further study indicated that QYSL inhibited growth of lung tumor by promoting T cell activation and promoting macrophage polarization to M1 type. We found QYSL could markedly inhibit M2 macrophage related protein expression and promote M1 macrophage related protein expression. Additionally, STAT6 and MTOR expression were reduced in QYSL group. **Conclusions:** Overall, this study indicated that QYSL potentially inhibited the growth of lung cancer by promoting T cell activation and M2 macrophage polarization to M1, which was found to be associated with the STAT6 and MTOR signaling pathway.

KEYWORDS

Lung cancer; TME; traditional Chinese medicine; QYSL

1 Introduction

Lung cancer is a common and malignant tumor in adults [1–3]. According to the latest cancer statistics published in 2020 by the International Cancer Institute, lung cancer mortality is the highest [4]. Lung cancer ranks first in the incidence and mortality of the top five malignant tumors in China [5–7]. Currently, the treatment of lung cancer is mainly surgical resection, followed by adjuvant chemotherapy, radiotherapy, targeted therapy and immunotherapy [8–10]. However, in clinical treatment, targeted therapy and



immunotherapy still face problems such as drug resistance and adverse reactions [11–14]. Therefore, new therapeutic strategies are still urgently needed.

The occurrence and development of lung cancer is closely related to tumor microenvironment (TME) [15–17]. In the process of anti-tumor immune response, tumor cells can evade the attack of immune cells by inhibiting T cell activation. In recent years, immune checkpoint inhibitors, which have been widely used at home and abroad, have achieved remarkable progress of the treatment of tumors by blocking the inhibitory signal of T cell activation in the tumor microenvironment [18–20]. Therefore, it is particularly important to explore the mechanism affecting the activation of T cells, reactivate the immune response effect of T cells on tumors, and further enhance the efficacy of immunotherapy. Tumor associated macrophages (TAM) in tumor tissue plays an important role in regulating the tumor microenvironment. Different roles played by their subtypes M1 and M2 are related to T cell function, M1 macrophages upregulated the immune response and M2 macrophages suppresses the immune response. Therefore, inducing M2 TAM to M1 polarization is beneficial to regulating the activation of T cells [21–23].

Traditional Chinese medicine has advantages in tumor treatment, which not only inhibit tumor growth, but also reduce drug side effects. Its efficacy has been proven by a large number of clinical practices and experimental studies [24–27]. As a clinical prescription for the treatment of lung cancer, the main ingredients of qiyusanlong (QYSL) prescription are astragalus (*Astragalus membranaceus* (Fisch.) Bunge), polygonatum odoratum (*Polygonatum odoratum* (Mill.) Druce), tianlong (Scolopendra), dilong (Pheretima), Solanum nigrum (*Solanum nigrum* L.), Hedyotis diffusa (*Hedyotis diffusa* Willd), coix seed (Coicis Semen), zedoary (*Euphorbiae helioscopiae*), zedoary turmeric (*Curcuma rhizoma*), and Fritillaria (*Fritillariae Cirrhosae Bulbus*). QYSL can significantly inhibit tumor growth and improve the quality of life of lung cancer patients. Studies have shown that QYSL can balance lung cancer mice helper T cell 1/2 (Th1/Th2) and inhibit tumor cell immune escape. Further studies found that 21 potential biomarkers were associated with the function of QYSL and the sphingolipid metabolic pathway was significantly associated with Th1-mediated anti-tumor immunity. However, there is no relevant report on whether it affects the tumor associated macrophage. In this study, we want to explore the inhibitory effect of QYSL on tumor growth of lung cancer tumor-bearing mice from the perspective of immune microenvironment, as well as the regulation of M2/M1 macrophage polarization and T cells, which will provide a new theoretical basis of future clinical research of QYSL.

2 Materials and Methods

2.1 Antibody

STAT6 Rabbit mAb (#5397), STAT3 Rabbit mAb (#12640), STAT1 Rabbit mAb (#14994), PTEN Rabbit mAb (#9188), MTOR Rabbit mAb (#2983), β -catin Mouse mAb (#3700), CD8 α (RPA-T8) FITC-conjugated mAb (#55397), CD4 (RPA-T4) FITC-conjugated mAb (#48705), F4/80 FITC-conjugated mAb (#52267), were purchased from Cell Signaling Technology (Danvers, MA, USA). CD3 APC-conjugated mAb (FAB4841A), CD69 PE-conjugated mAb (FAB2386P), CD206 APC-conjugated mAb (FAB2535A) were purchased from RD (McKinley, MN, USA). CD45 PE-conjugated mAb (ab269346), CD86 APC-conjugated mAb (ab218757) were purchased from Abcam (Cambridge, CB, UK).

