

Effect of *Malus asiatica* Nakai Leaf Flavonoids on the Prevention of Esophageal Cancer in C57BL/6J Mice by Regulating the IL-17 Signaling Pathway

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Xiang-Bo Jia*
Quan Zhang*
Lei Xu
Wen-Jian Yao
Li Wei

Department of Thoracic Surgery,
Zhengzhou Key Laboratory of Surgical
Treatment for End-Stage Lung Diseases,
Henan Provincial People's Hospital,
People's Hospital of Zhengzhou
University, Zhengzhou 450003, Henan,
People's Republic of China

*These authors contributed equally to
this work

Background: The aim of this study was to observe the preventive effect of flavonoids extracted from *Malus asiatica* Nakai leaves (FMANL) on esophageal cancer in mice, especially the ability of FMANL to regulate the interleukin 17 (IL-17) signaling pathway during this process.

Materials and Methods: The C57BL/6J mice were treated with 4-nitroquinoline *N*-oxide (4NQO) to induce esophageal cancer, and the visceral tissue index and the serum and esophageal tissue indexes of mice were used to verify the effect of FMANL.

Results: The experimental results showed that FMANL can effectively control the changes in visceral tissue caused by esophageal cancer. FMANL could increase the cytokine levels of interleukin 10 (IL-10), monocyte chemoattractant protein 1 (MCP-1) and decrease the cytokine levels of tumor necrosis factor alpha (TNF- α), interferon- γ (IFN- γ), interleukin 6 (IL-6), and interleukin 12p70 (IL-12p70) in serum of mice with esophageal cancer. FMANL could also reduce CD3⁺, CD4⁺, and CD8⁺ and enhance CD19⁺ mouse peripheral blood lymphocytes. The results of qPCR and Western blot analysis showed that FMANL could down-regulate the mRNA and protein expression levels of IL-17, interleukin 23 (IL-23), interleukin 1 beta (IL-1 β), chemokine (C-X-C) ligand 1 (CXCL1), chemokine (C-X-C) ligand 2 (CXCL2), S100 calcium-binding protein A8 (S100A8), S100 calcium-binding protein A9 (S100A9), matrix metalloprotein 9 (MMP-9), and matrix metalloprotein 13 (MMP-1) in mice with esophageal cancer. High-performance liquid chromatography (HPLC) detection showed that FMANL contained 10 chemicals, including rutin, hyperoside, isoquercitrin, dihydroquercetin, quercitrin, hesperidin, myricetin, baicalin, neohesperidin dihydrochalcone, and quercetin.

Conclusion: It could be concluded that FMANL can effectively prevent experimentally induced esophageal cancer in mice, and its effects might be obtained from 10 compounds present in FMANL.

Keywords: *Malus asiatica* Nakai leaves, flavonoid, esophageal cancer, C57BL/6J mice, mRNA IL-17 signaling pathway

Introduction

Malus asiatica Nakai (MAN) is a kind of *Malus mill* plant in the family *Rosaceae*, which contains nitrogen, phosphorus, potassium, and other mineral elements, as well as a variety of vitamins and flavonoids.¹ *Rosaceae* plants have a wide range of pharmacological functions, such as anti-cancer, anti-tumor, liver-protecting, anti-aging, anti-mildew, antibacterial, lipid-lowering and cholesterol-lowering effects.²⁻⁵ In South China, MAN leaves (MANL) are used as drinks similar to tea.⁶ In addition, MANL are also used for food preservation, and the MANL juice can

Correspondence: Li Wei
Email 270065823@qq.com

prevent Ciba (a cake food made of glutinous rice) from deterioration for several months.⁷

Flavonoids are abundant in natural plants, especially in leaves.⁸ As the core component of the biological activity of some plant leaves, plant flavonoids have a strong anti-oxidative effect, and they also exert the functions of enhancing immunity, preventing heart and brain blood diseases, and reducing blood lipids and cholesterol, which play an important role in human fat metabolism.^{9–11}

Esophageal cancer is a common digestive tract tumor, and about 300,000 people die of esophageal cancer every year in the world.¹² The occurrence of esophageal cancer may be related to many factors, such as heredity, environment, and lifestyle, but the exact pathogenesis has not been clarified. Immune cells, fibroblasts, structural molecules, and inflammatory cytokines in the tumor microenvironment can promote the development of esophageal cancer. After activation and expansion, CD4⁺ T cells differentiate into Th1, Th2, and Th17 cell subsets with different cytokines and effector functions.¹³ Interleukin-17 (IL-17), a key cytokine in this family, is mainly produced by Th17 cells.¹⁴ IL-17 plays an important role in a variety of human tumors, and a study has shown that IL-17 also plays a key role in esophageal carcinogenesis.^{15,16} 4-Nitroquinoline *N*-oxide (4NQO) is a carcinogen, which can induce esophageal carcinogenesis in C57BL/6J mice, and it is often used as an experimental esophageal cancer model.¹⁷

MANL are also often used as a tea-like drink or medicine in China, but the related product has not been fully developed and utilized due to lack of in-depth research and development. Some famous tea products in the world have been fully studied, including the extraction of functional components and product development. In this study, FMANL were used as the research object to study the role of FMANL in the prevention of esophageal cancer in vivo. At the same time, molecular biological detection technology was used to explain the mechanism of MANL.

Materials and Methods

Flavonoids from Extraction of *Malus asiatica* Nakai Leaves (FMANL)

MANL (Yichang Fengxiang Biotechnology Co., Ltd., Yichang, Hubei, China) were crushed and screened. Then, they were weighed to 200 g and put into a beaker. Further, 70% ethanol was added according to the liquid to material ratio of 20:1, and it was bathed in water at 60°C

for 3 h. After filtration, the extracted liquid was passed through the FL-3 macroporous resin. The resin was then eluted with 70% ethanol until it was colorless, and the first and second resin passing liquids were combined. The resinous liquid was evaporated to dryness by using a rotary evaporator, and the FMANL extract was obtained.

Determination of FMANL Composition

Dry rutin, hyperoside, isoquercitrin, dihydroquercetin, quercitrin, hesperidin, myricetin, baicalin, neohesperidin dihydrochalcone, and quercetin were weighed accurately in a 5 mL centrifuge tube, and 2 mL methanol solution was added respectively and then mixed, as the standard stock solution. Under the condition of liquid chromatography (Ultimate3000; Thermo Fisher Scientific, Inc., Waltham, MA, USA), the components and contents of flavonoids were determined. The chromatographic column was Thermo Scientific accucore C18 (4.6 mm × 150 mm, 2.6 μm), the gradient elution mobile phase C was acetonitrile, the mobile phase B was 0.5% glacial acetic acid aqueous solution, the flow rate was 0.5 mL/min, the column temperature was 35°C, the detection wavelength was 360 nm, and the injection volume was 10 μL.

