# Postprint: Qi-tonifying and Phlegm-resolving Method Alleviates Tumor-induced Skeletal Muscle Injury by Reducing Inflammatory Infiltration

**Authors:** Wu Yingzhao, Ziqian Luo, Y. Zhongjia, Pi Dajin, Cui Jiaqi, Lin Lizhu, Ouyang Mingzi, Chen Qianjun, Ouyang Mingzi, Chen Qianjun

**Date:** 2025-07-16T00:00:00+00:00

## Abstract

Background Cancer patients often experience symptoms such as muscle wasting, but the underlying pathological mechanisms remain incompletely understood. Objective To investigate the pathological mechanisms of cancer-associated skeletal muscle injury and to study the intervention effects of Yiqi Chutan therapy on cancer-associated skeletal muscle injury. Methods From October 2020 to May 2021, thirty 8-week-old female C57BL/6 mice were used. Twenty mice were randomly selected to establish a tumor-bearing mouse model via subcutaneous injection of LLC cells. After successful modeling, they were divided into a tumor group (n=10), a Yiqi Chutan therapy group (YQCT group, n=10), and a control group (n=10). The YQCT group was administered Yifei Sanjie pills by gavage (3 $g \cdot kg^{-1} \cdot d^{-1}$), while the control and tumor groups received equal volumes of normal saline for 21 days. Twenty-four hours after the final administration, behavioral assessments were performed using the open field test and elevated plus maze test; gastrocnemius muscle tissues were collected for histopathological analysis to observe pathological injury changes; transcriptomic analysis was conducted to observe differentially expressed genes; and enzyme-linked immunosorbent assay (ELISA) was used to detect tissue inflammatory factor levels. RAW264.7 cells in the logarithmic growth phase were randomly divided into a mouse serum control group (RAW264.7 group), a mouse tumor serum group (RAW264.7-LPS group), and a mouse Yiqi Chutan therapy serum group (YQCT group). The RAW264.7 group was treated with medium containing 10% control mouse serum, the RAW264.7-LPS group with medium containing 10% tumor group mouse serum + 100 ng/mL LPS, and the YQCT group with medium containing 10% YQCT mouse serum + 100 g/L LPS for 24 hours. ELISA was used to detect inflammatory factor levels in RAW264.7 cells. After the above-treated RAW264.7 cells were co-cultured with mouse C2C12 cells for 48 hours, a lysosomal red fluorescence probe kit

was used to detect autophagolysosome levels in C2C12 cells. Results The comparison of total movement distance in the open field test and elevated plus maze test among the three groups of mice showed statistically significant differences (P<0.001), with the tumor group lower than the control group and the YQCT group higher than the tumor group (P<0.001). Histopathological results revealed that compared with the control group, muscle cells in the tumor group exhibited obvious injury, while the degree of injury was reduced in the YQCT group. Transcriptomic analysis results showed that gene expression trends were relatively consistent between the control and YQCT groups, while the tumor group showed opposite trends. Immunofluorescence staining results demonstrated that compared with the control group, infiltration of M1-type macrophages, neutrophils, T lymphocytes, and B lymphocytes in the gastrocnemius muscle increased in the tumor group; compared with the tumor group, infiltration of M2-type macrophages increased while infiltration of other pro-inflammatory cells decreased in the YQCT group. ELISA detection results showed that levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in the tumor group were higher than those in the control group, while levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in the YQCT group were lower than those in the tumor group (P<0.001). Cell experiment results showed that levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in the RAW264.7-LPS group were higher than those in the control group, while levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ in the YQCT group were lower than those in the RAW264.7-LPS group (P<0.001), and activated RAW264.7 cells could cause increased autophagolysosomes in C2C12 cells. Conclusion Yiqi Chutan therapy reduces tumor-induced inflammatory cell infiltration in skeletal muscle, thereby alleviating inflammatory injury of skeletal muscle and protecting the motor function of tumor-bearing mice.

## Full Text

# Yiqi Chutan Method Alleviates Tumor-Related Skeletal Muscle Injury by Reducing Inflammatory Infiltration

**WU Yingchao**[1,2,3,4,5], **LUO Ziqian**[2,4], **YI Zhongjia**[6], **PI Dajin**[6], **CUI Jiaqi**[6], **LIN Lizhu**[1], **OUYANG Mingzi**[6*], **CHEN Qianjun**[1,2,3,4,5*]

[1]Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China
[2]The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China
[3]State Key Laboratory of Traditional Chinese Medicine Syndrome, Guangzhou 510120, China
[4]Department of Oncology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China
[5]Guangdong Academy of Traditional Chinese Medicine, Guangzhou 510120, China
[6]College of Traditional Chinese Medicine, Jinan University, Guangzhou 510632,

China

*Corresponding authors: OUYANG Mingzi, Associate Professor/Associate Chief Physician of Traditional Chinese Medicine; E-mail: mingzioy@jnu.edu.cn CHEN Qianjun, Chief Physician of Traditional Chinese Medicine/Doctoral Supervisor; E-mail: cqj55@163.com

