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The therapeutic effect of phellopterin on colitis-associated cancer and its effects on TLR4/NF-κB pathway and macrophage M2 polarization

Xiaoping Xu[1#], Yue Su[2#], Yuan Pan[1], Minmin Shen[1], Daochen Liu[1], Zhaohui Liu[1], Dong Chen[1], Jia Wu[3*]

[1] Department of Anorectal Surgery, First People's Hospital of Linping District, Hangzhou, 311100, Zhejiang Province, China
[2] Department of Colorectal Surgery, Beijing Anorectal Hospital (Beijing Erlonglu Hospital), Beijing, 100120, China
[3] Department of Pharmacy, First People's Hospital of Linping District, Hangzhou, 311100, Zhejiang Province, China

[#]These authors contributed equally to this work as co-first author.

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ABSTRACT

This study was conducted to investigate the effect and mechanism of phellopterin on colitis-associated cancer (CAC). For this purpose, the CAC mouse model was established by the AOM/DSS method, and the therapeutic effects of phellopterin in different doses were compared. The levels of interleukin-6 (IL-6), IL-1β, IL-10, and tumor necrosis factor-α (TNF-α) in peripheral blood were detected by ELISA. The changes in T lymphocyte subsets and the expressions of CD163, CD206, Arg-1, and Ym-1 in colonic macrophages were detected. The expression of TLR4 and NF-κB p65 in the colon was tested by Western blot. Results showed that as against the Model group, the body weight and survival rate of mice treated with phellopterin were increased, the disease activity index, hematochezia rate, and tumor formation rate were decreased, the colon length was increased, and the number of tumors and spleen index were decreased ($P<0.05$). As against the Model group, the proportion of CD4+ and CD8+ in the peripheral blood of phellopterin intervention mice increased, the content of IL-6, IL-1β, and TNF-α decreased, and the content of IL-10 increased. The expression of CD163, CD206, Arg-1, and Ym-1 in colonic macrophages was decreased. The protein expressions of TLR4 and NF-κB p65 in colon tissue were decreased ($P<0.05$). The effect of phellopterin intervention on CAC was dose-dependent. In conclusion, phellopterin can improve the symptoms and inflammatory response of CAC and inhibit the occurrence of colon cancer (CC) by inhibiting M2 polarization of macrophages and activation of the TLR4/NF-κB pathway.

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Introduction

Colitis cancer (CC) is one of the top three malignant tumors worldwide. The occurrence and development of CC are affected by a variety of factors, mainly related to patients' lifestyles, intestinal diseases, and family genetics (1). In addition, the abnormal expression of tumor-promoting/tumor suppressor genes is also an important factor inducing CC. Inflammatory bowel disease (IBD) is a recurrent chronic intestinal disease, which increases the risk of CC (2). With the repeated onset of inflammatory bowel disease, the course of the disease is prolonged, and CAC develops after prolonged treatment, which is the most serious complication of inflammatory bowel disease (3). Epidemiology shows that the risk of patients with inflammatory bowel disease developing into CC is about 1% after 8-10 years, and the risk increases to about 8% after 20 years, and about 20% after 30 years (4). The traditional treatment methods of CAC include surgery, radiotherapy, and chemotherapy, but the 5-year survival rate of patients is still only about 50% (5).