2.2 Animals

5 weeks old female C57BL/6N mice were purchased from Shanghai SLAC Co. (Shanghai, China) and housed in separate stainless steel cages (five mice per cage) at constant temperature (23°C) with a 12 h light/dark cycle had free access to water and food. It was raised in the animal room of the Key Laboratory of Xinan Medical Education Department of Anhui University of Chinese Medicine. This study has been authorized by the Animal Ethics Committee of Anhui University of Chinese Medicine (approval No. ahucm-mouse-2019013).

2.3 Cell Culture

The Lewis lung cancer cell line were purchased from the KeBai Biology (Nanjing, China), and cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 0.1% penicillin-streptomycin.

2.4 Drug Preparation

The suspension of QYSL prescription was obtained by impregnation, boiling, filtration and water bath concentration, The preparation steps are as follows: Astragalus (30 g), polygonatum odoratum (10 g), Tianlong (6 g), Dilong (6 g), Solanum nigrum (20 g), Hedyotis diffusa (20 g), coix seed (20 g), zedoary (6 g), zedoary turmeric (10 g) and Fritillaria (10 g), which were purchased from the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. After impregnation, boiling and filtration, QYSL prescription was prepared by concentrating in water bath until the crude drug content was 4.024 g/ml, and stored at 4°C.

2.5 Mouse Model

In this study, 20 SPF C57BL/6 mice were used to establish the tumor model. 1×10^6 Lewis lung cancer cells were injected into the subcutaneous space on the right flank of C57BL/6 mice. Animals were then randomized into treatment groups for efficacy studies. Daily treatment with the compound was initiated after 2 weeks. SPSS or QYSL was delivered daily by oral gavage. Body weight and tumor growth were measured every day after treatment. tumor volume was calculated: $V(\text{cm}^3) = \text{Length} * \text{Width}^2 / 2$.

2.6 RT-PCR

Total RNA was isolated from tissues using ultrapure RNA Kit (CW BIO, Beijing, China) according to the manufacturer's instructions. Then, RNA was reverse-transcribed into cDNAs using a Transcriptor First Strand cDNA Synthesis Kit (Roche). The relative expression of specific genes was determined using SYBR Green PCR Mastermix (TaKaRa, Dalian, China) with bio-rad real-time PCR system. The method of $2^{-\Delta\Delta Ct}$ was used to calculate the relative gene expression levels. Related primers were listed below (Table 1):

Table 1: Primers used for RT-PCR analysis

Gene	Primer
GAPDH-F	AGTATGACTCCACTCACGGCAA
GAPDH-R	TCTCGCTCCTGGAAGATGGT
TNF- α -F	CTAGTGGTGCCAGCCGATGGGTT
TNF- α -R	CAAATCGGCTGACGGTGTGGGTG
CXCL9-F	CCTAGTGATAAGGAATGCACGATG
CXCL9-R	CTAGGCAGGTTTGATCTCCGTTT
CCL17-F	CGAGAGTGCTGCCTGGATTACT
CCL17-R	GGTCTGCACAGATGAGCTTGCC
PTEN-F	TGAGTTCCCTCAGCCATTGCCT
PTEN-R	GAGGTTTCCTCTGGTCCTGGTA
IL-1 β -F	TCGCAGCAGCACATCAACAAGAG
IL-1 β -R	AGGTCCACGGGAAAGACACAGG

(Continued)

Table 1 (continued)	
Gene	Primer
IL-1a-F	TGATGAAGCTCGTCAGGCAGAAG
IL-1a-R	GCAGGTCATTAAACCAAGTGGTGC
IL-12-F	GACATCACCTGGACCTCAGACC
IL-12-R	CAGCATCTAGAAACTCTTTGACAGTG
MTOR-F	AGAAGGGTCTCCAAGGACGACT
MTOR-R	GCAGGACACAAAGGCAGCATTG
STAT1-F	GCCTCTCATTGTCACCGAAGAAC
STAT1-R	TGGCTGACGTTGGAGATCACCA
STAT3-F	CACCTTGGATTGAGAGTCAAGAC
STAT3-R	AGGAATCGGCTATATTGCTGGT
STAT6-F	CTCTGTGGGGCCTAATTTCCA
STAT6-R	GCATCTGAACCGACCAGGAAC

2.7 Flow Cytometry

The effect of QYSL on tumor microenvironment was examined. Cells were harvested and fixed, then stained with antibody. Cell distribution was examined with BD FACS Calibur cytometer. The data was analyzed with the Flow Jo software (Becton Dickinson, NJ, USA).