**Abstract**

**Background:** Cancer patients frequently experience sarcopenia and other muscle wasting symptoms, yet the underlying pathological mechanisms remain incompletely understood. **Objective:** This study investigated the pathological mechanisms of tumor-related skeletal muscle injury and the therapeutic effects of the Yiqi Chutan method on this condition. **Methods:** Between October 2020 and May 2021, thirty 8-week-old female C57BL/6 mice were utilized. Twenty mice were randomly selected to establish a tumor-bearing model via subcutaneous injection of LLC cells. Upon successful modeling, animals were divided into a tumor group (n=10) and a Yiqi Chutan group (YQCT group, n=10), with an additional control group (n=10). The YQCT group received Yifei Sanjie Pills via gavage ($3\ g \cdot kg^{-1} \cdot d^{-1}$) for 21 days, while the control and tumor groups received equal volumes of normal saline. Twenty-four hours after the final administration, behavioral assessments were conducted using the open field test and elevated plus maze test. Gastrocnemius muscle tissues were harvested for histopathological analysis to evaluate pathological changes. Transcriptomic analysis was performed to identify differentially expressed genes, and enzyme-linked immunosorbent assay (ELISA) was used to measure inflammatory cytokine levels. Additionally, RAW264.7 cells in logarithmic growth phase were randomly divided into a mouse serum control group (RAW264.7 group), mouse tumor serum group (RAW264.7-LPS group), and mouse Yiqi Chutan serum group (YQCT group). The RAW264.7 group was cultured in medium containing 10% serum from control mice, the RAW264.7-LPS group in medium containing 10% serum from tumor-bearing mice plus 100 ng/mL LPS, and the YQCT group in medium containing 10% serum from YQCT-treated mice plus 100 g/L LPS for 24 hours. ELISA was then used to detect inflammatory cytokine levels in RAW264.7 cells. After co-culturing these treated RAW264.7 cells with mouse C2C12 cells for 48 hours, lysosomal red fluorescent probe kits were employed to assess autophagolysosome levels in C2C12 cells.

**Results:** Significant differences were observed among the three groups in total movement distance in both the open field test and elevated plus maze test ($P<0.001$). The tumor group exhibited lower activity compared to the control group, while the YQCT group showed higher activity than the tumor group ($P<0.001$). Histopathological results revealed significant muscle cell damage in the tumor group compared to controls, with reduced damage observed in the YQCT group. Transcriptomic analysis demonstrated that gene expression

patterns were similar between the control and YQCT groups but opposite in the tumor group. Immunofluorescence staining showed increased infiltration of M1-type macrophages, neutrophils, T lymphocytes, and B lymphocytes in the gastrocnemius muscle of tumor group mice compared to controls. In contrast, the YQCT group exhibited increased M2-type macrophage infiltration and decreased infiltration of other pro-inflammatory cells compared to the tumor group. ELISA results indicated that IL-1$\beta$, IL-6, and TNF-$\alpha$ levels were higher in the tumor group than in controls, while these levels were lower in the YQCT group than in the tumor group ($P<0.001$). Cell experiments showed that the RAW264.7-LPS group had higher IL-1$\beta$, IL-6, and TNF-$\alpha$ levels than the control group, while the YQCT group had lower levels than the RAW264.7-LPS group ($P<0.001$). Moreover, activated RAW264.7 cells caused increased autophagolysosomes in C2C12 cells.

**Conclusion:** The Yiqi Chutan method reduces tumor-induced inflammatory cell infiltration in skeletal muscle, thereby alleviating inflammatory injury, preserving muscle function, and ultimately protecting the motor function of tumor-bearing mice.

**Keywords:** Yiqi Chutan method; transcriptomics; tumor; inflammation; skeletal muscle

---

## Introduction

The 2022 global cancer statistics report from the World Health Organization' s International Agency for Research on Cancer documented 19.96 million new malignant tumor cases and 9.737 million deaths worldwide. Epidemiological analysis revealed that the five most common cancers were lung, female breast, colorectal, prostate, and stomach, while the leading causes of cancer mortality were lung, colorectal, liver, female breast, and stomach cancers. The report projected that global cancer incidence will exceed 35 million cases annually by 2050 based on current demographic trends. Historically, due to the severe health threats posed by malignant tumors, their poor prognosis, and limited research resources, oncology research has focused predominantly on the tumors themselves, leading to the development of targeted interventions against tumor cell proliferation pathways. However, as economic development and increased life expectancy have raised cancer incidence in China, and as more effective treatments enable patients to live with tumors, the population of cancer survivors has grown substantially. Consequently, tumor-related adverse effects have gained increasing attention during both disease progression and clinical intervention.

Reports indicate that 30% of cancer patients experience tumor-related skeletal muscle injury characterized by fatigue, weight loss, and muscle mass reduction, with muscle mass loss affecting 23% of men and 10% of women. This phenomenon is particularly common in lung cancer patients. Previous research has inadequately addressed tumor-related adverse effects, causing studies on

tumor-induced skeletal muscle injury to lag significantly behind tumor-focused research and limiting effective clinical solutions. There is an urgent need to shift or expand research focus toward comprehensive approaches that address the entire spectrum of tumor occurrence, development, and treatment, promptly tackling emerging clinical problems from basic research to clinical application. Therefore, investigating the pathological mechanisms of tumor-related skeletal muscle injury and developing effective therapeutic interventions hold significant clinical importance.

