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Effect of Yifei Qinghua granule on the tumor microenvironment in lung cancer by regulating the inflammatory pathway

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ABSTRACT

To investigate the mechanism of action of inflammatory molecules regulating the tumor microenvironment and anti-tumor through Yifei Qinghua granules and phloroglucinol-containing serum intervening in the changes of tumor microenvironment in vitro in the co-culture of lung cancer cells and bone marrow cells. A549 lung adenocarcinoma cell line and ST2 bone marrow stromal cell line were selected and a transwell chamber was used to establish the co-culture system of the two kinds of cells. They were divided into normal saline, phloroglucinol, Qifei Qinghua granule, and phloroglucinol + Yifei Qinghua granule groups. They were given drug-containing serum interventions respectively. A549 cells and ST2 cells cultured separately were used as control. Flow cytometry was used to detect the proportions of MDSCs and Tregs in bone marrow cells of ST2 cells. ELISA was used to detect the levels of inflammatory factors in the culture supernatant. Western blot was used to detect the expressions of inflammatory pathways in A549 and ST2 cells. ST2 cells and A549 cells were co-cultured. The ratio of MDSCs and Treg in ST2 cells was increased. The levels of some inflammatory factors in the culture supernatant were increased. The expression level of the inflammatory pathway in ST2 cells was increased. However, the expression level of the inflammatory pathway in A549 cells had no obvious change. While Yifei Qinghua granule and phloroglucinol could partially reverse these changes. The combination of the two was more effective than a single drug. The conversion of cells to MDSCs and Treg was accelerated after the co-culture of ST2 cells and A549 cells. The combination of Yifei Qinghua granules with phloroglucinol can reshape the tumor microenvironment, prevent this phenomenon from occurring, reduce inflammatory secretion and inhibit tumor cell growth. This may be related to the inhibition of the expressions of TNF-α/IL-1- and NF-κB/STAT3 inflammatory pathways.

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Introduction

Lung cancer is still the leading cause of cancer death worldwide. At present, lung cancer has become the cancer with the highest incidence and mortality, far exceeding the sum of breast cancer, colon cancer and prostate cancer, ranking first in the world. In all stages of different subtypes of lung cancer, the patients with more than 10-year survival rates are less than 4%. The current treatment and treatments are not enough to reduce the mortality of this malignant tumor. Therefore, early detection and traditional Chinese medicine + chemotherapy may be the solution to change the accelerated mortality of lung cancer (1).

The tumor microenvironment is the necessary support for tumor occurrence, development and metastasis. Tumor cells interacting with various cells in the microenvironment can lead to rapid tumor growth and assist tumor cells in immune escape (2). There are many differences between the tumor microenvironment and the normal environment in the human body, mainly including hypoxia, low PH, interstitial hypertension, inflammation, advantages of angiogenic factors, immunosuppression, etc. Because of these characteristics, there are a large number of growth factors, cell chemokines and immuno-inflammatory responses caused by various proteolytic enzymes in the tumor microenvironment, which are conducive to tumor proliferation, invasion, adhesion, angiogenesis, reducing the sensitivity of radiotherapy and chemotherapy, and promoting the progress and metastasis of malignant tumors (3). Myeloid suppressor cells, derived from bone marrow precursor cells, are an important part of the tumor microenvironment and play an immunosuppressive role. When the body has inflammation, tumors, etc., it will rapidly increase and inhibit T lymphocytes, hindering immunotherapy (1-3).

Yifei Qinghua granule is the result of the national "Seventh Five Year Plan" research project "Clinical and Experimental Research on the Treatment of Advanced Primary Lung Cancer with Nourishing Qi and Nourishing Yin, Clearing Heat and Detoxifying Agents" and the national "Eighth Five Year Plan" research project "Clinical and Experimental Research on the Treatment of Advanced Lung Cancer with Feiliuping series drugs"(4). Yifei Qinghua granule was originally named Feiliuping and it was renamed Yifei Qinghua granule after being approved for listing. Studies have found that Yifei Qinghua granules