Induction of Gastric Injury in Mice

Forty 6-week-old SPF male C57BL/6J mice (20±2g, Chongqing Medical University, Chongqing, China) were fed adaptively for one week. The mice were divided into four groups; 10 mice in each group, and the groups were normal group, model group, low concentration FMANL group (FMANL-L), and high concentration FMANL group (FMANL-H). In the first eight weeks, mice in the normal group and the model group were only allowed to get food and water without any other treatment; mice in the FMANL-L and FMANL-H groups were treated with 50 mg/kg and 100 mg/kg FMANL by gavage. Then in addition to the normal group, drinking water of mice in the other three groups was replaced by 4NQO water with a concentration of 100 mg/L for ten weeks.¹⁸ Then the use of 4NQO water as drinking water was terminated, and all mice were given sterilized water as drinking water for four weeks. In the 14 weeks, mice in the FMANL-L and FMANL-H groups continued to receive FMANL at the corresponding concentration. Finally, all mice were fasted for 24 hours. Mice were killed by breaking their necks. Blood was obtained from their hearts and visceral tissues for use. Organ index was calculated by using the following formula: organ index = organ weight/body weight. This study was approved by the

Ethics Committee of Zhengzhou University (Zhengzhou, Henan, China) according to the guidelines for ethical review of experimental animal welfare (China National Standard GB/T 35,892–2018).

Detection of Serum Cytokines

The blood from mice was added to the blood vessels and kept at 4°C for 1 hour, and then the blood was centrifuged at 4000 rpm/min for 15 min. The upper serum layer was obtained, and the IL-6, IL-10, IL-12p70, MCP-1, TNF- α , and IFN- γ cytokine levels in mice were detected with the kits (Solarbio Life Sciences, Beijing, China).

Detection of Whole Blood Lymphocyte Typing

After blood centrifugation, the lower blood cells were obtained, and the cells were resuspended by 1 mL of 0.01 mol/LPBS. Each mouse was divided into four tubes and labeled. Each tube was successively added with 10 μ L diluted antibodies (IgG-FITC/IgG2a-PE, CD3-FITC/CD19-PE, CD3-FITC/CD4-PE, and CD3-FITC/CD8-PE) (Solarbio Life Sciences), and incubated at room temperature and darkness for 20 min. Each tube was added with 400 μ L of red blood cell lysate to lyse the red blood cells. The cells were vortex mixed and lysed on ice for 15 min at 4°C. Then, the lysate was centrifuged at 1000 rpm/min for 10 min, the supernatant was discarded, the precipitated cells were washed twice with 0.01 mol/L PBS, and the cells were resuspended with 500 μ L/tube of 1% paraformaldehyde. Finally, flow cytometry was used to detect the lymphocyte subsets in the peripheral blood of mice.

Quantitative PCR (qPCR) Detection

An esophageal tissue of moderate size was cut, RNAiso Plus was added to break the tissue in a tissue homogenizer, and RNA was extracted from tissue homogenizer lysate and amplified by reverse transcription cDNA and PCR. The amplification conditions were as follows: pre-denaturation at 95°C for 10 min; denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and extension at 72°C for 10 s, 40 cycles. The primer sequence is shown in Table 1 (Thermo Fisher Scientific, Inc.). Using GAPDH as the internal reference gene, the relative mRNA expression of each target gene was calculated with $2^{-\Delta\Delta CT}$.¹⁹

Table 1 Sequences of Primers Used in the qPCR Assay

Gene Name	Sequence
<i>IL-17</i>	Forward: 5'-TCAGCGTGCCAAACTGAG-3' Reverse: 5'-CGCCAAGGGAGTTAAAGACTT-3'
<i>IL-1β</i>	Forward: 5'-TCGCTCAGGGTCACAAGAAA-3' Reverse: 5'-CATCAGAGGCAAGGAGGAAAAC-3'
<i>IL-23</i>	Forward: 5'-TGTGCCCGTATCCAGTGTG-3' Reverse: 5'-AAAAGCCAGACCTTGCCGGA-3'
<i>CXCL1</i>	Forward: 5'-ACCCAAACCGAAGTCATAGC-3' Reverse: 5'-TGGGGACACCTTTTAGCATC-3'
<i>CXCL2</i>	Forward: 5'-TGAACAAAGGCAAGGCTAAGT-3' Reverse: 5'-GAGGCACATCAGGTACGATCC-3'
<i>S100A8</i>	Forward: 5'-AAATCACCATGCCCTCTACAAG-3' Reverse: 5'-CCCCTTTTATCACCATCGCAA-3'
<i>S100A9</i>	Forward: 5'-ACCACCATCATCGACACCTTC-3' Reverse: 5'-AAAGTTGCCAAGTGTGCTTC-3'
<i>MMP-9</i>	Forward: 5'-CTGGACAGCCAGACACTAAAG-3' Reverse: 5'-CTCGCGGCAAGTCTTCAGAG-3'
<i>MMP-13</i>	Forward: 5'-CTTCTTCTGTTGAGCTGGACTC-3' Reverse: 5'-CTGTGGAGGTCACTGTAGACT-3'
<i>GAPDH</i> (house-keeping gene, control gene)	Forward: 5'-AGGTCGGTGTGAACGGATTG-3' Reverse: 5'-GGGGTCGTTGATGGCAACA-3'

Western Blot Detection

Firstly, 100 mg esophageal tissue samples were homogenized with 1 mL RIPA and 10 μ L PMSF and centrifuged at 4°C for 5 min at 12,000 rpm/min. The intermediate protein layer solution was obtained, and the protein was quantified with the bicinchoninic acid (BCA) protein quantitative kit (Thermo Fisher Scientific, Inc.). Each group of samples was diluted to 50 μ g/mL, the diluted protein and sample buffer were mixed at 4:1 and heated at 100°C for 5 min to mix acrylamide, resolving buffer, starting buffer, tiled water, 10% APS, and TEMED to obtain the SDS-PAGE separation gel and the concentration gel (Thermo Fisher Scientific, Inc.) in proportion, and then they were poured into a rubber plate for use. Samples of prestained protein ladder were obtained, and these samples were injected into the sample hole of the rubber sheet, respectively. Then the SDS-PAGE gel containing protein was subjected to 50 min vertical gel electrophoresis. The PVDF membrane was activated with methanol for 1 min, and then it was closed with TBST solution containing 5% skimmed milk for 1 h. After sealing, the PVDF membrane (Thermo Fisher Scientific, Inc.) was cleaned with TBST and incubated with the first antibody at 25°C for 2 h,

and then the PVDF membrane was cleaned with TBST and incubated with the second antibody at 25°C for 1 h. Finally, the PVDF membrane was covered with SuperSignal West Pico plus and observed under iBright FI1000.²⁰

Statistical Analysis

The results of three parallel experiments were averaged, and then the statistical analysis software SAS 9.1 was used to analyze whether there was a significant difference between the experimental results of each group at the level of $P < 0.05$ by using one-way ANOVA.