2.8 Patients

A total of 4 lung cancer patients were included in this study. All patients provided written informed consents, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University and complied with the ethical guidelines of the Helsinki Declaration (1975 revision).

2.9 Western Blot

The tumor tissues were fully lysed with RIPA buffer, and the supernatants were collected after centrifugation. The protein concentration in the supernatant was determined using the BCA Assay Kit (Beyotime, China). An aliquot of 40 µg total protein from each sample was loaded onto 10% SDS-PAGE gels and transferred to PVDF membranes. PVDF membranes were blocked with 5% BSA for 1 h at room temperature and incubated with primary antibodies. The membranes were visualized using an enhanced chemiluminescence detection system (Tanon, China). The qualification of the protein bands was carried out with Image J software by densitometry.

2.10 Statistical Analysis

Student's *t* test was used for comparisons among the different groups. All statistical tests were two-tailed, and a *p* value of less than 0.05 was considered statistically significant.

3 Results

3.1 QYSL Inhibited Growth of Lung Cancer Tumor in a Subcutaneous Mouse Model

QYSL decoction, as a clinical prescription for the treatment of lung cancer, can significantly improve the quality of life of lung cancer patients. In this study, we mainly explored the mechanism of QYSL from the aspect of tumor immunity. Firstly, subcutaneous mouse model was established by subcutaneous injection of

Lewis lung cancer cells to explore the role of QYSL in tumor growth *in vivo*. The results were shown in Fig. 1A. In vehicle group, the tumor gradually increased in a time-dependent manner. However, treatment with QYSL potently suppressed the tumor growth. Tumor growth inhibition reached 26.2% on day 14 after QYSL administration (Fig. 1B). These results indicate that QYSL potently inhibited the proliferation of lung cancer *in vivo*. We also observed that QYSL had no effect on the body weight (Fig. 1C). The result indicated that QYSL had high safety. Overall, these results showed that QYSL potently inhibited the growth of lung cancer *in vivo*.

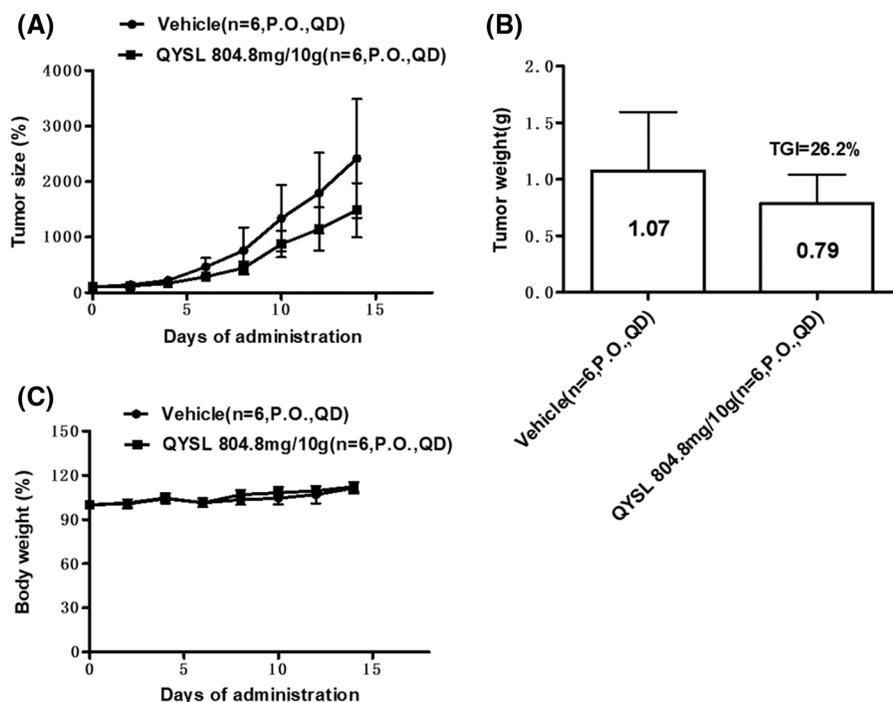


Figure 1: QYSL inhibited growth of lung cancer tumor in a subcutaneous mouse model. (A) *In vivo* effect of QYSL on Lewis lung tumors. Tumor growth curves of Lewis tumors treated with vehicle control or drugs. (B) Comparison of final tumor weights in each group after a 14 d treatment period. Numbers in columns indicate the mean tumor weight in each group. (C) Body weight measurements of transplanted mice