The Yiqi Chutan method is a classical therapeutic approach for non-small cell lung cancer, formulated by national TCM master Zhou Daihan based on decades of clinical experience. The classical prescription consists of eight herbs: Maozhaocao (Ranunculi Ternati Radix), Chao Jiangcan (Bombyx Batryticatus), Zhongjiefeng (Sarcandrae Herba), Fabanxia (Pinelliae Rhizoma Praeparatum), Shancigu (Cremastrae Pseudobulbus Pleiones Pseudobulbus), Zhebeimu (Fritillariae Thunbergii Bulbus), Lingzhi (Ganoderma), and Xiyangshen (Panacis Quinquefolii Radix), which has been prepared as a hospital preparation for long-term clinical use. Clinical studies have confirmed that the Yiqi Chutan method achieves favorable efficacy in lung cancer treatment, effectively prolonging progression-free survival, median survival, and overall survival. Additionally, clinical research has demonstrated that this method improves quality of life and alleviates tumor-related adverse effects such as fatigue and weight loss during survival with tumor, though its mechanisms remain incompletely elucidated. Preliminary basic research has confirmed that the Yiqi Chutan method can effectively prevent muscle injury during tumor progression and chemotherapy, but its precise biological mechanisms require further investigation. Therefore, systematically studying the pathways through which the Yiqi Chutan method intervenes in tumor-related skeletal muscle injury holds important theoretical value for expanding the application of Chinese medicine in supportive cancer care.

---

## Materials and Methods

**Cells and Experimental Animals** Mouse LLC lung cancer cells, mouse RAW264.7 mononuclear macrophages, and mouse C2C12 myoblasts were maintained in the Ouyang Mingzi research group at the College of Traditional Chinese Medicine, Jinan University. For animal experiments, thirty 8-week-old SPF-grade female C57BL/6 mice (body weight $18\$\pm 1g)wereprovidedbyBeijingHuafukangBiologicalTechnologyCo.,Ltd.[LicenseNo.:SCXK(Beijing)2019-0008].Animalswerehousedincontrolledanimalrooms(5micepercage)understandardco$ $temperature23\pm 2°C, humidity60\pm 10\pm\$2$ Pa, with free access to food and water, and a 12-hour light/dark cycle (lights on 6:00-18:00). This study was approved by the Institutional Animal Care and Use Committee of Jinan University (Approval No.: IACUC-20200923-06).

**Experimental Equipment** Cell culture incubator [Thermo Fisher Scientific (USA), Model 371]; mouse behavioral analysis platform with EthoVision XT 14 software [Noldus Information Technology (Beijing) Co., Ltd.]; fluorescence microscope [Olympus (Japan), Model IX71]; transmission electron microscope [Hitachi (Japan), Model HT7800/HT7700]; ultrasonic homogenizer (Ningbo Xinzhi Biotechnology Co., Ltd., Model JY92-IIN); low-speed centrifuge [Eppendorf (Germany), Model 5702R].

**Experimental Reagents** DMEM high-glucose medium [Gibco (USA), Cat# 11965092]; fetal bovine serum [Gibco (USA), Cat# 10270106]; penicillin/streptomycin [Gibco (USA), Cat# 10378016]; Yifei Sanjie Pills (First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangdong Pharmaceutical Preparation No. Z20190015000); Hematoxylin-Eosin staining kit (Shanghai Acmec Biochemical Co., Ltd., Cat# AG1120); One-step TUNEL apoptosis detection kit (Shanghai Beyotime Biotechnology, Cat# C1086); rabbit anti-inducible nitric oxide synthase (iNOS) monoclonal antibody [Cell Signaling Technology (USA), Cat# 13120T]; rabbit anti-mannose receptor (CD206) monoclonal antibody [Cell Signaling Technology (USA), Cat# 24595T]; rabbit anti-integrin $\alpha$M chain (CD11b) monoclonal antibody [Cell Signaling Technology (USA), Cat# 17800T]; rabbit anti-T lymphocyte surface antigen (CD3) monoclonal antibody [Cell Signaling Technology (USA), Cat# 78588T]; anti-rabbit leukocyte differentiation antigen 20 (CD20) monoclonal antibody [Cell Signaling Technology (USA), Cat# 70168T]; goat anti-rabbit IgG antibody [Cell Signaling Technology (USA), Cat# 4412S]; mouse IL-1$\beta$ ELISA kit (Hangzhou Lianke Biotechnology Co., Ltd., Cat# EK201B-48); mouse IL-6 ELISA kit (Hangzhou Lianke Biotechnology Co., Ltd., Cat# EK206/3-48); mouse TNF-$\alpha$ ELISA kit (Hangzhou Lianke Biotechnology Co., Ltd., Cat# EK282HS-48); lipopolysaccharide (LPS, Shanghai Acmec Biochemical Co., Ltd., Cat# AC12037); lysosomal red fluorescent probe kit (Shanghai Beyotime Biotechnology, Cat# C1046).

**Cell Culture** Mouse LLC lung cancer cells, RAW264.7 mononuclear macrophages, and C2C12 myoblasts were cultured in DMEM high-glucose medium supplemented with 10% fetal bovine serum and antibiotics (penicillin 100 U/mL, streptomycin 100 g/mL). Cells were maintained at 37°C with 5% $CO_2$ in a humidified incubator, with fresh medium replacement every 48 hours. Cells were passaged upon reaching 90% confluence, and all subsequent experiments utilized cells in logarithmic growth phase.