Results

Composition Analysis of FMANL

The HPLC assay found that FMANL contained rutin, hyperoside, isoquercitrin, dihydroquercetin, quercitrin, hesperidin, myricetin, baicalin, neohesperidin dihydrochalcone, and quercetin (Figure 1). The respective contents

were 2.18 mg/g, 127.16 mg/g, 84.06 mg/g, 1.34 mg/g, 339.38 mg/g, 14.03 mg/g, 2.69 mg/g, 21.14 mg/g, 1.94 mg/g, and 18.99 mg/g.

Survival Analyses of Mice

In the course of the experiment, the normal mice grew normally, and the mice induced esophageal cancer showed poor appetite and decreased exercise. At the same time, two mice in the model group died before the end of the experiment, and the other mice did not die in advance.

Visceral Index

The body weight and visceral weight of mice were determined (Table 2). Compared with the normal group, the body weight of the model group was decreased. The indexes of kidney, heart, and lung in the model group were significantly ($P < 0.05$) higher than those in the normal group. FMANL

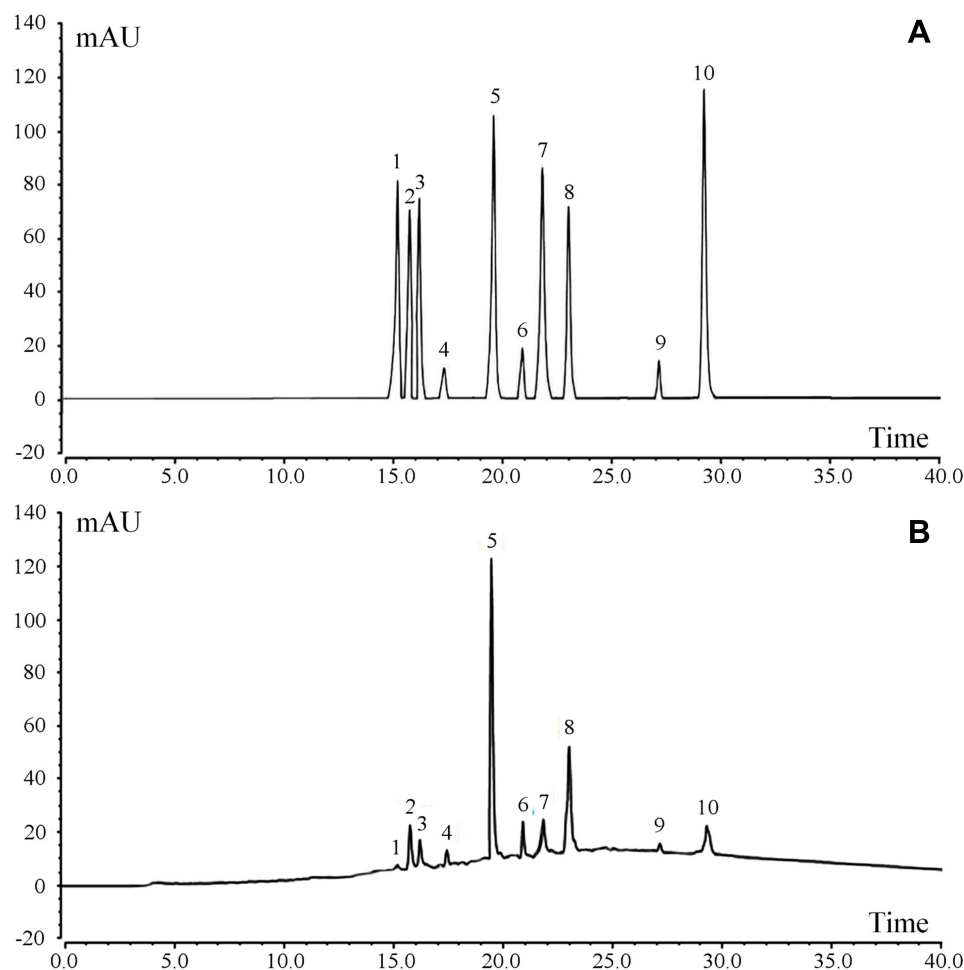


Figure 1 Flavonoids extract constituents of *Malus asiatica* Nakai leaves. **(A)** Standard chromatograms; **(B)** Flavonoids of *Malus asiatica* Nakai leaves chromatograms. 1: rutin; 2: hyperoside; 3: isoquercitrin; 4: dihydroquercetin; 5: quercitrin; 6: hesperidin; 7: myricetin; 8: baicalin; 9: neohesperidin dihydrochalcone; 10: quercetin.

Table 2 Effect of Flavonoids of *Malus asiatica* Nakai Leaves on Organ Index in 4NQO-Induced Esophageal Cancer Mice

Group	Body Weight (g)	Kidney Index	Spleen Index	Cardiac Index	Lung Index	Liver Index
Normal	26.85±0.45 ^a	0.012±0.001 ^d	0.0028±0.0003 ^a	0.0049±0.0002 ^d	0.0058±0.0001 ^d	0.0352±0.0010 ^a
Model	14.97±0.87 ^d	0.020±0.002 ^a	0.0025±0.0003 ^a	0.0086±0.0007 ^a	0.0132±0.0008 ^a	0.0335±0.0016 ^a
FMANL-L	18.09±0.37 ^c	0.017±0.001 ^b	0.0026±0.0001 ^a	0.0070±0.0004 ^b	0.0102±0.0010 ^b	0.0346±0.0006 ^a
FMANL-H	22.13±0.52 ^b	0.014±0.001 ^c	0.0027±0.0005 ^a	0.0057±0.0004 ^c	0.0074±0.0004 ^c	0.0349±0.0005 ^a

Notes: ^{a-d}Mean values with different letters in the same row are significantly different ($p < 0.05$) according to Duncan's multiple-range test. FMANL-L group: 50 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose; FMANL-H group: 100 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose.

could inhibit these changes, and the effects of high concentration FMANL (FMANL-H) were better.