Traditional Chinese medicine (TCM) has the advantages of multiple targets, low recurrence rate, and few side effects. Recent studies have found that TCM can cooperate with chemotherapy and other treatment methods to improve the therapeutic effect (6). In addition, TCM can also become the main intervention method in the empty window period after surgery and radiotherapy and chemotherapy. Previous studies have confirmed that Shaoyao decoction in inflammatory bowel disease and CAC (7). A previous study found that the Decoction of Seven Ingredients Including White Peony Root could inhibit the classical inflammatory pathway TLR4/MyD88/NF-κB pathway, Thus, it can alleviate 2,4, 6-trinitrobenzene sulfonic acid-induced inflammatory response and relieve intestinal inflammatory symptoms (8). Moreover, it has been found that paeony lactone, one of the effective components of paeony, can regulate the expression of CDX2 through AMPK, thus alleviating the inflammatory response and apoptosis of enteritis (9). Common aucklandia root is an important component of the Decoction of Seven Ingredients Including White Peony Root, while phellopterin is one of the active ingredients of common aucklandia root. It confirmed that phellopterin has anti-tumor activity and can arrest the cell cycle to the G2/M phase (10). In addition, phellopterin can inhibit the secretion of TNF-α, transforming growth factor-β (TGF-β), and IL-4 (11). However, the role of phellopterin in colitis or CC has not

* Corresponding author. Email: huoconghui3250@163.com

been reported.

Therefore, in this article, the CAC mouse model was prepared to analyze the anti-tumor effect and mechanism of phellopterin in CAC, to provide an experimental basis for the development of phellopterin as an anti-CAC drug and to provide a reference for the study of TCM in the prevention and treatment of CAC.

Materials and Methods

Experimental materials

Phellopterin (Chengdu Phytoelite Biological Technology Co., Ltd.); AOM/DSS (Wuhan Antgene Biological Technology Co., LTD.); CD4+ and CD8+ antibodies (Invitrogen); IL-6, IL-1β, TNF-α, IL-10 ELISA kit (Shanghai mlbio); PrimeScript™ RT reagent Kit (Perfect Real Time) and TB Green® Premix Ex Taq™ II (Tli RNaseH Plus), Bulk (Beijing Takara Bio Technology Co., LTD.); BCA protein Quantitative Detection Kit (Shanghai Beyotime Biotechnology Co., LTD.); TLR4, NF-κB p65, GAPDH primary and secondary antibodies (Abcam).

CAC model construction and grouping treatment

One hundred C57BL/6 mice were randomly grouped, with 20 mice in each group. They were divided into blank control (Ctrl group), CAC Model (Model group), Low-phellopterin, Middle-phellopterin, and High-phellopterin groups. Except for the Ctrl group, the mice in the other groups were intraperitoneally injected with the chemical mutagen AOM (1 mg/mL, 12 mg/kg). After 2 days, they were orally administrated with the inflammatory agent DSS (3%) for 7 days, the mice were given normal sterile water to recover for 14 days, and then the mice were given 3% DSS for 7 days. The cycle was repeated twice, a total of 3 cycles. Throughout the experiment, the mice had ad libitum access to food and water. The phellopterin groups were treated with different doses of phellopterin by gavage based on model establishment. The Low-phellopterin group was given 0.5 mg/kg phellopterin, the Middle-phellopterin group 1.0 mg/kg phellopterin, and the High-phellopterin group 2.0 mg/kg phellopterin by intragastric administration.

Evaluation of physical signs and disease activity

During the experiment, changes in body weight and food intake of mice were monitored, and general conditions of mice such as hair, activity, defecation, and hematochezia were observed, and the death of mice was recorded. Mouse body weight was measured at a fixed time each day. The presence of fecal occult blood was determined using the benzidine method. The disease activity index of the mice was calculated adopting the equation: (fractional weight loss + fractional stool trait + fractional stool blood)/3. At the end of the experiment, blood was collected by eyeball blood sampling method, and serum was separated for later use. Spleens were separated and weighed. The colon tissues of mice were isolated, and the location, size, number, and weight of tumors were observed. The length and weight of the colon were tested. The changes in body weight, colon length, number and volume of tumors were recorded.

Detection of peripheral blood T lymphocyte subsets

The mice serum was collected, and 20 μL of CD4+, CD8+, and other antibodies were added, respectively. Following shaking, the cells were kept in the dark and incubated for 20 min at ambient temperature. Subsequently, 2 mL of 1× hemolysin was added to each tube, mixed, incubated at ambient temperature in the dark for 10 min, centrifugation at 1,000 rpm for 5 min, and the supernatant was discarded. One mL of 0.01 M phosphate buffer was put into each tube, mixing, the mixture was centrifuged at 1,000 rpm for 5 min, and the supernatant was discarded. 0.3 mL of 0.01 M phosphate buffer was put into the flow tube, mixing, detection inside 1 h, analyzing the results by Flowjo software.