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can significantly reduce the symptoms of cough, phlegm, chest pain, shortness of breath, fatigue and other symptoms of lung cancer patients, inhibit the growth and metastasis of tumors, reduce the toxic and side effects caused by chemotherapy, and improve the quality of life. Animal experiments have confirmed that Yifei Qinghua granules can inhibit the expression of lung tumor cells, reduce the adhesion of tumor cells to the basement membrane and extracellular matrix, inhibit the invasion and metastasis of cancer cells, and reduce the expression of matrix metalloproteinases. Phloroglucinol has a good anti-tumor effect, and as an active component of clinical anticancer drugs, it plays an important role in the treatment of malignant tumors (5). Our previous study found that Yifei Qinghua granule could reduce the levels of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (tumor necrosis factor, TNF- α) and cytokines in the serum of mice. Therefore, through the co-culture system of A549 lung adenocarcinoma cells and ST2 bone marrow stromal cells, we explored the changes in the biological behavior of tumor cells under co-culture conditions, and the effects of Yifei Qinghua granule and phloroglucinol on them, so as to further explore the interaction between inflammatory pathway in the tumor microenvironment and tumor cells to study the mechanism of Yifei Qinghua granule and phloroglucinol in inhibiting inflammation and reducing the recruitment of tumor cells to the body.

Materials and Methods

Cell culture and experimental drugs

A549 lung adenocarcinoma cell line was purchased from Tianjin Wuqing District HALS experimental equipment sales center, and cultured in 1640 medium containing 10% fetal bovine serum, 1% HEPES, 50IU/ml penicillin, and 100U/ml streptomycin, with 5% CO_2 and 37 ℃ constant temperature for culture. The purchasing unit of bone marrow stromal cell line ST2 was the same as above, cultured in DMEM medium containing 10% fetal bovine serum, penicillin 50iu/ml and streptomycin 100u/ml, with 5% CO_2 and 37 ℃ constant temperature for culture. Yifei Qinghua granules were purchased from Guang'anmen Hospital, Chinese Academy of Traditional Chinese Medicine, and phloroglucinol was purchased from Shanxi Pude Pharmaceutical Co., Ltd.

Reagents

FITC-labeled Ly-6G / Ly-6C flow antibody (553127) and PE-labeled CD11b flow antibody (553311) were purchased from BD company in the United States. FITC-labeled CD4 monoclonal antibody (11-0041-82) and PE-labeled CD25 monoclonal antibody (12-0251-82) were purchased from Thermo Fisher, USA. β- actin rabbit monoclonal antibody (ab115777) was purchased from American abcam company, IL-1 β rabbit polyclonal antibody (PAA563Mu01), TNF-α rabbit polyclonal antibody (PAA133Mu01), NF-κ B rabbit polyclonal antibody (PAB824Hu01) and STAT3 rabbit polyclonal antibody (PAB743Mi01) were purchased from Wuhan Yunclone Technology Company. IL-1 β (DLB50), IL-6 (D6050), IL-10 (d1000b), TNF- α (DTA00c), TGF- β 1 (DB100b), IFN- γ The ELISA kits of (DIF50) and IL-13 (D1300b) were purchased from R&D company of the United States.

Instruments

The instruments included an incubator (Blue Pard, China); flow cytometry (American BD company); small electrophoresis system (BIO-RAD, USA); Gel imaging system (BIO-RAD, USA); microplate reader (BioTek, USA).

Preparation of medicated serum

Forty SPF-grade male Wistar healthy rats, aged 8-9 weeks old, with a body mass of (300 ± 20) g, were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., with license number SCXK (Beijing) 2016-0001. They were randomly divided into a phloroglucinol group, a Yifei Qinghua granule group, a phloroglucinol + Yifei Qinghua granule group and a blank group with 4 rates, and the other three groups contained 10 rats in each. All groups were fed adaptively for 24 hours before administration. Blood was taken from the abdominal aorta of rats within 1-2 hours after the last administration, centrifuged at 4 ℃ at low temperature (3000 rpm, 5 minutes), then the upper serum was taken and subpackaged. Then the serum was stored in a -80 ℃ refrigerator and inactivated in a 56 ℃ water bath for 30 minutes before acting on the cell culture system.

Phloroglucinol group

This experiment adopted the method of preparing medicated serum of phloroglucinol, and small doses of continuous administration were used to ensure the plasma concentration of drugs. The dosage was given according to the standard of 25mg/kg, intraperitoneal injection was taken for 1ml / time, once a day, for 3 consecutive days.

Yifei Qinghua granule group

The oral dosage of Yifei Qinghua granule for normal adults was 20g Bid; the equivalent dose of rats = 20000mg/60kg*6.3 = 2100mg/kg bid. The previous experiment found that the double dose group had the strongest effects on inhibiting the proliferation, migration and invasion of A549 cells compared with the equivalent dose group and the four times dose group, so the double dose was used as the dosage, and 200ml normal saline + 84g Yifei Qinghua granule was used to prepare the gastric perfusion solution, and 10ml/kg was used for gastric perfusion.