Serum Cytokine Levels in Mice

The cytokine levels of IL-6, IL-12p70, MCP-1, TNF- α , and IFN- γ in the model group were significantly ($P < 0.05$) higher than those in the normal group (Table 3), only the level of IL-10 was lower than that in the normal group. The levels of IL-10, IL-6, IL-12p70, MCP-1, TNF- α , and IFN- γ in mice with FMANL-treated esophageal cancer were higher than those in the untreated model group. The FMANL-H group showed the highest levels of IL-10, IL-6, IL-12p70, MCP-1, TNF- α , and IFN- γ .

Peripheral Blood Lymphocyte Counts in Mice

As shown in Table 4, compared with the normal group, CD3⁺, CD19⁺, CD4⁺, and CD8⁺ cells in the model group were increased significantly ($P < 0.05$); as a result, the ratio of CD4⁺/CD8⁺ was decreased significantly ($P < 0.05$), while the ratio of CD3⁺/CD9⁺ was increased significantly. FMANL could bring the number of CD3⁺, CD4⁺, CD8⁺ and CD19⁺ cells close to that in the normal group, and with the increase in concentration, the ability to improve anticancer effects.

mRNA and Protein Expression Levels in Esophageal Tissue of Mice

The mRNA (Figure 2) and protein (Figure 3) expression levels of IL-17, IL-23, IL-1 β , CXCL1, CXCL2, S100A8,

S100A9, MMP-9, and MMP-13 in the esophageal tissue of the normal group were significantly ($P < 0.05$) lower than those in the esophageal tissue of the model group. The above-mentioned expression levels in the FMANL groups were also significantly ($P < 0.05$) lower than those in the model group, and the above-mentioned expression levels in the FMANL-H group were only higher than those in the normal group.

Discussion

After the development of cancer, the patient's body is greatly affected, the weight is reduced, and the internal organs are also affected.²¹ Animal experiments have also shown that the visceral index changed after carcinogenesis, and the visceral index gradually approached the normal state after carcinogenesis was inhibited.²² Patients suffering from cancer, especially esophageal cancer, due to the reduction of dietary intake, the body absorption function damage and other factors such as weight will be significantly reduced. The animal experiment also showed a similar situation, in which the weight of cancer affected animals decreased significantly, resulting in a decrease in visceral index.¹⁷ In this study, FMANL could prevent esophageal cancer, reduce the pathological changes caused by esophageal cancer in mice, and effectively inhibit the weight loss and visceral tissue changes caused by esophageal cancer in mice.

Modern experiments have shown that natural plants have a wide range of biological effects and these effects

Table 3 Serum Cytokine Levels of Flavonoids of *Malus asiatica* Nakai Leaves in 4NQO-Induced Esophageal Cancer Mice (pg/mL)

Group	IL-10	IL-6	IL-12p70	MCP-1	TNF- α	IFN- γ
Normal	4.54±0.25 ^c	2.55±0.18 ^d	6.50±0.44 ^d	29.95±1.34 ^d	12.02±1.09 ^d	2.08±0.12 ^d
Model	2.28±0.14 ^d	8.35±0.33 ^c	14.11±0.78 ^c	48.38±0.79 ^c	21.41±1.34 ^c	4.43±0.15 ^c
FMANL-L	10.59±0.96 ^b	17.35±0.87 ^b	26.97±1.30 ^b	57.53±1.21 ^b	26.42±1.14 ^b	5.04±0.14 ^b
FMANL-H	22.01±1.15 ^a	25.89±1.01 ^a	35.20±1.42 ^a	65.72±1.63 ^a	36.23±1.7 ^a	5.98±0.24 ^a

Notes: ^{a-d}Mean values with different letters in the same row are significantly different ($p < 0.05$) according to Duncan's multiple-range test. FMANL-L group: 50 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose; FMANL-H group: 100 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose.

Table 4 Peripheral Blood Lymphocyte Counts in 4NQO-Induced Esophageal Cancer Mice Treated with Flavonoids of *Malus asiatica* Nakai Leaves in (pg/mL)

Group	CD3 ⁺	CD19 ⁺	CD3 ⁺ /CD19 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺
Normal	42.81±1.26 ^d	42.92±0.98 ^a	1.00±0.03 ^d	17.22±0.83 ^d	15.39±0.75 ^d	1.12±0.06 ^a
Model	66.63±1.04 ^a	25.23±1.05 ^d	2.64±0.13 ^a	24.22±0.70 ^a	28.78±0.57 ^a	0.84±0.04 ^c
FMANL-L	56.60±1.07 ^b	33.80±0.98 ^c	1.67±0.06 ^b	21.57±0.78 ^b	23.60±0.72 ^b	0.91±0.05 ^{bc}
FMANL-H	47.08±1.21 ^c	38.56±0.50 ^b	1.22±0.02 ^c	18.88±0.67 ^c	19.21±1.23 ^c	0.98±0.07 ^b

Notes: ^{a-d}Mean values with different letters in the same row are significantly different ($p < 0.05$) according to Duncan's multiple-range test. FMANL-L group: 50 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose; FMANL-H group: 100 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose.

are closely related to the cytokine network.²³ IL-10 plays multiple roles in the regulation of tumor immunity, inflammatory response, and autoimmune response. It can stimulate T cells, especially CD8⁺ cells; stimulate B cells to mature, proliferate, and generate antibodies; and increase the ability of killing tumor cells.²⁴ The anti-tumor mechanism of TNF- α is complex; it is mainly through apoptosis and necrosis. TNF- α can be used as an anticancer factor to inhibit cancer.²⁵ IFN- γ can inhibit tumor angiogenesis, growth, and apoptosis.²⁶ MCP-1 has a significant inhibitory effect on tumor growth, which is related to its concentration. MCP-1 has chemotactic activity on monocytes, can activate monocytes and macrophages, and inhibit tumor cell growth.²⁷ Appropriate amount of IL-6 can participate in the immune regulation of an inflammatory response and the activation of immune cells; but after the disorder, it will affect the normal immune regulation of the body, and may cause tumorigenesis.²⁸ IL-12p70 can increase the immune response of T cells and NK cells, induce IFN- γ secretion and regulate the balance of the Th1/Th2 type immune response, and improve the immune ability of the body to the tumor.²⁹ In this study, the levels of cytokines IL-6, IL-12p70, MCP-1, TNF- α , and IFN- γ in the model group were higher than those in the normal group, which might be related to a certain degree of stress immunoregulation in mice. The body participates in the immunoregulation of an inflammatory response to fight against tumor cells. Levels of cytokines IL-10, IL-6, IL-12p70, MCP-1, TNF- α , and IFN- γ in the FMANL groups were significantly higher than those in the normal group and the model group. Combined with the results of body weight, organ weight and index in the FMANL groups, it was found that FMANL could play a role in the prevention and treatment of esophageal cancer by improving the levels of these cytokines and the immune function of the body.