3.2 QYSL Induces an Anti-Cancer Immune Environment in Lung Cancer

Immune microenvironment is very important to tumor occurrence, development, and drug response, especially activated T cells and macrophages. Current studies have confirmed that M2 macrophages can promote tumor growth, and M1 cells can inhibit tumor growth. Therefore, we want to explore the effects of QYSL on T cells and macrophages. In order to examine the effects of QYSL on T cell and macrophage, flow cytometry was used to examine expression of cluster of differentiation 3 (CD3), CD69, and CD8 for CD8⁺ T cell, and examine expression of CD86 and CD206 for macrophages. The results shown that total T cells and CD8⁺ T cells were significantly increased in QYSL group (Figs. 2A and 2B). CD8⁺ T cells are the main effector cells in the body against tumors and other pathogenic microorganisms. Our results demonstrate that QYSL could exert tumor-killing effects by activating CD8 T cells. We also found that compared with vehicle group, the ratio of M2/M1 was significantly decreased in QYSL group (Fig. 2C). To further confirmed the effect of QYSL on macrophage, we examined the related factors expressed by M1 and M2 macrophages. The result shown that

M1 macrophage related factors were increased, such as tumor necrosis factor α (TNF- α), interleukin 1 (IL-1), and IL-12, M2 macrophage related factors were decreased, such as CXC chemokine ligand 9 (CXCL-9) and CC chemokine ligand 17 (CCL-17) (Figs. 2D–2I). M2 macrophages promote tumor growth, while M1 macrophages inhibit tumor growth. These results indicated that QYSL inhibits tumor growth by promoting T cell activation and promoting macrophage polarization to M1 type.