Yifei Qinghua granule + phloroglucinol group

Yifei Qinghua granule was given by gavage combined with phloroglucinol by intraperitoneal injection. The dosage is the same as described below.

The blank group was given the same amount of normal saline.

Co-culture system and grouping

The co-culture system of A549 cells and ST2 cells were established by using a transwell chamber with a filter membrane aperture of 0.4 μm (purchased from corning company (3412) in the United States). The cells in the upper and lower chambers could interact with each other through cytokines. A549 was seeded in the lower chamber of the transwell, and ST2 cells were seeded in the upper chamber of the transwell. The upper chamber and the lower chamber were intervened with serum in the blank group and medicated serum at the ratio of 3:5 of the two-chamber medium capacity for 24h, respectively, as normal

saline group (S), phloroglucinol group (H), Yifei Qinghua granule (F) and phloroglucinol + Yifei Qinghua granule (H + F) group. A549 cells cultured in the lower chamber alone and supplemented with serum in the blank group (A) and ST2 cells cultured in the upper chamber alone and supplemented with serum in the blank group (T) were used as control.

Flow cytometry was used to detect the proportions of MDSCs and CD4 + CD25 + Treg in ST2 cells of the upper chamber

The culture medium in the transwell upper chamber of cells in all groups except group A and the digested ST2 cells were transferred to the centrifuge tube together, and then the supernatant was discarded by centrifugation at 1000rpm. The centrifuge tube was buckled on the absorbent paper to discard the supernatant as much as possible, and then the cells were suspended in the above medium. After counting with the counting plate, the supernatant was discarded by centrifugation, and the cells were adjusted to 2×10^6/ml, then put on ice for standby.

The prepared ST2 cells were into four test tubes, and the number of cells in each tube was adjusted to 1×10^6 pieces, and 1ml 1 × PBS suspension cells were added, then 9ml 1 × PBS was added, and those were mixed and centrifuged to discard the supernatant, and this operation was repeated twice. The first tube cells were added with 0.25 μl FITC labeled Ly-6G / Ly-6C antibody, the second tube cells were added with 0.25 μl, the third tube was used as the experimental group and added the above two antibodies and the fourth tube was not added with any antibodies. After incubating at 4 ℃ in the dark for 30 minutes, each tube of cells was added with 1ml 1 × PBS suspension cells, then added with 9ml 1 × PBS, after mixing, the cells were centrifuged to discard the supernatant. This step for washing was repeated twice. 500 μl 1 × PBS suspension cells were added in each tube and the proportion of MDSCs was detected by flow cytometry.

The previous steps were the same as above and 4 tubes of ST2 cells were prepared. 0.125ug of FITC-labeled CD4 antibody was added to the first tube cell, 0 06μg of PE-labeled CD25 antibody was added to the second tube cell, the third tube cell was added with the above two antibodies, and the last tube cell was not added with any antibodies. After incubating at 4 ℃ in the dark for 30min, each tube of cells was added with 500 μ L precooled flow cytometry staining buffer to wash the cells, and then the cells were centrifuged to deposit the cells and then the supernatant was discarded. Then another 500 μl flow cytometry staining buffer was added to resuspend the cells, and the proportion of Treg was detected by flow cytometry.

ELISA was used to detect the expression of inflammatory factors in the supernatant of the Transwell lower chamber culture

Because the pore size of the filter membrane between the upper and lower chambers of the transwell was 0.4um, and cytokines could pass through, it could be considered that the levels of cytokines in the upper and lower chambers were the same. Therefore, the culture supernatant was extracted from the lower chamber with more capacity to detect the expressions of cytokines. The operation was in strict accordance with the instructions of the ELISA kit, the reading of the microplate reader was set at 450nm, the standard curve was established, and then the test results were calculated.

Western blot was used to detect the expressions of inflammatory proteins in A549 cells and ST2 cells

ST2 cells in all groups except group A and A549 cells in all groups except group T were collected. The total protein was extracted with RIPA lysate, the protein concentration was determined by the BCA method, and the membrane was transferred by the SDS-PAGE electrophoresis wet transfer method. After blocking, the corresponding primary antibody (dilution ratio of 1:1000) and secondary antibody were used for antigen-antibody reaction, and the ECL luminescence method was used for color development. The expression of protein bands was observed after exposure in the exposure system for 3min.