The decrease in CD3⁺ T cells will affect the induction and activation of the immune response and the inhibition

of cellular immunity, which is the main factor of disease and tumor.³⁰ CD19⁺ is one of the most important membrane antigens involved in B cell activation and proliferation. CD19⁺ cells increase in B-lymphocyte system malignant tumors.³¹ CD4⁺ T cells can promote the anti-tumor effect of effector cells. If the proportion of CD4⁺ T cells is reduced, the induction of immune response will be reduced, the body will be susceptible to infection and the specific anti-tumor effect will be reduced.³² CD8⁺ T cells cause the target cells of cancer cells to dissolve and die, which can inhibit the effect of other lymphocytes and prevent an excessive immune response.³³ The low ratio of CD3⁺/CD19⁺ will lead to a decrease in immunity and recovery ability. The imbalance of CD4⁺/CD8⁺ ratio may cause immune dysfunction and tumor.³⁴ This study showed that FMANL could improve the imbalance of immune function and immunosuppression, and thus regulate and prevent esophageal cancer.

It has been found that IL-17 promoted the progression of non-small cell lung cancer (NSCLC) through the STAT3/NF- κ B/Notch 1 signaling pathway.³⁵ Another study found that some tumors grew faster in IL-17 knockout mice.³⁶ A clinical report showed that IL-17 was highly expressed in primary liver cancer, and it might be involved in the carcinogenesis of liver cancer.³⁷ IL-23 and IL-1 β can promote the differentiation of Th17 cells and the production of IL-17. IL-17 can promote the development of inflammation and cancer by inducing the production of CXCL1, CXCL2, S100A8, S100A9, MMP-9, and other factors.¹⁷ Activated dendritic cells in the tumor microenvironment produce IL-23 and IL-1 β , which stimulate the initial CD4⁺ T cells to differentiate into Th17 cells and increase IL-17 production.³⁸ Th17 may be involved in the invasion and metastasis of esophageal squamous cell carcinoma, and it may be related to poor prognosis.³⁹ S100 calcium-binding protein family plays a role between tumor cells and stromal cells, promoting the formation of the inflammatory tumor microenvironment, thus promoting the growth and metastasis of primary

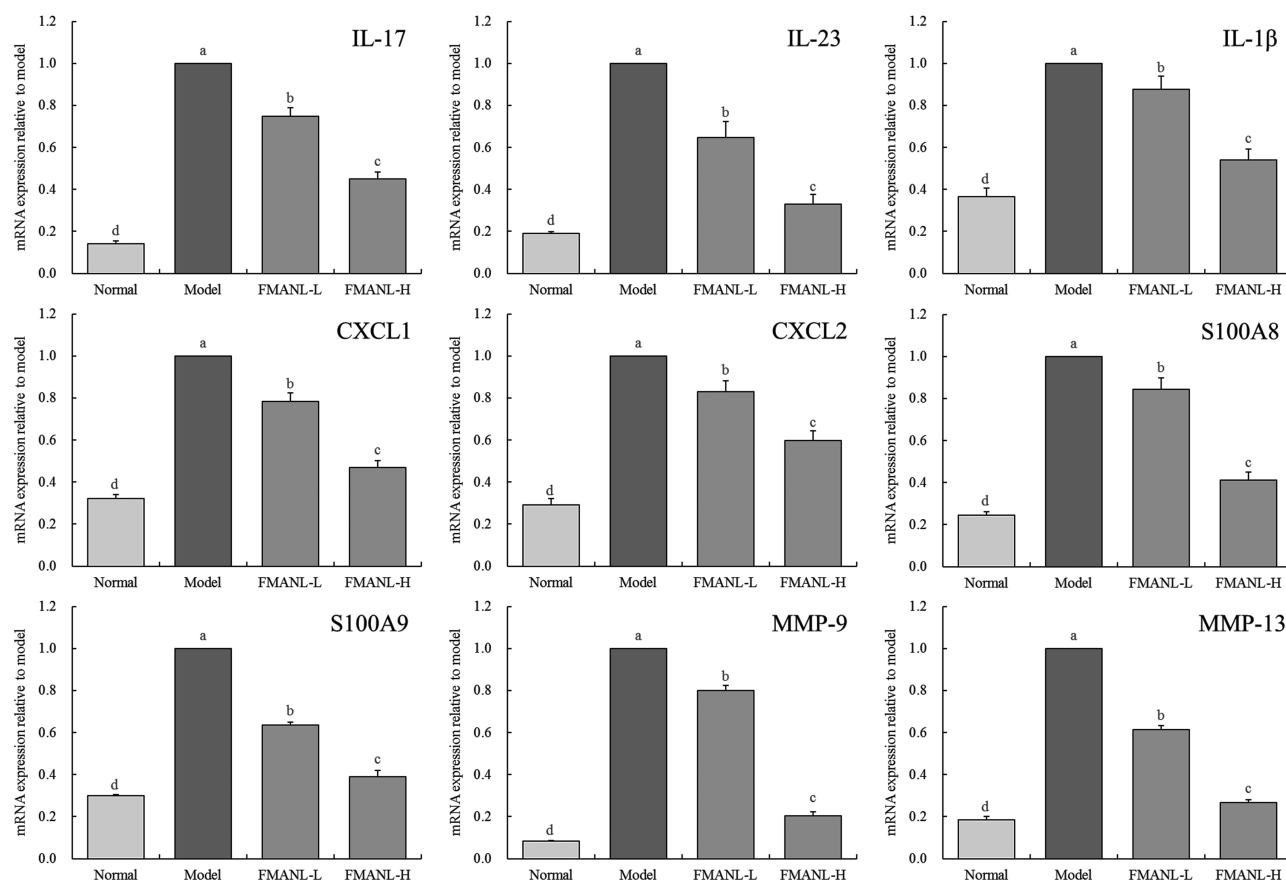


Figure 2 mRNA expression of IL-17, IL-23, IL-1 β , CXCL1, CXCL2, S100A8, S100A9, MMP-9, and MMP-13 in esophageal tissue of mice. Values presented are the mean \pm standard deviation. ^{a-c}Mean values with different letters in the bars are significantly different ($p < 0.05$) according to Duncan's multiple-range test. FMANL-L group: 50 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose; FMANL-H group: 100 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose.

tumors.⁴⁰ MMPs are a kind of secreted proteases, which are involved in tissue remodeling, angiogenesis, tumor invasion, and metastasis. The expression of MMP-9 is increased in esophageal cancer, which is related to lymph node metastasis and invasion depth.⁴¹ FMANL could down-regulate the expression levels of IL-17, IL-23, IL-1 β , CXCL1, cxcl2, S100A8, S100A9, MMP-9, and MMP-13 in esophageal tissues of mice with esophageal cancer, thus prevent esophageal cancer.