**Tumor Mouse Model Construction and Grouping** Following our established tumor-bearing animal model protocol, thirty female C57BL/6 mice were acclimated for one week. Ten mice were randomly assigned to the control group, while the remaining twenty received subcutaneous injection of 1$\times$10^{5}$ LLC cells in the right axillary region to establish tumor-bearing models. Seven days post-injection, the twenty tumor-bearing mice were randomly divided into tu-

mor and Yiqi Chutan (YQCT) groups (n=10 each). The YQCT group received Yifei Sanjie Pills via gavage (3 $g \cdot kg^{-1} \cdot d^{-1}$) for 21 days, while control and tumor groups received equal volumes of normal saline. Behavioral assessments and sample collection were performed 24 hours after the final administration.

**Cell Model Establishment, Grouping, and Intervention** Logarithmic-phase RAW264.7 mononuclear macrophages were randomly divided into three groups: mouse serum control group (RAW264.7 group), mouse tumor serum group (RAW264.7-LPS group), and mouse Yiqi Chutan serum group (YQCT group). The RAW264.7 group was treated with medium containing 10% serum from control mice, the RAW264.7-LPS group with medium containing 10% serum from tumor-bearing mice plus 100 ng/mL LPS, and the YQCT group with medium containing 10% serum from YQCT-treated mice plus 100 g/L LPS for 24 hours. ELISA was then used to measure inflammatory cytokine levels in RAW264.7 cells. Subsequently, the treated RAW264.7 cells were co-cultured with mouse C2C12 cells for 48 hours, after which lysosomal red fluorescent probe kits were used to detect autophagolysosome levels in C2C12 cells.

**Behavioral Testing Open Field Test:** Following established protocols, mice were placed in a 40 cm × 40 cm × 30 cm open field apparatus and recorded for 5 minutes under low-light conditions. Total movement distance was analyzed using Noldus EthoVision XT 14 software.

**Elevated Plus Maze Test:** Using a standard elevated plus maze paradigm with a central platform (5 cm × 5 cm) connecting two open arms (30 cm × 5 cm × 0.5 cm) and two closed arms (30 cm × 5 cm × 15 cm), mouse behavior was recorded for 5 minutes. Total movement distance was quantified using EthoVision XT 14 software.

**Histopathological Analysis** At the experimental endpoint, mice were euthanized via intraperitoneal injection of sodium pentobarbital (50 mg/kg), followed by whole blood collection and gastrocnemius muscle harvesting. All biological samples were preserved according to standard protocols.

**Hematoxylin-Eosin (HE) Staining:** Following our previous methodology, fresh gastrocnemius tissues were fixed in 4% paraformaldehyde for 24 hours, then processed through tissue trimming, graded dehydration, and paraffin embedding. Continuous 4 m sections were prepared, deparaffinized in xylene, rehydrated through graded alcohols, stained with hematoxylin (5 seconds), differentiated in hydrochloric acid alcohol (25 seconds), and counterstained with eosin (5 seconds). After dehydration and clearing, sections were mounted with neutral resin and examined under light microscopy at consistent anatomical levels.

**Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL):** Paraffin sections were prepared as described above. Following our established protocol, sections were incubated with permeabilization

solution at 37°C for 8 minutes, washed three times with PBS (3 minutes each), treated with proteinase K at 37°C for 20 minutes, washed again three times with PBS, then incubated with TDT and dUTP mixture (1:9) at 37°C for 1 hour. After PBS washes, sections were mounted with fluorescent mounting medium containing DAPI and examined under fluorescence microscopy at consistent tissue levels.

**Transmission Electron Microscopy (TEM):** According to established protocols, fresh gastrocnemius tissues were primarily fixed in 2.5% glutaraldehyde for 4 hours, rinsed three times with 0.01 M PBS (15 minutes each), post-fixed in 1% osmium tetroxide for 2 hours, then thoroughly washed with PBS. Samples underwent graded acetone dehydration, Epon812 resin infiltration, and programmed polymerization (37°C for 6 hours → 45°C for 12 hours → 63°C for 24 hours). Ultrathin 70 nm sections were prepared, double-stained with uranyl acetate and lead citrate, and examined via transmission electron microscopy to assess ultrastructural features of muscle fibers.

**Immunofluorescence (IF):** Paraffin sections were prepared as described above. Following our previous methodology, after antigen retrieval, sections were blocked with peroxidase (20 minutes at room temperature) and serum (10% goat serum, 20 minutes at room temperature), then incubated with primary antibodies against iNOS, CD206, CD11b, CD3, and CD20 overnight at 4°C. After washing, sections were incubated with fluorescent secondary antibodies (2 hours at room temperature, protected from light) and mounted with DAPI-containing fluorescent mounting medium. Fluorescence microscopy was performed at consistent tissue levels to observe structural changes.

**Transcriptomic Analysis of Mouse Gastrocnemius Muscle** Fresh gastrocnemius muscle samples were processed and total RNA was extracted using the RNAprep pure Tissue Kit (Cat# DP431, Tiangen Biotech). RNA integrity and quantity were assessed using the Agilent 2100 bioanalyzer system. Qualified total RNA was used for library construction, and sequencing was performed on approved libraries to obtain sequence information. Reference genome sequences and annotations were obtained from genomic databases, and reference genome indices were constructed using HISAT2 v2.0.5 for alignment with paired-end clean reads. Feature Counts (1.5.0-p3) was used to calculate read counts mapped to each gene. DESeq2 (1.20.0) was employed for differential expression analysis between two groups to identify differentially expressed transcripts (DETs), with $P<0.05$ set as the significance threshold. Transcriptomic data were analyzed for differential expression and enrichment using the OmicShare platform.