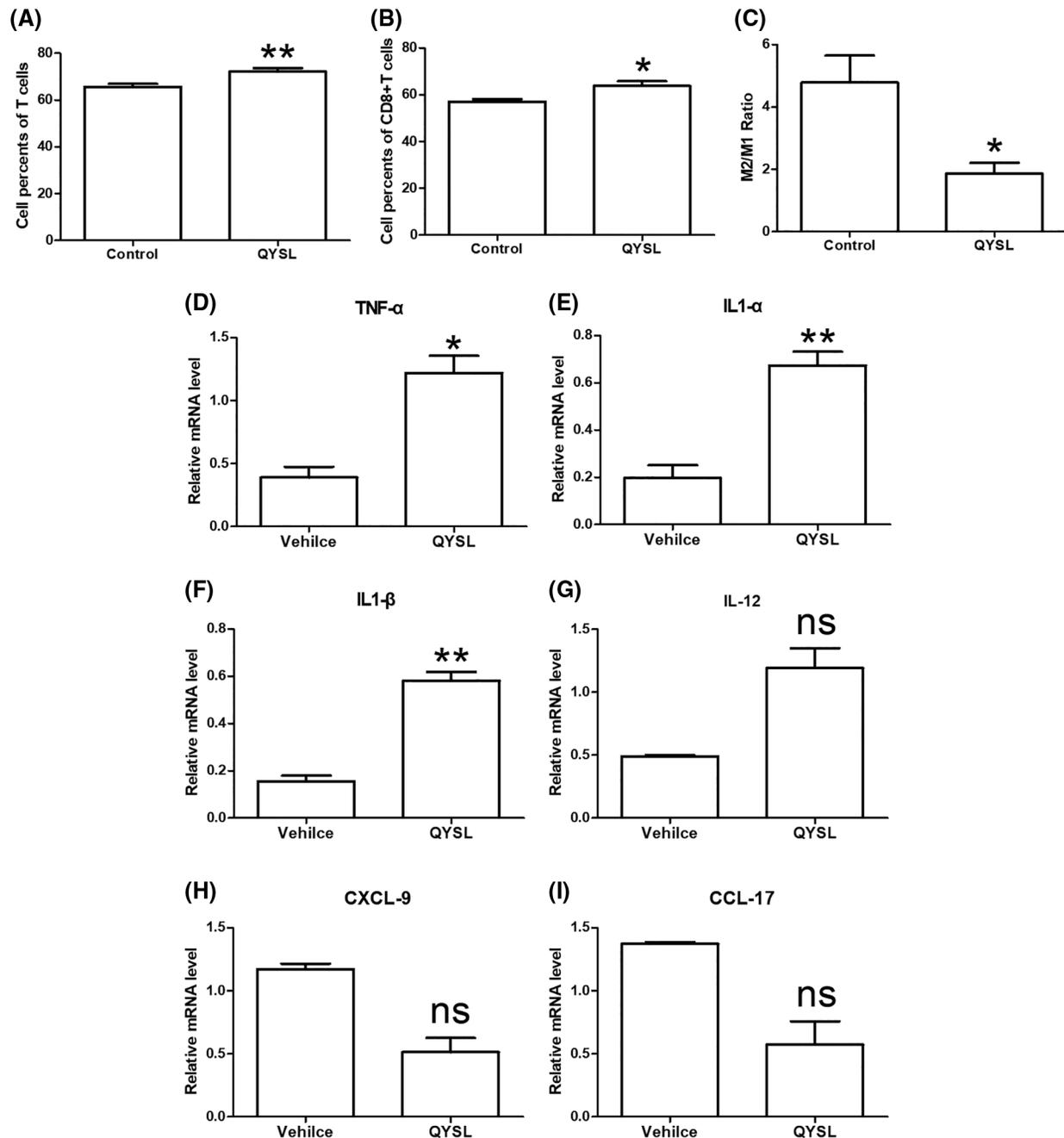


Figure 2: QYSL induces an anti-cancer immune environment in lung cancer. Mice with subcutaneous tumors were dealt with SPSS and QYSL. (A) Total T cell, (B) CD8+ T cell, (C) macrophage, (D–G) M1 macrophage related factors, and (H, I) M2 macrophage related factors in tumor tissue were measured by flow cytometry. Results are mean \pm SD of three independent experiments; ns, not statistically significant; (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$

3.3 QYSL Inhibits M2 Macrophage Related Signaling Pathways

JAK/STAT and MTOR are known to be important molecules involved in M2 polarization. To investigate the molecular mechanism underlying QYSL decreasing M2 macrophages, we examined the effect of QYSL on STAT1, STAT3, STAT6, PTEN and MTOR. We found that QYSL potently inhibits mRNA level of MTOR and STAT6 (Fig. 3A). We also examined the level of protein with western blot. We also observed that the level of MTOR and STAT6 protein were significantly decreased in QYSL group (Figs 3B and 3C). These results suggested that QYSL promoting macrophage polarization to M1 type possibly through inhibiting MTOR and STAT6 expression.

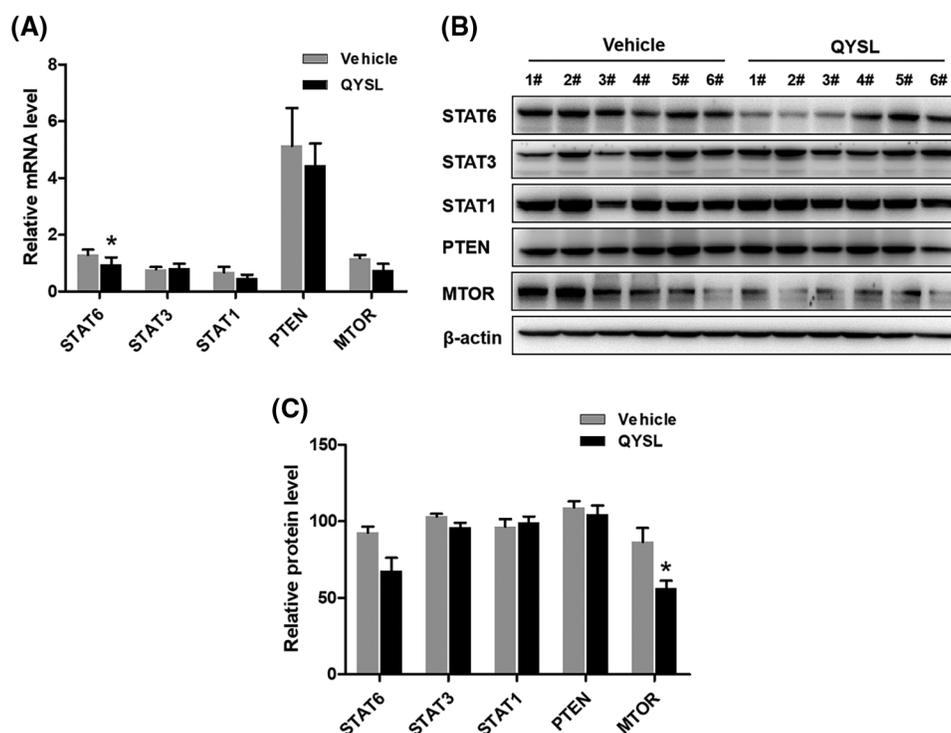


Figure 3: QYSL inhibits M2 macrophage related signaling pathways. Mice with subcutaneous tumors were dealt with SPSS and QYSL. (A) mRNA levels of indicated proteins in tumor tissue were measured by RT-PCR. Results are mean \pm SD of three independent experiments. ns, not statistically significant, (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$. (B) The expression of indicated proteins in tumor tissue was measured by western blot. 1#–6# represent tumor samples taken from mice. (C) The level of indicated proteins is quantified by image J

3.4 QYSL Combined with Anti-Tumor Agents Significantly Prevented Tumor Progression in Clinical

QYSL is a commonly used and important formula for the treatment of lung cancer in our department, which has been proved to have good anti-tumor effect. In this study, we share four clinical cases.