Rutin has antioxidant and anti-inflammatory effects, and it can reduce inflammation and promote cell repair.⁴² Hyperoside has many functions, such as anti-tumor, anti-aging, anti-depression, and anti-inflammatory effects, and it can also regulate the circulatory system and immune system.⁴³ Isoquercitrin can significantly prevent oxidative damage and inhibit some cancer cells, including bladder cancer cells.⁴⁴ It has been shown that dihydroquercetin is a very effective anticancer compound, which has very good inhibitory effects on liver cancer, gastric cancer, and cervical cancer.⁴⁵ Quercitrin can reduce the infiltration

of macrophages and granulocytes in inflammatory tissues, and thus prevent and inhibit cancer.⁴⁶ Hesperidin has obvious inhibitory effects on human lung cancer, rectal cancer, renal cancer, and breast cancer cells. It not only lacks mutagenicity, but it also antagonizes the mutagenicity of other chemotherapy drugs, which can be used for cancer prevention.⁴⁷ Myricetin has the functions of scavenging-free radicals and antioxidation, and it can also affect the activation and proliferation of lymphocytes, thus regulating cancer.⁴⁸ Baicalin has good anti-oxidative and anti-inflammatory effects, and it has certain inhibitory effects on breast cancer and blood malignant tumor.⁴⁹ Neohesperidin dihydrochalcone also has a good anti-oxidative effect, and this effect might play a role in cell repair and anticancer.⁵⁰ Quercetin is a multi-target substance, which can be used as a pump inhibitor, and it is related to a variety of cancer prevention mechanisms. It can inhibit the growth of a variety of malignant tumors, such as gastric cancer, breast cancer, liver cancer, cervical cancer, colon cancer, ovarian cancer, and gallbladder

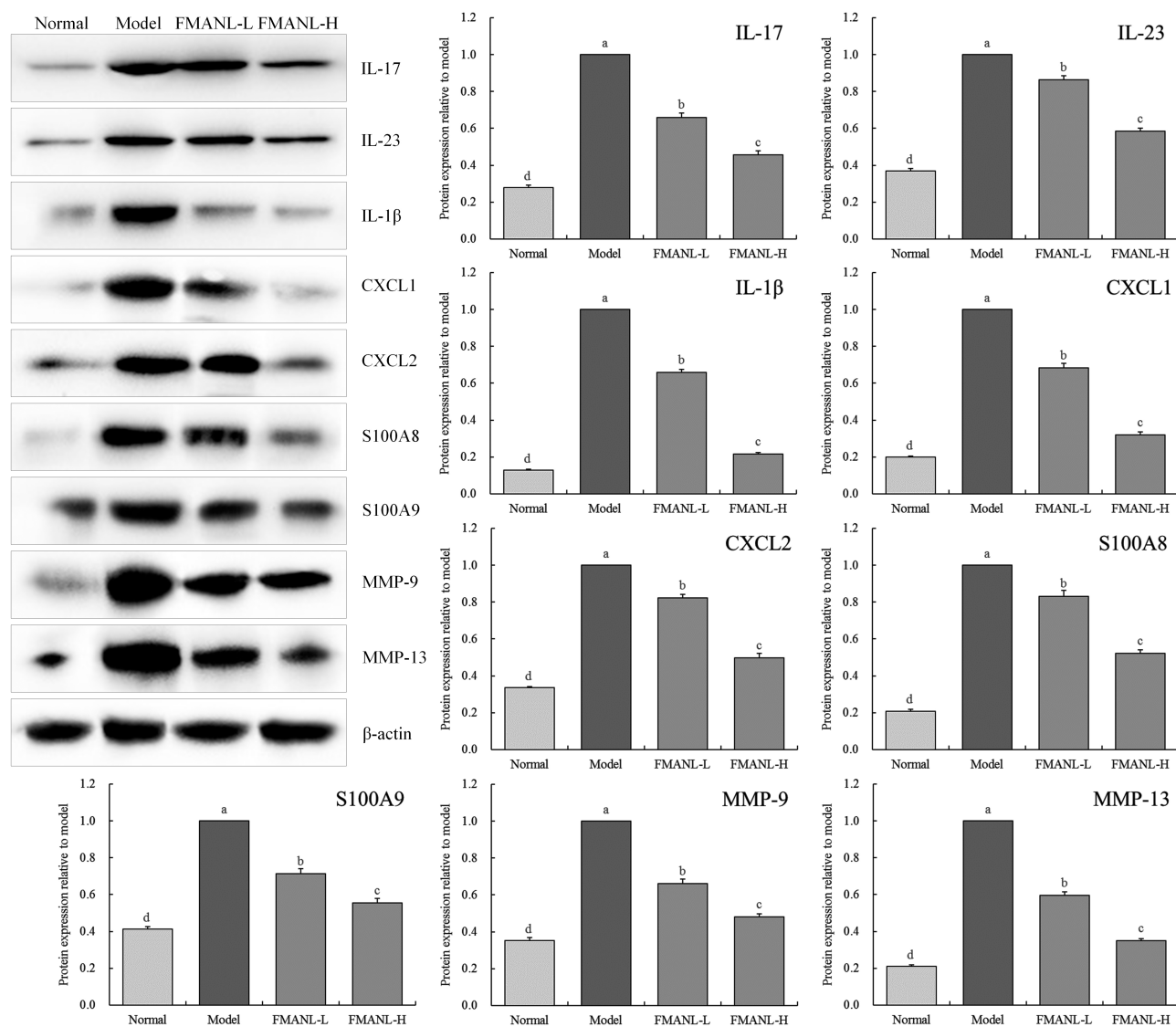


Figure 3 Protein expression of IL-17, IL-23, IL-1 β , CXCL1, CXCL2, S100A8, S100A9, MMP-9, and MMP-13 in esophageal tissue of mice. Values presented are the mean \pm standard deviation. ^{a-d}Mean values with different letters in the bars are significantly different ($p < 0.05$) according to Duncan's multiple-range test. FMANL-L group: 50 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose; FMANL-H group: 100 mg/kg flavonoids of *Malus asiatica* Nakai leaves dose.

cancer, and it has an impact on the apoptosis of malignant cells.⁵¹ The 10 bioactive substances present in FMANL might not only have their own preventive effect on esophageal cancer, but they also have a synergistic effect to increase the mutual anti-cancer effect.