Detection of inflammatory factors in peripheral blood

The mouse serum was collected, and the standard preparation was carried out according to the instructions of the ELISA kit. After dilution, 50 μL of the standard was placed in the reaction well, and 50 μL of biotin-labeled antibody was added, shaken evenly, incubation for 1 h at 37℃. Following washing, the samples were dried, and 80 μL HRP-labeled affinity streptomycin was added. It was shaken evenly, and incubated at 37℃ in the dark for 30 min. Following washing, the mixture was dried, 50 μL each of working solutions A and B were added, shaken evenly, and incubated in the dark for 10 min at 37℃. 50 μL of termination solution was added, and the microplate reader was employed to measure the absorbance at 450 nm. The concentrations of IL-6, IL-1β, TNF-α, and IL-10 were calculated.

Quantitative reverse transcription PCR assay

Macrophages were isolated from the colon tissue of mice and inoculated in a DMEM medium having 10% FBS. The cells were placed in an incubator containing 5% CO_2 at 37℃ for 2 h and then fresh medium was replaced to incubate for another 48 h. The colonic macrophages in the logarithmic growth phase were isolated and identified by immunofluorescence single standard method. Trizol reagent was adopted to extract total RNA from cells, and the working solution was prepared based on guiding of the reverse transcription kit, and reverse transcription for cDNA was carried out. Then, a 20 μL reaction system was set up, and the expression of CD206, CD163, Arg1, ym-1, and GAPDH was tested. CD206, upstream: 5'-CTCTGTTCAGCTATTGGACGC-3'; Downstream: 5'-CGGAATTTCTGGGATTCAGCTTC-3'. CD163, 5'-ATGGGTGGACACAGAATGGTT-3'; 5'-CAGGAGCGTTAGTGACAGCAG-3'. Arg1, 5'-CTCCAAGCCAAAGTCCTTAGAG-3'; 5'-AGGAGCTGTCATTAGGGACATC-3'. ym-1, 5'-CAGGTCTGGCAATTCTTCTGAA-3'; 5'-GTCTTGCTCATGTGTGTAAGTGA-3'. GAPDH, 5'-AGGTCGGTGTGAACGGATTTG-3'; 5'-TGTAGACCATGTAGTTGAGGT-3'. In the final experimental data, the relative gene expression of the targets was detected by the $2^{-\Delta\Delta Ct}$ method, GAPDH as the reference gene.

Western blot analysis

100 mg of mouse colon tissue was cut into pieces and fully ground with liquid nitrogen. The protein was extracted according to the RIPA method, and the protein concentration was detected by the BCA kit. The corresponding concentrations of separating glue and concentrating glue were prepared and subjected to SDS-PAGE gel electro-

phoresis. The membrane was transferred by wet method and blocked with 5% skim milk powder blocking solution for 2 h at ambient temperature. Then, first antibodies against TLR4 (1:2000), NF-κB p65 (1:2000), and GAPDH (1:2000) were added, and the cells were incubated overnight at 4°C. Then HRP-labeled IgG (1:10000) secondary antibody was added and incubated for 2 h at room temperature. ECL chemiluminescence staining was adopted for visualization, which was exposed and photographed under a gel imaging system. GAPDH as an internal control, the relative expression levels of TLR4 and NF-κB p65 proteins were tested.

Statistical analysis

SPSS 22.0 software was employed for statistical analysis. All results were expressed as mean ± standard deviation. One-way ANOVA was adopted for contrast among groups, and the LSD method was adopted for pairwise contrast among groups. The difference among groups was considered statistically significant when $P<0.05$.