A 61 years old female patient had cough and expectoration for more than 2 months. Chest enhanced computed tomography (CT) showed a space occupying lesion in the lower lobe of the left lung, considering lung cancer, with multiple metastasis of mediastinum, bilateral hilar and bilateral supraclavicular lymph nodes. The pathological results showed that the metastatic small cell carcinoma of the right supraclavicular lymph node was likely to originate from the lung. Immunohistochemical results were CK (+), CK7 (–), TTF-1 (+), napsina (–), CK5/6 (–), p63 (–), p40 (–), NSE (+), CD56 (+),

CGA (-), syn (+), Cdx-2 (-), CK20 (-), ALK (-), Ki67 (60% +). This patient had received a regimen with Etoposide injection 0.1 g for 9 days, Carboplatin Injection 300 mg for 3 days, combined with Sintilimab 200 mg immunotherapy and QYSL prescription. Finally, this patient was in partial remission (Fig. 4A).

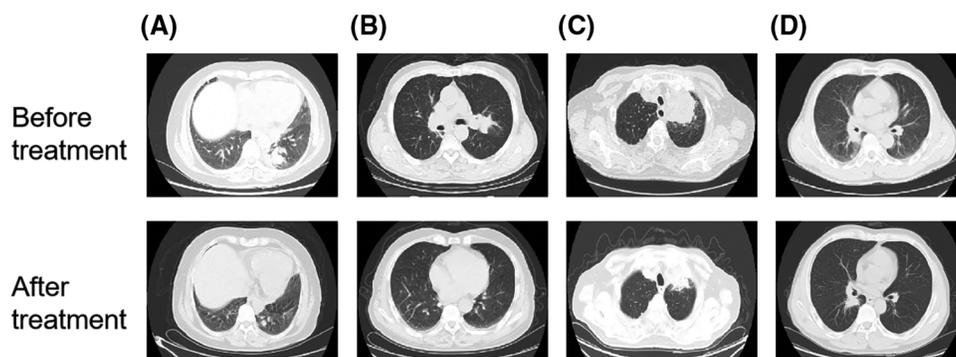


Figure 4: QYSL combined with anti-tumor agents significantly prevented tumor progression in clinical. (A–D) CT image comparison of lung cancer patients before and after combined treatment with QYSL

A 73 years old male patient went to Anhui provincial hospital because no obvious inducement to lose weight. Chest and abdominal CT showed that the right upper lobe mediastinum was occupied with obstructive pneumonia. Considering the possibility of tumor, further examination is recommended. The pathological results of bronchoscopy showed that scattered heterology epithelial cells were found, which was consistent with squamous cell carcinoma. Immunohistochemical results were p40 (+), CK7 (-), TTF-1 (-), Ki67 (60% +). After that, radiotherapy for lung lesions and lymphatic drainage area was started. Gross tumor volume (GTV): 6000cGy/30f, 200cGy/f. The patient received radiotherapy for 22 times and refused to continue radiotherapy because adverse radiation reactions. After discharge, the patient went to our outpatient department for intermittent QYSL prescription treatment. On August 03, 2020, the patient rechecked chest CT. The results showed that the right lung cancer with right pulmonary obstructive pneumonia had unclear boundary with the superior vena cava, and the mass shrank. On August 12, 2020, the patient was admitted to our department. Considering that the patient was lung squamous cell carcinoma and the gene test was negative, the patient had received a regimen with recombinant human endostatin 210 mg 2 ml/h micropump, tegafur bid d1-21, Sintilimab 200 mg, combined with QYSL prescription. Finally, This patient was in partial remission (Fig. 4B).