In this study, the preventive effect of FMANL on esophageal cancer in vivo was studied. The results showed that FMANL had a preventive effect on the development of esophageal cancer in mice, and the effect was positively correlated with the concentration of FMANL. FMANL could regulate IL-17 and related factors to prevent esophageal cancer. FMANL contained 10 chemical components which were the main source of esophageal cancer prevention. It could be seen that FMANL is a kind of active

component with esophageal cancer prevention effect. The results of this study provide a theoretical basis for further study of FMANL.

Disclosure

The authors have no conflict of interest to declare.

References

- Zhu K, Huang G, Xie J, et al. Preventive effect of flavonoids from Wushan Shencha (*Malus doumeri* leaves) on CCl₄-induced liver injury. *Food Sci Nutr*. 2019;7(11):3808–3818. doi:10.1002/fsn3.1243
- Lee YH, Jung MG, Kang HB, et al. Effect of anti-histone acetyltransferase activity from *Rosa rugosa* Thunb. (Rosaceae) extracts on androgen receptor-mediated transcriptional regulation. *J Ethnopharmacol*. 2008;118(3):412–417. doi:10.1016/j.jep.2008.05.006

3. Bilia AR, Nieri E, Braca A, et al. Screening of Mediterranean Rosaceae plants for their molluscicidal and piscicidal activities. *Phytother Res*. 2000;14(2):126–129. doi:10.1002/(SICI)1099-1573(200003)14:2<126::AID-PTR553>3.0.CO;2-V
4. Tonin TD, Thiesen LC, de Oliveira Nunes ML, et al. Rubus imperialis (Rosaceae) extract and pure compound niga-ichigoside F1: wound healing and anti-inflammatory effects. *Naunyn Schmiedeberg's Arch Pharmacol*. 2016;389:1235–1244. doi:10.1007/s00210-016-1285-8
5. Riaz M, Zia-Ul-Haq M, Ur-Rahman N, et al. Neuropharmacological effects of methanolic extracts of Rubus fruticosus L. *Turk J Med Sci*. 2014;44(3):454–460. doi:10.3906/sag-1211-1
6. Li C, Liu C, Zhang J, et al. Evaluation of in vitro bio-activities effects of WST (Wushanshencha). *Appl Sci*. 2019;9(7):1325. doi:10.3390/app9071325
7. Mu X, Zhao X, Li GJ, et al. Preventive effect of Wushan shencha on gastric injury in SD-rats. *J ZhongKai Univ Agri Technol*. 2014;2014(2):57–59. doi:10.3969/j.issn.1674-5663.2014.02.014
8. Zhao X, Zhu K, Yi R, et al. Total flavonoid from Ba lotus leaf protected the reserpine-induced gastric ulcer in mice. *Biomed Res*. 2017;28(1):345–352.
9. Hu Y, Hou Z, Yi R, et al. Tartary buckwheat flavonoids ameliorate high fructose-induced insulin resistance and oxidative stress associated with the insulin signaling and Nrf2/HO-1 pathways in mice. *Food Funct*. 2017;8(8):2803–2816. doi:10.1039/C7FO00359E
10. Liu B, Feng X, Zhang J, et al. Preventive effect of Anji White tea flavonoids on alcohol-induced gastric injury through their antioxidant effects in Kunming mice. *Biomolecules*. 2019;9(4):137. doi:10.3390/biom9040137
11. Liu B, Zhang C, Zhang J, et al. Wu Shan Shen Cha (Malus asiatica Nakai. leaves)-derived flavonoids alleviate alcohol-induced gastric injury in mice via an anti-oxidative mechanism. *Biomolecules*. 2019;9(5):169. doi:10.3390/biom9050169
12. Huang F-L, Yu S-J. Esophageal cancer: risk factors, genetic association, and treatment. *Asian J Surg*. 2018;41(3):210–215. doi:10.1016/j.asjsur.2016.10.005
13. Zhao J, Liu J, Denney J, et al. TLR2 involved in naive CD4+ T cells rescues stress-induced immune suppression by regulating Th1/Th2 and Th17. *Neuroimmunomodulation*. 2015;22(5):328–336. doi:10.1159/000371468
14. Song Y, Yang JM. Role of interleukin (IL)-17 and T-helper (Th)17 cells in cancer. *Biochem Biophys Res Commun*. 2017;493(1):1–8. doi:10.1016/j.bbrc.2017.08.109
15. Tanaka T, Kawabata K, Sugie S. 4-nitroquinoline 1-oxide-induced tongue and esophagus carcinogenesis in obese and diabetic TSOD mice. *World J Oncol*. 2017;8(4):97–104. doi:10.14740/wjon1038w
16. Yin J, Wang L, Shi Y, et al. Interleukin 17A rs4711998 A>G polymorphism was associated with a decreased risk of esophageal cancer in a Chinese population. *Dis Esophagus*. 2014;27(1):87–92. doi:10.1111/dote.12045
17. Guo MX, Zheng YJ, Tang B, et al. Expressions of IL-17 signaling pathway-related factors mRNA in esophageal tissue of mouse with esophageal cancer. *J Zhengzhou Univ (Med Sci)*. 2019;54(4):492–495. doi:10.13705/j.issn.1671-6825.2019.02.046
18. Lyu CT, Dong ZB, Chen YL. Effects of Liujunzi decoction on quality of life and immune regulation of mice with esophageal carcinoma induced by 4NQO. *China J Trad Chin Med Pharm*. 2016;31(7):2831–2834.
19. Zhou Y, Tan F, Li C, et al. White Peony (fermented Camellia sinensis) polyphenols help prevent alcoholic liver injury via antioxidant. *Antioxidants*. 2019;8(11):524. doi:10.3390/antiox8110524
20. Liu L, Tan T, Liu L, et al. Exploring the antioxidant effects and periodic regulation of cancer cells by polyphenols produced by the fermentation of grape skin by Lactobacillus plantarum KFY02. *Biomolecules*. 2019;9(10):575. doi:10.3390/biom9100575
21. Bruggeman AR, Kamal AH, LeBlanc TW, et al. Cancer cachexia: beyond weight loss. *J Oncol Pract*. 2016;12(11):1163–1171. doi:10.1200/JOP.2016.016832
22. Ferràu F, Spagnolo F, Cotta OR, et al. Visceral adiposity index as an indicator of cardiometabolic risk in patients treated for craniopharyngioma. *Endocrine*. 2017;58(2):295–302. doi:10.1007/s12020-016-1196-y
23. Zhu K, Tan F, Mu J, et al. Anti-obesity effects of Lactobacillus fermentum CQPC05 isolated from Sichuan pickle in high-fat diet-induced obese mice through PPAR- α signaling pathway. *Microorganisms*. 2019;7(7):194. doi:10.3390/microorganisms7070194
24. Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol*. 2003;3(3):199–210. doi:10.1038/nri1027
25. Zheng L, Wang W, Ni J, et al. Role of autophagy in tumor necrosis factor- α -induced apoptosis of osteoblast cells. *J Investig Med*. 2017;65(6):1014–1020. doi:10.1136/jim-2017-000426
26. Xia H-L, Li C-J, Hou X-F, et al. Interferon- γ affects leukemia cell apoptosis through regulating Fas/FasL signaling pathway. *Eur Rev Med Pharmacol Sci*. 2017;21(9):2244–2248.
27. Hultgren EM, Patrick ME, Evans RL, et al. SUSD2 promotes tumor-associated macrophage recruitment by increasing levels of MCP-1 in breast cancer. *PLoS One*. 2017;12(5):e0177089. doi:10.1371/journal.pone.0177089
28. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295. doi:10.1101/cshperspect.a016295
29. Huang Z-Q, Wang J-L, Pan -G-G, et al. Association of single nucleotide polymorphisms in IL-12 and IL-27 genes with colorectal cancer risk. *Clin Biochem*. 2012;45(1–2):54–59. doi:10.1016/j.clinbiochem.2011.10.004
30. Al Omar SY, Marshall E, Middleton D, et al. Increased numbers but functional defects of CD56+CD3+ cells in lung cancer. *Int Immunol*. 2012;24(7):409–415. doi:10.1093/intimm/dxr122
31. Sato S, Steeber DA, Tedder TF. The CD19 signal transduction molecule is a response regulator of B-lymphocyte differentiation. *Proc Natl Acad Sci USA*. 1995;92(25):11558–11562. doi:10.1073/pnas.92.25.11558
32. Wakabayashi O, Yamazaki K, Oizumi S, et al. CD4+ T cells in cancer stroma, not CD8+ T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. *Cancer Sci*. 2003;94(11):1003–1009. doi:10.1111/j.1349-7006.2003.tb01392.x
33. Naito Y, Saito K, Shiiba K, et al. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res*. 1998;58(16):3491–3494.
34. Blaeschke F, Stenger D, Kaeuferle T, et al. Induction of a central memory and stem cell memory phenotype in functionally active CD4+ and CD8+ CAR T cells produced in an automated good manufacturing practice system for the treatment of CD19+ acute lymphoblastic leukemia. *Cancer Immunol Immunother*. 2018;67(9967):1053–1066. doi:10.1007/s00262-018-2155-7
35. Wang R, Yang L, Zhang C, et al. Th17 cell-derived IL-17A promoted tumor progression via STAT3/NF- κ B/Notch1 signaling in non-small cell lung cancer. *Oncimmunology*. 2018;7(11):e1461303. doi:10.1080/2162402X.2018.1461303
36. Numasaki M, Fukushi J, Ono M, et al. Interleukin-17 promotes angiogenesis and tumor growth. *Blood*. 2003;101(7):2620–2627. doi:10.1182/blood-2002-05-1461
37. Hurtado CG, Wan F, Housseau F, et al. Roles for interleukin 17 and adaptive immunity in pathogenesis of colorectal cancer. *Gastroenterology*. 2018;155(6):1706–1715. doi:10.1053/j.gastro.2018.08.056
38. Barrie A, Khare A, Henkel M, et al. Prostaglandin E2 and IL-23 plus IL-1 β differentially regulate the Th1/Th17 immune response of human CD161(+) CD4(+) memory T cells. *Clin Transl Sci*. 2011;4(4):268–273. doi:10.1111/j.1752-8062.2011.00300.x
39. Guéry L, Hugues S. Th17 cell plasticity and functions in cancer immunity. *Biomed Res Int*. 2015;2015:314620. doi:10.1155/2015/314620
40. Lukanidin E, Sleeman JP. Building the niche: the role of the S100 proteins in metastatic growth. *Semin Cancer Biol*. 2012;22(3):216–225. doi:10.1016/j.semcancer.2012.02.006