Statistical analysis

SPSS 18.0 software was used for data analysis. The measurement data were described as mean ± standard deviation ($x \pm s$) and repeated measurement analysis of variance was used for comparison between groups under different conditions. Paired t-test and χ^2 test was used for the measurement data and count data respectively. All statistical tests were performed with bilateral tests, and the difference was statistically significant when $P < 0.05$.

Results

The proportions of MDSCs and $CD4^+CD25^+$Treg in ST2 cells

The proportions of MDSCs and Treg in ST2 cells cultured alone (group T) were the lowest in all groups, but after the co-culture with A549 lung adenocarcinoma cells (Group S), the proportions of MDSCs and Treg were increased significantly, while Yifei Qinghua granule medicated serum (Group F) had no significant effect on the increased proportions of MDSCs and Treg, while the proportions of MDSCs and Treg were decreased after the treatment of phloroglucinol medicated serum (Group H). However, the decrease of MDSCs was not statistically significant compared with group S ($P > 0.05$), but the decrease of Treg was more significant, which was statistically significant compared with group S ($P < 0.05$). The proportions of MDSCs and Treg in group H + F were significantly decreased, which was statistically significant compared with group S($P < 0.05$), as shown in Figures 1

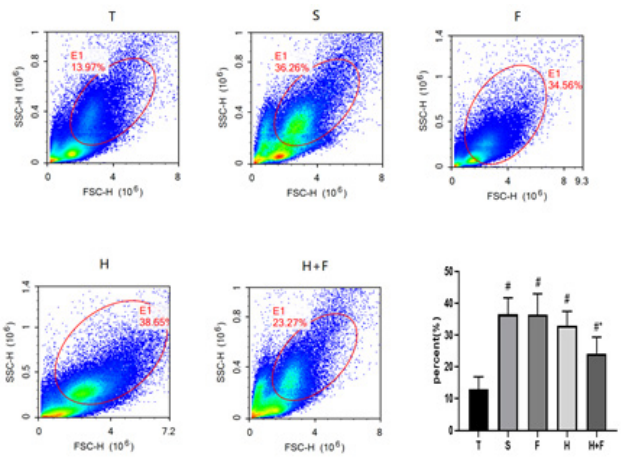

Figure 1. The proportion of MSDCs in ST2 cells. Note: Compared with group T, # indicates p<0.05; compared with s group, * indicates p<0.05.

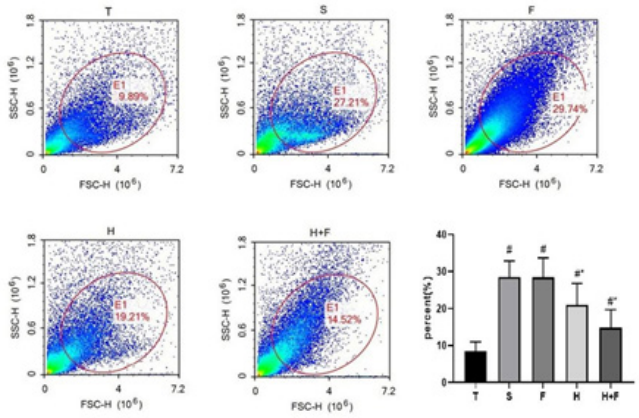

Figure 2. The proportion of Treg in ST2 cells. Note: S: normal saline group, H: phloroglucinol group, F: Yifei Qinghua granule group, H+F: phloroglucinol + Yifei Qinghua granule group, A: A549 cells cultured separately in the lower chamber and added with rat serum in the blank group, T: ST2 cells cultured separately in the upper chamber and added with rat serum in the blank group. Compared with group T, # indicates p<0.05; compared with s group, * indicates p<0.05.

and 2.

The expressions of inflammatory cytokines in cultural supernatant of Transwell lower chamber

The levels of inflammatory factors in group S were generally higher than those in groups A and T and the levels of IFN- α, IL-6, TGF- β 1 and IFN- γ in group H were significantly decreased compared with group S, with a significant difference (P<0.05). The levels of IL-6 and TGF β1 in group F were significantly lower than those in group S, with a significant difference(P < 0.05). The levels of various cytokines in the H + F group were significantly lower than those in group S (P < 0.05), and except for IL-13, the levels of various cytokines in the H + F group were the lowest compared with those in groups H and F (P < 0.05). See Table 1.