The 84 years old male patient had chest pain without obvious inducements, which was more obvious on the left. Chest CT showed a space occupying lesion in the posterior segment of the upper lobe tip of the left lung (maximum diameter 5.9 cm), considering lung cancer. On September 02, 2021, the patient was admitted to our department. Lung puncture pathology showed that it was squamous cell carcinoma. After consultation, the thoracic surgery and radiotherapy department would consider that the patient was old, with chronic bronchitis, emphysema and pulmonary bullae, which was not suitable for surgery and radiotherapy. The gene detection showed that there was no mutation in EGFR, ALK, ROS1, and NTRK. Then, this patient had received a regimen with Tislelizumab 100 mg d0, ENDOSTAR 210 mg was continuously micropumped for 7 days Q3W, combined with QYSL prescription. Finally, this patient was in partial remission (Fig. 4C).

A 58 years old male patient coughed and expectorated for more than 1 month. Bronchoscopy showed that new organisms blocked the lumen of the middle bronchus of the right lung. The new biopsies of the middle bronchus of the right lung were sent for pathological examination. The results showed that it was moderately to poorly differentiated squamous cell carcinoma. Immunohistochemical results were CK (+),

p63 (+), p40 (+), CK5/6 (+), CK7 (-), TTF-1 (-), napsin-a (-), Ki67 (70% +), ALK (d5f3) (-). This patient had received a regimen with ENDOSTAR continuous micropump, Tislelizumab 200 mg ivgtt, tegafur 40 mg bid po, anti-vascular combined with immune regimen, combined with QYSL prescription. The patient was in stable condition during treatment (Fig. 4D).

Overall, the clinical application results show that the combination of QYSL and other drugs can achieve better clinical therapeutic effect. And because of the strengthening effect of traditional Chinese medicine, the side effects of patients treated with QYSL are significantly reduced.

4 Discussion

As the most common malignant tumor, lung cancer has the characteristics of easy metastasis and recurrence [28–32]. Therefore, how to prolong the life of patients and improve the quality of life has become an urgent problem to be solved.

The current treatment of lung cancer mainly includes surgery, radiotherapy, chemotherapy, targeted therapy and immunotherapy. In addition, traditional Chinese medicine also plays an important role in the clinical treatment of lung cancer. Sui et al. [33] found *Selaginella* could activate the mitochondrial apoptosis pathway and reduce Ki67 expression to inhibit the growth of NSCLC cells. Xu et al. [34] found *Lianjia Shengjiefang* could regulate EGFR and p53 signaling pathway to inhibit the proliferation of lung cancer cells. Lee et al. [35] found *Calotropis gigantea* could stimulate the intrinsic and extrinsic signal transduction pathways of NSCLC cells to induce apoptosis. Reno et al. [36] found *Tripterygium wilfordii* could inhibit focal adhesion kinase, which can lead to dysregulation of the migration mechanism. Our previous study shows that QYSL could induce autophagy through mTOR signaling pathway, and inhibit Wnt/ β -catenin pathway in NSCLC cells and xenografts to inhibit the progress of NSCLC [37,38].

Modern studies have found that in the process of anti-tumor immunity, TME is the main place for the body's immune system to exert anti-tumor immune response. According to the theory of "cancer immune editing", infiltrating immune inflammatory cell occupies an important position in TME, and their functions are diverse. By reshaping the tumor microenvironment, tumor cell immune escape can be inhibited [39].

CD8⁺ T cell can kill antigen expressed cells, which is an important effector cell for anti-infection, acute allograft rejection and killing tumor cells. Effector killer T cells exist in normal bodies as inactive resting T cells. Therefore, it can differentiate into effector killer T cell (TC) only after antigen activation and Th cell synergy. The activation of killer T cells also requires dual signaling. Firstly, T cell receptor (TCR) binds to the complex of major histocompatibility complex (MHC) molecules and antigen peptides on the target cell membrane, and delivers the first signal through the CD3 complex molecule. Accessory molecules such as CD2, LFA-1, CD8 and CD28 can be combined with corresponding ligand molecules such as LFA-3, ICAM-1, MHC class I molecules and B7 molecules on TC cells. It not only enhanced the adhesion between TC cells and target cells, but also delivered costimulatory signal to TC cells to activate them [40,41]. In this study, we investigated the effect of QYSL on CD8 T cells. The result showed that QYSL significantly promoted the activation of CD8 T cells. It indicated that QYSL can inhibit tumor growth by promoting CD8 T cell-mediated immune response.

In TME, M1 and M2 macrophages play different important roles through a variety of mechanisms. M1 macrophages highly express IL-12 and IL-23, produce killer molecules such as nitric oxide (NO), reactive oxygen species (ROS), a variety of proinflammatory cytokines (IL-1, IL-6, IL-13 and TNF- α), and chemokines (CCL2, CCL3, CXCL-10) [42]. It was found that M1 macrophage polarization can significantly up-regulate the expression of CXCL10 and IL-6, cause acute inflammatory environment, and then reshape immune microenvironment, so as to inhibit the aggregation of inhibitory cells and enhance anti-tumor immune response [43]. We investigated the effect of QYSL on M1 macrophage related factors.