41. Huang H. Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: recent advances. *Sensors*. 2018;18(10):E3249. doi:10.3390/s18103249
42. Gautam R, Singh M, Gautam S, et al. Rutin attenuates intestinal toxicity induced by methotrexate linked with anti-oxidative and anti-inflammatory effects. *BMC Complement Altern Med*. 2016;16(1):99. doi:10.1186/s12906-016-1069-1
43. Jin XN, Yan EZ, Wang HM, et al. Hyperoside exerts anti-inflammatory and anti-arthritis effects in LPS-stimulated human fibroblast-like synoviocytes in vitro and in mice with collagen-induced arthritis. *Acta Pharmacol Sin*. 2016;37(5):674–686. doi:10.1038/aps.2016.7
44. Huang G, Tang B, Tang K, et al. Isoquercitrin inhibits the progression of liver cancer in vivo and in vitro via the MAPK signalling pathway. *Oncol Rep*. 2014;31(5):2377–2384. doi:10.3892/or.2014.3099
45. Rogovskii VS, Matiushin AI, Shimanovskii NL, et al. Antiproliferative and antioxidant activity of new dihydroquercetin derivatives. *Eksp Klin Farmakol*. 2010;73(9):39–42.
46. Cincin ZB, Unlu M, Kiran B, et al. Molecular mechanisms of quercitrin-induced apoptosis in non-small cell lung cancer. *Arch Med Res*. 2014;45(6):445–454. doi:10.1016/j.arcmed.2014.08.002
47. Ahmadi A, Shadboorestan A. Oxidative stress and cancer; the role of hesperidin, a citrus natural bioflavonoid, as a cancer chemoprotective agent. *Nutr Cancer*. 2016;68(1):29–39. doi:10.1080/01635581.2015.1078822
48. Jung SK, Lee KW, Byun S, et al. Myricetin suppresses UVB-induced skin cancer by targeting Fyn. *Cancer Res*. 2008;68(14):6021–6029. doi:10.1158/0008-5472.CAN-08-0899
49. Wang XF, Zhou QM, Du J, et al. Baicalin suppresses migration, invasion and metastasis of breast cancer via p38MAPK signaling pathway. *Anticancer Agents Med Chem*. 2013;13(6):923–931. doi:10.2174/18715206113139990143
50. Su C, Xia X, Shi Q, et al. Neohesperidin dihydrochalcone versus CCl₄-induced hepatic injury through different mechanisms: the implication of free radical scavenging and Nrf2 activation. *J Agric Food Chem*. 2015;63(22):5468–5475. doi:10.1021/acs.jafc.5b01750
51. Jeong J-H, An JY, Kwon YT, et al. Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. *J Cell Biochem*. 2009;106(1):3–82. doi:10.1002/jcb.21977

OncoTargets and Therapy

Dovepress

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>