**ELISA Detection of Inflammatory Cytokines in Tissues and Cells** Mouse IL-1$\beta$, IL-6, and TNF-$\alpha$ ELISA kits were used according to manufacturer instructions to detect inflammatory cytokine levels in gastrocnemius muscle tissues and RAW264.7 cells.

**Detection of Autophagolysosome Levels in Cells** Lysosomal red fluorescent probe kits were used according to manufacturer instructions to detect autophagolysosome levels in C2C12 cells.

**Statistical Analysis** SPSS 13.0 software was used for statistical analysis. Normally distributed continuous data are presented as mean ± standard deviation ($\bar{x} \pm s$). Comparisons among multiple groups were performed using one-way ANOVA, with pairwise comparisons conducted using LSD-t tests. Data visualization was performed using GraphPad Prism 9 software. $P<0.05$ was considered statistically significant.

---

## Results

**2.1 Behavioral Comparisons Among the Three Groups** Significant differences were observed among the three groups in total movement distance in both the open field test and elevated plus maze test ($P<0.001$). The tumor group exhibited significantly lower activity compared to the control group, while the YQCT group showed significantly higher activity compared to the tumor group ($P<0.001$) [FIGURE:1, TABLE:1].

**TABLE 1** Comparison of Total Movement Distances Among Three Groups of Mice ($\bar{x} \pm s$, mm)

| Group | n | Open Field Test | Elevated Plus Maze Test |
|---|---|---|---|
| Control | 10 | 21,919.10 ± 2,325.29 | 8,914.00 ± 968.61 |
| Tumor | 10 | 3,018.06 ± 1,189.86 | 1,135.94 ± 222.16 |
| YQCT | 10 | 20,172.66 ± 3,261.84 | 8,165.38 ± 872.03 |

Note: YQCT = Yiqi Chutan method; $P<0.05$ vs. control group; $P<0.05$ vs. tumor group.

**2.2 Comparison of Gastrocnemius Muscle Structure Among Three Groups** HE staining revealed that the control group maintained intact muscle structure with compact, orderly arranged muscle fibers. In contrast, the tumor group showed loose muscle structure with disorganized fiber arrangement. The YQCT group demonstrated significantly reduced muscle damage compared to the tumor group, with intact and orderly fiber structure and notably fewer inter-fiber spaces [Figure 2: see original paper]A. TUNEL staining showed significant muscle cell damage in the tumor group compared to controls, with reduced damage in the YQCT group [Figure 2: see original paper]B. TEM analysis revealed marked morphological changes in the tumor group, including disorganized fiber arrangement, widened inter-fiber spaces, and increased

mitochondrial autophagosomes. The YQCT group showed significant improvement compared to the tumor group, with substantially reduced mitochondrial autophagosomes [Figure 2: see original paper]C. These findings suggest that the Yiqi Chutan method can ameliorate skeletal muscle injury in tumor-bearing mice, potentially enhancing muscle strength, energy reserves, and metabolic capacity.

**2.3 Comparison of Differentially Expressed Genes in Gastrocnemius Muscle Transcriptomes** Transcriptomic analysis of gastrocnemius muscle tissues demonstrated intra-group homogeneity and inter-group heterogeneity among control, tumor, and YQCT groups [Figure 3: see original paper]A, C. Compared to controls, the tumor group showed 642 upregulated and 480 downregulated genes [Figure 3: see original paper]B. Compared to the tumor group, the YQCT group exhibited 1,054 upregulated and 768 downregulated genes [Figure 3: see original paper]D. Notably, 217 differentially expressed genes in the tumor group were reversed by Yiqi Chutan intervention [Figure 3: see original paper]E. Hierarchical clustering of these 217 genes revealed similar expression trends between control and YQCT groups, with opposite patterns in the tumor group [Figure 3: see original paper]F.

**2.4 Enrichment Analysis of Differentially Expressed Genes** GO enrichment analysis of the 217 differentially expressed genes revealed enrichment in biological processes and molecular functions including regulation of leukocyte proliferation, immune system processes, lymphocyte proliferation regulation, mononuclear cell proliferation regulation, positive regulation of cytokine production, mitochondrial inner membrane protein complexes, and cytokine binding [Figure 4: see original paper]A. KEGG pathway analysis showed enrichment in oxidative phosphorylation, complement and coagulation cascades, and notably, the immune system [Figure 4: see original paper]B. Reactome pathway analysis demonstrated enrichment in innate immune system, neutrophil degranulation, respiratory electron transport, and exocytosis of secretory granule membrane proteins [Figure 4: see original paper]C.

**2.5 Immunofluorescence Staining Analysis of Gastrocnemius Muscle** Immunofluorescence staining revealed significantly increased infiltration of M1-type (pro-inflammatory) macrophages, neutrophils, T lymphocytes, and B lymphocytes in the tumor group compared to controls. In contrast, the YQCT group showed significantly increased M2-type (anti-inflammatory) macrophage infiltration and decreased infiltration of other pro-inflammatory cells compared to the tumor group [Figure 5: see original paper].

**2.6 Comparison of IL-1$\beta$, IL-6, and TNF-$\alpha$ Levels in Gastrocnemius Muscle** ELISA results demonstrated significant differences in IL-1$\beta$, IL-6, and TNF-$\alpha$ levels among the three groups ($P<0.001$). The tumor group exhibited

higher levels of these cytokines compared to controls, while the YQCT group showed lower levels compared to the tumor group (P<0.001) .