Results

Effect of phellopterin on signs, disease activity, and survival rate of model mice

Weight changes and disease activity index of mice in different groups were observed as given in Figure 1A. The weight of mice in the Ctrl group remained stable, while the weight of mice in Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups decreased gradually over time. The Model group exhibited the most significant weight loss, and on day 45, the body weight of mice in Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups was lower as against Ctrl group; The body weight in Low-phellopterin, Middle-phellopterin, and High-phellopterin groups was higher as against Model group; The body weight in Middle-phellopterin and High-phellopterin groups was higher as against Low-phellopterin group, and the body weight in High-phellopterin group was higher as against Middle-phellopterin group; Similarly, the disease activity index of mice in Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups increased gradually over time (Figure 1B). On day 45, the disease activity index of these groups was higher as against the Ctrl group; The disease activity index of the Low-phellopterin, Middle-phellopterin, and High-phellopterin groups was lower as against the Model group; Additionally, the disease activity index in Middle-phellopterin and High-phellopterin groups was lower as against Low-phellopterin group, and the disease activity index in High-phellopterin group was lower as against Middle-phellopterin group. ($P<0.05$).

Figure 2A and Figure 2B demonstrate that the hematochezia rate and tumor formation rate were 0.0% in the Ctrl group and 100.0% in the Model group. In the Low-phellopterin, Middle-phellopterin, and High-phellopterin groups, the hematochezia rates were 80.0%, 70.0%, and 55.0%, respectively, while the tumor formation rates were 75.0%, 65.0%, and 40.0%, respectively. In Figure 2C, except for the Ctrl group, all four groups of mice experienced fatalities (samples were collected immediately after death and stored at -80°C). At 15 weeks, the survival rates were 50.0% in the Model group, 60.0% in the Low-phellopterin group, 70.0% in the Middle-phellopterin group, and 85.0% in the High-phellopterin group. The survival rate in the Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups was lower as against the Ctrl group; The hematochezia rate and tumor formation rate of the Low-phellopterin, Middle-phellopterin, and High-phellopterin groups were lower as against Model group, while the three groups had a higher survival rate. The Middle-phellopterin and High-phellopterin had a lower hematochezia rate and tumor formation rate, as well as a higher survival rate as against the Low-phellopterin. The High-phellopterin presented lower hematochezia and tumor formation rates and a higher survival rate relative to the Middle-phellopterin ($P<0.05$).

Effects of phellopterin on the organs of the model mice

Figure 3 illustrates that, in comparison to the Ctrl group, the colon length decreased and the spleen index increased in the Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups; In contrast, the Low-phellopterin, Middle-phellopterin, and High-phellopterin groups had an increased colon length and decreased number of tumors and spleen index relative to the Model group; Fur-

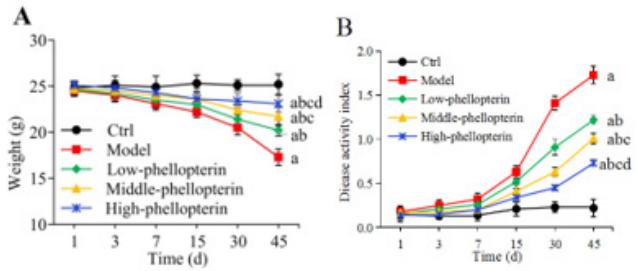

Figure 1. Changes in body weight (A) and disease activity index (B) of mice. Note: relative to Ctrl group, [a] $P<0.05$; relative to Model group, [b]$P<0.05$; relative to Low-phellopterin, [c]$P<0.05$; relative to Middle-phellopterin, [d]$P<0.05$.