The expressions of inflammatory proteins of A549 and ST2 cells

The expressions of TNF-α, STAT3, NF-κB, IL-1β and other iso-inflammatory proteins in Group S were significantly increased compared with group T, with a significant difference (P < 0.05). The expressions of groups F and H were lower than those in group S, with an obvious decrease in TNF-α, STAT3, and NF-κB and the difference was significant compared with group S (P > 0.05). But the expression of IL-1 β was also deceased, there was no significant difference compared with group S (P < 0.05). The expressions of these four proteins mentioned above in the H + F group were significantly lower than those in group S (P < 0.05), and more obvious in TNF-α, STAT3, and IL-1β compared with group F and group H. See Figure 3 (left).

The expressions of TNF- α, STAT3, NF- κB and IL-1 β of A549 cells in group S were not changed significantly compared with group A. After the treatment with Yifei Qinghua granule (Group F) and (or) phloroglucinol (Group H), only the expressions of TNF-α and STAT3 in Group F were significantly higher than those in Group S (P < 0.05), and there was no significant difference in other groups compared with group S. See Figure 3 (right).

Discussion

In recent years, with the deepening of research, tumor research has gradually expanded from the tumor cells themselves to the internal environment of tumor cells. Many studies have shown that the tumor microenvironment plays an important role in the occurrence, development and metastasis of tumors. There are many inflammatory factors and growth factors in the tumor microenvironment. Various body cells around the tumor and at the distal end of the body, such as vascular endothelial cells, pericytes, macrophages, dendritic cells, and bone marrow-derived

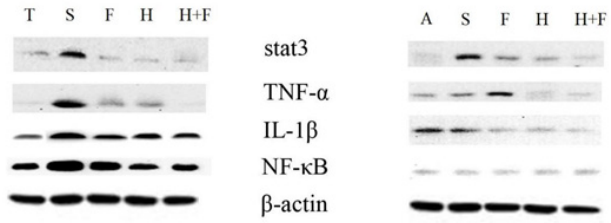

Figure 3. The expressions of inflammatory-related proteins detected by Western blot. Note: S: normal saline group, H: phloroglucinol group, F: Yifei Qinghua granule group, H+F: phloroglucinol + Yifei Qinghua granule group, A: A549 cells cultured separately in the lower chamber and added with rat serum in the blank group, T: ST2 cells cultured separately in the upper chamber and added with rat serum in the blank group. Compared with the T/A group, # indicates p <0.05; compared with group s, * indicates P < 0.05.

Table 1. The expression of inflammatory cytokines in cultural supernatant of Transwell lower chamber in each group.

Indexes	A	T	S	H	F	H+F
IL-1β	6.86±0.23	5.39±0.37	10.38±0.25$^{\#\&}$	10.01±0.41$^{\#\&}$	9.45±0.55$^{\#\&}$	6.86±0.38*
IL-6	15.46±0.54	24.12±1.23$^{\#}$	53.62±3.95$^{\#\&}$	13.78±0.74$^{\&*}$	22.57±2.82$^{\#*}$	16.92±2.36*
IL-10	8.35±0.64	5.33±0.51$^{\#}$	347.64±46.02$^{\#\&}$	328.71±25.87$^{\#\&}$	324.43±41.77$^{\#\&}$	191.24±22.43$^{\#\&*}$
TNF-α	48.57±6.14	36.2±4.89$^{\#}$	458.52±64.37$^{\#\&}$	251.91±28.41$^{\#\&*}$	487.22±39.74$^{\#\&}$	202.57±35.41$^{\#\&*}$
TGF-β1	223.51±35.52	168.43±24.15$^{\#}$	1564.78±143.8$^{\#\&}$	429.63±34.87$^{\#\&*}$	935.82±136.38$^{\#\&*}$	268.11±43.24$^{\&*}$
IFN-γ	18.44±3.11	13.50±3.34	65.29±6.41$^{\#\&}$	32.18±4.15$^{\#\&*}$	34.13±1.66$^{\#\&}$	26.92±4.87$^{\#\&*}$
IL-13	105.78±14.99	69.14±4.13$^{\#}$	894.45±111.24$^{\#\&}$	850.95±49.35$^{\#\&}$	763.56±89.14$^{\#\&}$	658.44±47.22$^{\#*}$

Note: S: normal saline group, H: phloroglucinol group, F: Yifei Qinghua granule group, H+F: phloroglucinol + Yifei Qinghua granule group, A: A549 cells cultured separately in the lower chamber and added with rat serum in the blank group, T: ST2 cells cultured separately in the upper chamber and added with rat serum in the blank group. Compared with group A, # indicates p < 0.05; compared with T group, & indicates P < 0.05; compared with group S, * indicates P < 0.05.