**TABLE 2** Comparison of IL-1$\beta$, IL-6, and TNF-$\alpha$ Levels Among Three Groups of Mice ($\bar{x} \pm s$, ng/L)

| Group | IL-1$\beta$ | IL-6 | TNF-$\alpha$ |
|---|---|---|---|
| Control | 2.17 ± 0.42 | 11.10 ± 1.45 | 17.07 ± 1.22 |
| Tumor | 11.93 ± 2.36 | 29.30 ± 5.31 | 33.23 ± 2.98 |
| YQCT | 4.17 ± 1.30 | 12.40 ± 1.60 | 16.37 ± 3.31 |

Note: IL = interleukin, TNF = tumor necrosis factor; P<0.05 vs. control group; P<0.05 vs. tumor group.

**2.7 Comparison of IL-1$\beta$, IL-6, and TNF-$\alpha$ Levels in Cell Models** LPS-activated RAW264.7 cells were used to establish an inflammatory cell activation model. Significant differences in IL-1$\beta$, IL-6, and TNF-$\alpha$ levels were observed among the three cell groups (P<0.001). The RAW264.7-LPS group showed higher cytokine levels than the control group, while the YQCT group exhibited lower levels than the RAW264.7-LPS group (P<0.05) .

**TABLE 3** Comparison of IL-1$\beta$, IL-6, and TNF-$\alpha$ Levels Among Three Cell Models ($\bar{x} \pm s$, ng/L)

| Group | IL-1$\beta$ | IL-6 | TNF-$\alpha$ |
|---|---|---|---|
| RAW264.7 | 36.87 ± 3.92 | 44.92 ± 8.22 | 83.50 ± 9.75 |
| RAW264.7-LPS | 62.00 ± 3.83 | 1,249.20 ± 107.96 | 328.80 ± 35.60 |
| YQCT | 36.93 ± 3.07 | 132.37 ± 63.47 | 129.57 ± 28.25 |

Note: P<0.05 vs. RAW264.7 group; P<0.05 vs. RAW264.7-LPS group.

**2.8 Comparison of Autophagolysosome Levels in Cell Models** Co-culture of RAW264.7 and C2C12 cells demonstrated that activated RAW264.7 cells induced increased autophagolysosomes in C2C12 cells, indicating that pro-inflammatory cell infiltration directly causes muscle cell damage. After Yiqi Chutan intervention to modulate RAW264.7 cell activation, C2C12 cell damage was significantly attenuated [Figure 6: see original paper].

## Discussion

From the perspective of traditional Chinese medicine theory, lung cancer pathogenesis manifests as deficiency in the root and excess in the branch, with lung-spleen qi deficiency forming the pathological basis that influences the entire disease course, while phlegm-toxin represents the branch excess. Phlegm accumulation promotes tumor formation, and tumor progression exacerbates phlegm production, creating a vicious cycle with dual pathological properties as both causative factor and pathological product. Phlegm obstruction leads to organ dysfunction and qi stagnation, causing secondary pathological changes. Long-standing cancer with phlegm invasion of the muscle surface impairs qi flow and nutrient distribution, damaging muscle tissue. Thus, tumor progression and tumor-related skeletal muscle injury share etiological associations. The eight-herb Yiqi Chutan formula, dominated by the method of cultivating earth to generate metal, restores transportation and transformation function through spleen-strengthening and qi-tonifying, promotes qi-blood generation, and disperses lung phlegm while detoxifying and dispersing masses—precisely addressing the etiology and pathogenesis of tumor-related skeletal muscle injury. Elucidating the mechanism of Yiqi Chutan method in treating tumor-related skeletal muscle injury holds significant importance for guiding clinical medication.

Modern medical research indicates that tumor growth and treatment generate substantial inflammatory factors and mediators that enter systemic circulation, creating a hyper-inflammatory state that may represent a crucial trigger for tumor-related adverse effects. Previous studies have confirmed that tumor-induced chronic inflammation causes symptoms such as fatigue. Furthermore, prolonged high inflammatory infiltration in muscle leads to abnormal energy metabolism and eventual muscle cell damage. Normal energy metabolic function is fundamental for maintaining motor function. This study found that during tumor progression, pro-inflammatory cell infiltration and inflammatory cytokine levels in skeletal muscle were significantly higher than in non-tumor-bearing states, causing inflammatory injury to muscle cells and ultimately impairing motor function in tumor-bearing mice.

Guided by TCM Yiqi Chutan theory, this study confirmed that the herbal formula Yifei Sanjie Pills promotes M2-type macrophage polarization in skeletal muscle of tumor-bearing mice, reduces infiltration of pro-inflammatory cells and levels of pro-inflammatory cytokines, effectively alleviating tumor-related skeletal muscle injury caused by chronic hyper-inflammatory states and preserving normal muscle function. Clinically, severe skeletal muscle injury with intense pain is typically managed with analgesics such as ibuprofen sustained-release capsules or diclofenac sodium sustained-release tablets, which however can reduce muscle growth rate and slow injury recovery. Similar to these reported findings, our study demonstrates that the Yiqi Chutan method alleviates tumor-related skeletal muscle injury by reducing inflammatory infiltration, suggesting potential synergistic and toxicity-reducing effects with conventional treatments. These findings not only preliminarily reveal the pathological mech-

anisms of tumor-related skeletal muscle injury but also propose an effective and safe therapeutic strategy that may address current treatment gaps and provide a potential approach for comprehensive cancer patient management.