The result showed that QYSL significantly promoted the expression of TNF- α , IL-1, and IL-12. These results suggest that QYSL inhibits tumor growth by promoting the polarization of M1 macrophages.

M2 macrophages can promote tumor progression by releasing various cytokines and signal molecules. M2 macrophages can release VEGF, PDGF and other factors to stimulate the formation of abnormal blood vessels and lymphatic vessels. M2 macrophages can release matrix metalloproteinase 2 (MMP2), MMP9 and other molecules to degrade extracellular matrix and promote tumor escape and progression. And, M2 macrophages also secrete IL-6 to promote the growth of tumor stem cells, and leads to the production of chemoresistant cancer cells. On the other hand, M2 macrophages inhibit the anti-tumor immune function of the body through a variety of mechanisms. Arginase-1 (Arg-1), IL-10, CCL17, CCL24, and TGF- β were highly expressed in M2 macrophages, iNOS, IL-6 and TNF- α were low expressed in M2 macrophages. Arg-1 can mediate the depletion of L-arginine, resulting in the loss of CD3 ζ chain expression in T cell receptor complex, thus inhibiting the activation of effector T cells [44]. IL-10 and TGF- β secreted by macrophages can further interfere with the function of cytotoxic CD8 $^+$ T and CD4 $^+$ T cells, so as to inhibit the self-renewal of TME. M2 macrophages can also regulate PI3K γ pathway to inhibit the activation of cytotoxic CD8 $^+$ T cells [45]. In addition, M2 macrophages also inhibit the infiltration of CD8 $^+$ T cells by reducing the expression of CXCL9 and CXCL10, and can induce dysfunction of tumor infiltrating effector T cells by expressing PD-1 ligand. It is correlated with the prognosis of patients with malignant tumor, such as lung cancer [46].

In this study, we found that the M2 macrophages were increased in tumor and QYSL inhibited tumor growth in the tumor mice model through decreasing activation of M2. We further investigated the mechanism of QYSL regulating macrophage. RT-PCR results showed that QYSL significantly down-regulated the expression of M2 macrophage related genes, such as CXCL-9 and CCL-17. STAT6 and MTOR play important roles in the polarization of M2 macrophages. To further demonstrate whether QYSL inhibited M2-type macrophage polarization by affecting polarization-related signaling pathways, we used RT-PCR and western blot experiments to detect the expressions of STAT1, STAT3, STAT6, PTEN and MTOR proteins. Our results showed that QYSL inhibited the expression of STA6 and MTOR, effectively inhibiting M2 macrophage polarization. Overall, QYSL inhibits tumor growth by inhibiting polarization of M2 macrophages and regulating tumor immune microenvironment.

At present, there are more and more clinical applications of traditional Chinese medicine. The combination of traditional Chinese medicine and anti-tumor drugs can often relieve clinical symptoms, improve the quality of life and the efficacy of chemotherapeutic drugs in clinical practice. It can be seen from the cases provided by us that QYSL combined with anti-tumor drugs has a good therapeutic effect on small cell lung cancer with poor prognosis, especially for poorly differentiated small cell lung cancer. In recent years, for patients with advanced cancer, our department has used QYSL combined with chemotherapy or immunotherapy or radiotherapy to greatly alleviate the side effect of radiotherapy, chemotherapy and immunotherapy, and at the same time enhance the patient's physique, while ensuring shrinkage of tumor. This lays a solid foundation for radical surgery.

In conclusion, QYSL can inhibit the tumor growth of lung cancer by enhancing the activation of CD8 T cell and polarization of M2 macrophages to M1 macrophages.

5 Study Limitation

Which component in QYSL plays a key role has not been studied.

6 Conclusion

This study found that QYSL potently inhibited the growth of lung cancer by promoting T cell activation and M2 macrophage polarization to M1, which was found to be associated with the STAT6 and MTOR signaling pathway.

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Author Contributions: PL and ZGL designed the research. CY and HW developed the methodology. CY, HW, ANJ, JBT, JZ, and MZ performed the experiments and collected the data. CY wrote the manuscript. CY and HW provided the experiment materials. PL and ZGL revised the manuscript.

Ethics Approval and Informed Consent Statement: This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui medical university with the No. PJ2021-08-08. Written informed consent was obtained from the patients for publication of the related data.

Availability of Data and Materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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