cells, are activated and recruited by various inflammatory factors in the tumor microenvironment and become myeloid suppressor cells, regulatory T cells, tumor-related macrophages, tumor-related fibroblasts, etc(6). After being recruited by tumor cells, these cells will, in turn, secrete more inflammatory factors and growth factors, thus inhibiting immunity, and promoting angiogenesis and epithelial-mesenchymal transition, leading to tumor progression and metastasis. Therefore, the inflammatory factors and tumor-related cells in the tumor microenvironment promote and influence each other, and they are mutually causal and complementary in the formation and maintenance of the inflammatory microenvironment.

Among these cells in the tumor microenvironment, there are many studies related to Treg and MDSCs, and many evidences have shown that they play an important role in inhibiting immunity, stimulating angiogenesis, and promoting tumor invasion and metastasis. Treg can secrete inhibitory cytokines such as IL-10 and TGF- β 1 to form a local microenvironment promoting tumor to inhibit T cell function(7); they can express granzyme A and kill T-cell antigen-presenting cells through the perforation factor pathway(8); through the expression of cd399, ATP and ADP can be transformed into cAMP, and further transformed into adenosine with immunosuppressive effect(9); they can also directly inhibit the anti-tumor activity of NK cells mediated by NKG2D, thereby inhibiting immunity(10). Like Treg, MDSCs can secrete immunosuppressive factors such as IL-10 and TGF-β1 and they can also decompose and consume L arginine and tryptophan in the microenvironment through highly expressing Arg-1, and iNOS, which are necessary for T cell activation (11-12); MDSCs can also generate reactive oxygen species to inhibit the activation of T cells. In addition, MDSCs can also up-regulate the immune checkpoint inhibitors represented by programmed death-1 (PD1) /PD1 ligand (PD-L1) inhibitors to prevent the activation of T cells and block their anti-tumor immunity (13,14). Other studies have shown that MDSCs can induce the proliferation of Treg, and Treg can also promote the proliferation of MDSCs. The interaction between the two promotes tumor immune escape (15). In addition, Treg and MDSCs can also promote tumor progression through non-immune pathways, such as promoting angiogenesis, matrix degradation and tumor invasion (16).

Lung cancer has been recorded in traditional Chinese medicine for a long time, among which "dyspneal hypochondrium mass", "rushing respiration", "lung accumulation", and "pulmonary gangrene" belong to the category of TCM disease names of lung cancer. Leading thoughts to make an argument based on emptiness is one of the dominant ideas in pathogenesis, which is exactly the so-called "the lack of healthy Qi, the presence of evil Qi, and the accumulation of evil Qi". The deficiency of positive Qi and the six evils of external pathogens take advantage of the deficiency, resulting in the imbalance of Yin and Yang of Qi and blood in Zang and Fu organs, occurring pathological changes such as Qi stagnation and blood stasis, accumulation of phlegm and dampness, and accumulation of heat and toxin, and with the time goes by, the cancer pathogen occurs and the block forms. The "positive" not only refers to the delicate substances such as Qi, blood, Yin and Yang, body fluid, but also includes the balance of Yin and Yang, and the normal functions of Qi, and blood, and Zang Fu meridians; it includes not only the disease resistance and self-recovery ability of the human body but also the normal cells, tissues and organs of the human body. The normal tissues and organs of the human body belong to "Yin", and their physiological functions, self-adjustment function and anti-evil ability belong to Yang, which are all components of the positive Qi of the human body. Cancer pathogen arises from a deficiency of vital energy, which is different from general disease pathogens. It is severe and can infect normal cells, tissues and other positive Qi belonging to "Yin" to make them used for itself, so that their physiological functions and anti-evil abilities can not be normally played, but in turn, they help cancer pathogen attack vital energy, make deficiency of vital energy more obvious, and lead to the progress of cancer. As for the influence of cancer pathogens on positive Qi, it is equivalent to the recruitment ability of cancer cells to normal cells in modern medicine. It runs through the "poison" and "blood stasis" of the disease, that is, the inflammatory microenvironment of the tumor (17). Anti-tumor therapy not only needs to kill tumor cells directly, that is, to eliminate pathogenic factors, but also needs to change the inflammatory microenvironment of the tumor, that is, to detoxify and remove blood stasis, and reduce the recruitment of cancer pathogens to normal cells, so as to eliminate pathogenic factors and restore positive.