Notably, due to intestinal barrier limitations, most components of Yifei Sanjie Pills cannot enter the bloodstream to exert direct effects, and the formula may possess multiple pharmacological mechanisms involving multiple components and targets. Therefore, future studies should employ advanced big data artificial intelligence methods such as artificial neural networks, DeepSeek, and ChatGPT to screen for high-potential lead compounds from Yifei Sanjie Pills components to support subsequent pharmacological validation and drug development. Additionally, while Yifei Sanjie Pills have been primarily used for lung cancer treatment clinically, their application in alleviating tumor-related side effects lacks clinical evidence. Thus, future clinical studies are necessary to further clarify the clinical potential of Yifei Sanjie Pills.

In summary, the Yiqi Chutan method may alleviate tumor burden-induced inflammatory infiltration in skeletal muscle tissue by promoting M2-type macrophage polarization, thereby reducing inflammatory injury to muscle cells, preserving normal muscle function, and ultimately alleviating fatigue and other adverse symptoms caused by tumors. This study confirms the efficacy of the herbal formula Yifei Sanjie Pills in alleviating tumor-related skeletal muscle injury and validates the effectiveness of treatment guided by TCM Yiqi Chutan theory. These findings provide basic research evidence for expanding the clinical application of Yifei Sanjie Pills.

---

## Author Contributions

WU Yingchao: study design and manuscript writing; LUO Ziqian: animal experiments; YI Zhongjia: cell experiments; PI Dajin: data collection and organization; CUI Jiaqi: statistical analysis; LIN Lizhu: TCM theory guidance; OUYANG Mingzi: manuscript revision; CHEN Qianjun: quality control and overall responsibility for the article.

This article has no conflicts of interest.

---

## References

1. Zhang X, Yang L, Liu S, et al. Interpretation of the 2022 global cancer statistics report [J]. Chinese Journal of Oncology, 2024, 46(7): 710-721. DOI:10.3760/cma.j.cn112152-20240416-00152.
2. Passaro A, Al Bakir M, Hamilton EG, et al. Cancer biomarkers: Emerging trends and clinical implications for personalized treatment [J]. Cell, 2024, 187(7): 1617-1635. DOI:10.1016/j.cell.2024.02.041.

3. Kaur R, Bhardwaj A, Gupta S. Cancer treatment therapies: Traditional to modern approaches to combat cancers [J]. Molecular Biology Reports, 2023, 50(11): 9663-9676. DOI:10.1007/s11033-023-08191-0.
4. Lan G, Wu Y, Zhu Y. Trends and 20-year projections of incidence and mortality of digestive tract cancers in China from 1990-2021 [J]. Chinese Journal of Cancer Prevention and Treatment, 2025, 32(02): 84-92. DOI:10.16073/j.cnki.cjcpt.2025.02.04.
5. Ou M, Zhou H, Chen D, et al. Epidemiological characteristics and trends of malignant tumors among elderly people in Guangdong cancer registration areas from 2013-2017 [J]. China Cancer, 2023, 32(05): 348-359.
6. Tang Z, Huang H. Discussion on cancer treatment concepts: "tumor-free state" versus "survival with tumor" [J]. Journal of Guangzhou University of Traditional Chinese Medicine, 2023, 40(12): 3180-3185. DOI:10.13359/j.cnki.gzxbtcm.2023.12.035.
7. Williams GR, Dunne RF, Giri S, et al. Sarcopenia in the older adult with cancer [J]. Journal of Clinical Oncology, 2021, 39(19): 2068-2078. DOI:10.1200/JCO.21.00102.
8. Meza-Valderrama D, Marco E, Dávalos-Yerovi V, et al. Sarcopenia, malnutrition, and cachexia: Adapting definitions and terminology of nutritional disorders in older people with cancer [J]. Nutrients, 2021, 13(3): 761. DOI:10.3390/nu13030761.
9. Bozzetti F. Age-related and cancer-related sarcopenia: Is there a difference? [J]. Current Opinion in Clinical Nutrition and Metabolic Care, 2024, 27(5): 410-418. DOI:10.1097/MCO.0000000000001033.
10. Bossi P, Delrio P, Mascheroni A, et al. The spectrum of malnutrition/cachexia/sarcopenia in oncology according to different cancer types and settings: A narrative review [J]. Nutrients, 2021, 13(6): 1980. DOI:10.3390/nu13061980.
11. Zhou D, Lin L, Tian H, et al. Effect of Yiqi Huatan method on median survival time in elderly non-small cell lung cancer patients: A multicenter, prospective clinical cohort study [J]. World Chinese Medicine, 2014, 9(7): 833-838, 844. DOI:10.3969/j.issn.1673-7202.2014.07.003.
12. Zhou D, Lin L, Zhou Y, et al. Effect of Yiqi Chutan method in prolonging median survival of non-small cell lung cancer [J]. Journal of Traditional Chinese Medicine, 2005, 46(8): 600-602. DOI:10.13288/j.11-2166/r.2005.08.026.
13. Gao T, Chen Z, Lin L, et al. Meta-analysis of the effect of Yiqi Chutan method on survival in advanced non-small cell lung cancer patients [J]. Acta Chinese Medicine, 2018, 33(8): 1383-1387. DOI:10.16368/j.issn.1674-8999.2018.08.328.
14. Huang Y. Evaluation and survival analysis of Yiqi Chutan method in treating elderly advanced non-small cell lung cancer [D]. Guangzhou: Guangzhou University of Chinese Medicine, 2014.
15. Chen W. Effect of Yifei Sanjie Pills on quality of life in advanced non-small cell lung cancer patients [D]. Guangzhou: Guangzhou University of Chinese Medicine, 2023. DOI:10.27044/d.cnki.ggzzu.2023.000882.

16. Guan J, Ouyang M, Xiao Z, et al. Observation on the efficacy of Yiqi Chutan formula in preventing chemotherapy-related fatigue [J]. Liaoning Journal of Traditional Chinese Medicine, 2016, 43(6): 1211-1213. DOI:10.13192/j.issn.1000-1719.2016.06.033.
17. Wu Y, Zuo Q, Luo W, et al. Effect of Yifei Sanjie Pills in alleviating cancer-related muscle atrophy in mice [J]. Journal of Southern Medical University, 2023, 43(11): 1839-1849. DOI:10.12122/j.issn.1673-4254.2023.11.02.
18. Wu YC, Pi DJ, Zhou SY, et al. Yiqi Chutan formula reverses cisplatin-induced apoptosis and ferroptosis of skeletal muscle by alleviating oxidative stress [J]. Integrative Cancer Therapies, 2023, 22: 15347354231172117. DOI:10.1177/15347354231172117.
19. Wu YC, Pi DJ, Chen YL, et al. Yifei Sanjie Pills alleviate chemotherapy-related fatigue by reducing skeletal muscle injury and inhibiting tumor growth in lung cancer mice [J]. Evidence-Based Complementary and Alternative Medicine, 2022, 2022: 2357616. DOI:10.1155/2022/2357616.
20. Wu YC, Zhou SY, Pi DJ, et al. Deciphering the molecular mechanism of Yifei-Sanjie Pill in cancer-related fatigue [J]. Journal of Oncology, 2023, 2023: 5486017. DOI:10.1155/2023/5486017.
21. Ouyang M, Cui J, Wang H, et al. Kai-Xin-San alleviates doxorubicin-induced depression in mice by reducing iron death in prefrontal cortex [J]. Journal of Southern Medical University, 2024, 44(08): 1441-1449.
22. Wu YC, Pi DJ, Chen YL, et al. Ginsenoside Rh4 inhibits colorectal cancer cell proliferation by inducing ferroptosis via autophagy activation [J]. Evidence-Based Complementary and Alternative Medicine, 2022, 2022: 6177553. DOI:10.1155/2022/6177553.
23. Wu YC, Pi DJ, Zhou SY, et al. Ginsenoside Rh3 induces pyroptosis and ferroptosis through the Stat3/p53/NRF2 axis in colorectal cancer cells [J]. Acta Biochimica et Biophysica Sinica, 2023, 55(4): 587-600. DOI:10.3724/abbs.2023068.
24. Wu Y, Wu P, Tian H, et al. Whole transcriptome association analysis of the mechanism of Kai-Xin-San in alleviating chemotherapy-induced cognitive impairment in breast cancer [J]. China Journal of Chinese Materia Medica, 2024, 49(17): 4711-4722. DOI:10.19540/j.cnki.cjcmm.20240430.401.
25. Wu Y, Cui J, Wang H, et al. Kai-Xin-San activates AMPK signaling pathway to improve doxorubicin-induced neurotoxicity [J]. China Journal of Chinese Materia Medica, 2024, 49(24): 6763-6772. DOI:10.19540/j.cnki.cjcmm.20240712.708.
26. Mu H, Chen J, Huang W, et al. OmicShare tools: A zero-code interactive online platform for biological data analysis and visualization [J]. Imeta, 2024, 3(5): e228. DOI:10.1002/imt2.228.
27. Lin L, Sun L, Qi X. 40 years of lung cancer research: Construction of phlegm-toxin theory and application of Yiqi Chutan method [J]. Journal of Guangzhou University of Traditional Chinese Medicine, 2024, 41(10): 2601-2607. DOI:10.13359/j.cnki.gzxbtcm.2024.10.014.
28. Lyu W, Ouyang M, Ma X, et al. Kai-Xin-San attenuates doxorubicin-induced cognitive impairment by reducing inflammation, oxidative

stress, and neural degeneration in 4T1 breast cancer mice [J]. Evidence-Based Complementary and Alternative Medicine, 2021, 2021: 5521739. DOI:10.1155/2021/5521739.
29. Deans C, Wigmore SJ. Systemic inflammation, cachexia and prognosis in patients with cancer [J]. Current Opinion in Clinical Nutrition and Metabolic Care, 2005, 8(3): 265-269. DOI:10.1097/01.mco.0000165004.93707.88.
30. Bower JE. Cancer-related fatigue—mechanisms, risk factors, and treatments [J]. Nature Reviews Clinical Oncology, 2014, 11(10): 597-609. DOI:10.1038/nrclinonc.2014.127.
31. Rooney SI, Baskin R, Torino DJ, et al. Ibuprofen differentially affects supraspinatus muscle and tendon adaptations to exercise in a rat model [J]. American Journal of Sports Medicine, 2016, 44(9): 2237-2245. DOI:10.1177/0363546516646377.

(Received: April 8, 2025; Revised: May 29, 2025)
(Editor: LI Weixia)

*Note: Figure translations are in progress. See original paper for figures.*