Yifei Qinghua granule (AFDA approval number Z20050851) is a listed drug of "Feiliuping", an in-hospital preparation of Guang'anmen Hospital, Chinese Academy of Traditional Chinese Medicine. The prescription strictly focuses on the pathogenesis of "deficiency", "poison" and "blood stasis". Astragalus membranaceus, Codonopsis pilosula can invigorate the lung and replenish Qi, Radix Adenophorae and Radix Ophiopogonis are used to nourish Yin and benefit the lung targeting for "deficiency" of lung cancer, Hedyotis diffusa and Patrinia villosa are used to clear heat and dissipate blood stasis, and eliminate carbuncle and detoxification targeting for the "poison" and "blood stasis" of lung cancer, and combined with Bulbus Fritillariae Cirrhosae and Almonds can moisten the lung, relieve cough and resolve phlegm, Hairyvein agrimony is astringent and hemostatic, and the combination of various drugs has the effects of supplementing Qi and nourishing Yin, resolving phlegm and relieving cough, clearing heat, detoxifying and cooling blood, which has been clinically proved to be effective. The mechanism of its action had been extensively and deeply studied in the past, and it had been found that its regulation effect on the ratio of TXB2 and TXB2 / 6-keto-PGF1 α is helpful for the prevention and treatment of metastasis in lung cancer host; it also has a certain inhibitory effect on transplanted tumor and metastasis and its mechanism may be through inhibiting the expressions of CD44V6 and CD49 in tumor cells, reducing the adhesion of tumor cells to the basement membrane and extracellular matrix, and inhibiting the invasion and metastasis of cancer cells; it can significantly improve the function of DC and killing activity of NK cells; it can play an anti-tumor effect by regulating the antigen-presenting function of DC and its mechanism is related to inhibiting angiogenesis, up-regulating the expressions of DC related membrane molecules, inducing maturation, increasing the formation of immune synapses, promoting migration, and improving the killing activity of T cells.

We selected A549 adenocarcinoma cells and ST2

bone marrow stromal cells to observe the changes in the levels of inflammatory factors and the intervention effects of Yifei Qinghua granules and phloroglucinol after their co-culture, and further reveal the internal mechanism of the increased in the proportion of Treg and MDSCs after their co-culture by detecting the inflammatory pathway. The results suggested that after co-culture of A549 cells with ST2 bone marrow cells, the levels of TNF- α, IL-6, TGF- β 1, IFN- γ, IL-13, IL-1 β, IL-10 and other inflammatory factors in cultural supernatant were significantly increased compared with A549 cells and ST2 bone marrow cells cultured alone, while Yifei Qinghua granule and phloroglucinol used alone could reduce the levels of some cytokines such as TNF α, TGF- β, and IFN- γ, but it had little effect on other factors. The combination of Yifei Qinghua granule and phloroglucinol had a strong reversal effect on the increase of the above cytokines and showed the strongest inhibitory effect on most factors except IL-6. TNF- α, IL-6, IL-13, and IL-1 β are the main factors that induce the expansion, recruitment and activation of MDSCs(16,17). Similarly, studies have shown that IFN- γ can promote the expansion and function of monocyte-like and granulocyte-like MDSCs (18). If the signaling pathway of IFN- γ is blocked, it can weaken the inhibitory function of T lymphocytes mediated by MDSCs(19). Therefore, we believe that the changes of these cytokines are one of the reasons for the lowest proportion of MDSCs in the H+F group. MDSCs can secrete TGF- β, IL-10 and other cytokines to induce the production of Treg (20). With the decrease of the proportion of MDSCs, the levels of TGF- β, and IL-10 are also decreased, resulting in a significant decrease in the proportion of Treg in the H + F group (21).

In order to further clarify the source of the above inflammatory factors, we detected the protein expressions of the two key inflammatory pathways, namely TNF- α/ Il-1- and NF- κB/ STAT3 of ST2 cells and A549 cells in the co-culture system by Western blot method. Current studies have shown that tumor-related inflammatory factors play an important role in the recruitment and transformation of Treg and MDSCs, while NF- κ B/STAT3 is the main regulatory pathway of inflammation in vivo (22-23). The results showed that after ST2 cells were co-cultured with A549, the protein expressions of TNF- α, IL-1, NF- κ B and STAT3 were significantly higher than those of ST2 cells cultured alone. After the treatment with Yifei Qinghua granule and phloroglucinol, this increase could be partially corrected, and the correction effect of Yifei Qinghua granule combined with phloroglucinol was the strongest. However, the changes of the above inflammatory proteins of A549 cells in the co-culture system were not obvious. After treatment with Yifei Qinghua granule, the expressions of TNF- α and STAT3 were higher than those of A549 cells and co-cultured A549 cells. These results suggest that the inflammatory factors in the tumor microenvironment are not necessarily mainly secreted by tumor cells, and the related cells recruited and

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References

1. Chandnani N, Choudhari VS, Talukdar R, et al. Depletion of enhancer zeste homolog 2 (EZH2) directs transcription factors associated with T cell differentiation through epigenetic regulation of Yin Yang 1(YY1) in combating non-small cell lung cancer (NSCLC). Med Oncol 2023;40(7):185.
2. Yang W, Zhang F, Deng H, et al. Smart Nanovesicle-Mediated Immunogenic Cell Death through Tumor Microenvironment Modulation for Effective Photodynamic Immunotherapy. ACS Nano 2020;14(1):620-631.
3. Yan ZX, Li L, Wang W, et al. Clinical Efficacy and Tumor Microenvironment Influence in a Dose-Escalation Study of Anti-CD19 Chimeric Antigen Receptor T Cells in Refractory B-Cell Non-Hodgkin's Lymphoma. Clin Cancer Res 2019;25(23):6995-7003.
4. Li FF, Wu H, Zhou B. Effect of yifei qinghua granule on VEGF, bFGF, angiostatin, and endostatin in Lewis lung cancer mice: an experimental study. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2013 ;33(8):1086-92.
5. Kwon YH, Jung SY, Kim JW, et al. Phloroglucinol inhibits the bioactivities of endothelial progenitor cells and suppresses tumor angiogenesis in LLC-tumor-bearing mice. PLoS One 2012;7(4):e33618.
6. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 2012;21(3):309-22.
7. Liuhaiyang, kongyingjun, lishimin, et al. Research progress of regulatory T cells in lung cancer. J Clinic Lung 2020;25 (03):455-458.
8. Sula Karreci E, Eskandari SK, Dotiwala F,et al. Human regulatory T cells undergo self-inflicted damage via granzyme pathways upon activation. JCI Insight 2017;2(21):e91599.
9. Jenabian MA, Seddiki N, Yatim A, et al. Regulatory T cells negatively affect IL-2 production of effector T cells through CD39/adenosine pathway in HIV infection. PLoS Pathog 2013;9(4):e1003319.
10. Kinoshita T, Ishii G, Hiraoka N, et al. Forkhead box P3 regulatory T cells coexisting with cancer associated fibroblasts are correlated with a poor outcome in lung adenocarcinoma. Cancer Sci 2013;104(4):409-15.
11. Groth C, Hu X, Weber R, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. Br J Cancer 2019;120(1):16-25.
12. Prendergast GC, Malachowski WJ, Monday a, et al.indoleamine2, 3 -dioxygenase and its therapeutic intervention in cancer. Int Rev Cell Mol Biol 2018 (336):175-203.
13. Ohl K, Tenbrock K. Reactive Oxygen Species as Regulators of MDSC-Mediated Immune Suppression. Front Immunol 2018;9:2499.
14. Lu C, Redd PS, Lee JR, et al. The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. Oncoimmunol 2016;5(12):e1247135.
15. Noman MZ, Desantis G, Janji B, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med 2014;211(5):781-90.
16. Carole Siret, AUR é lie Collignon, Fran ç oise Silvy, et al.deciding the crosstalk between myloid derived suppressor cells and regulatory T cells in pancreatic ductal adrenarcinoma. Front Immunol 2020;10:3070
17. Fanhuiting, Lin Hongsheng. Research progress of myeloid derived suppressor cells and malignant tumors. Modern Oncol 2020;28 (12):2150-2154.
18. Geng Liang, Hua Baojin.To explore the anticancer mechanism of fuzhengpeiben principle from the perspective of affecting the

recruitment ability of cancer cells. J Traditional Chin Med 2011;26 (6):646-648.
19. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol. 2009;182(8):4499-506.
20. Gallina G, Dolcetti L, Serafini P, et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. J Clin Invest 2006;116(10):2777-90.
21. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 2012;30:531-64.
22. Li L, Yu R, Cai T, et al. Effects of immune cells and cytokines on inflammation and immunosuppression in the tumor microenvironment. Int Immunopharmacol 2020;88:106939.
23. Fukumura D, Xavier R, Sugiura T, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell 1998;94(6):715-25.