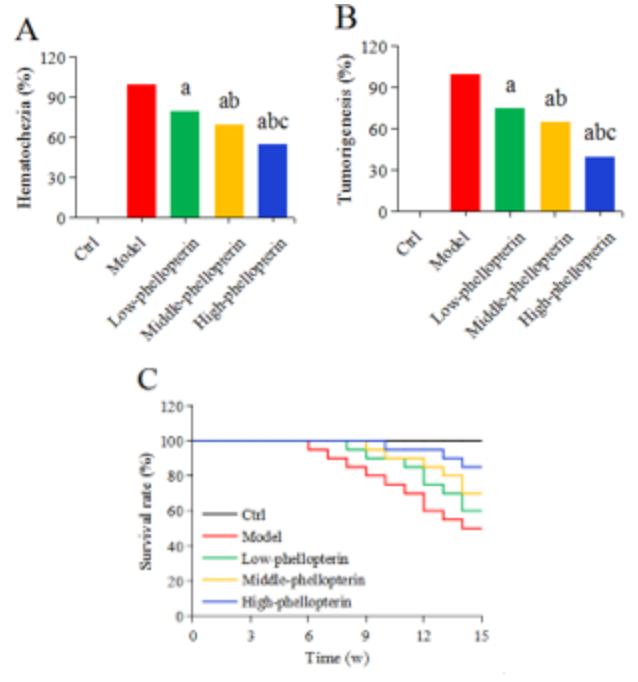

Figure 2. Differences in hematochezia rate (A), tumor formation rate (B), and survival curve (C) of mice. Note: in contrast to the Model group, [a] $P<0.05$; in contrast to Low-phellopterin, [b]$P<0.05$; in contrast to Middle-phellopterin, [c]$P<0.05$.

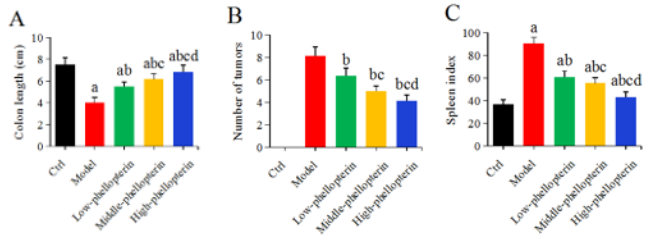

Figure 3. Differences in colon length (A), number of tumors (B), and spleen index (C). Note: as opposed to the Ctrl group, [a] $P<0.05$; as opposed to the Model group, [b]$P<0.05$; as opposed to Low-phellopterin, [c]$P<0.05$; as opposed to Middle-phellopterin, [d]$P<0.05$.

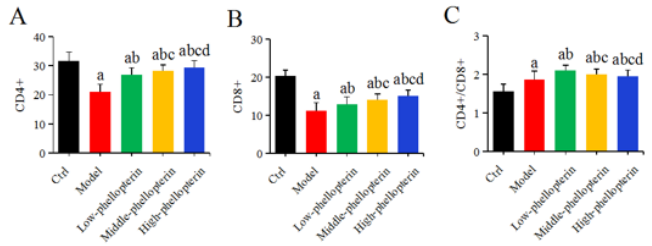

Figure 4. Differences in CD4+ (A), CD8+ (B), and CD4+/CD8+ (C) in peripheral blood. Note: in contrast to the Ctrl group, [a] $P<0.05$; in contrast to the Model group, [b]$P<0.05$; as against Low-phellopterin, [c]$P<0.05$; as against Middle-phellopterin, [d]$P<0.05$.

thermore, the Middle-phellopterin and High-phellopterin groups had an increased colon length and decreased number of tumors and spleen index relative to the Low-phellopterin group; The High-phellopterin group demonstrated an increased colon length and decreased number of tumors and spleen index relative to the Middle-phellopterin group ($P<0.05$).

Effect of phellopterin on the level of T lymphocyte subsets in model mice

Based on Figure 4, the Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups all exhibited a significant decrease in peripheral blood CD4+ and CD8+ levels. However, CD4+/CD8+ was increased in these groups; In comparison to the Model group, the Low-phellopterin, Middle-phellopterin, and High-phellopterin groups suggested an increase in peripheral blood CD4+ and CD8+ levels, while CD4+/CD8+ was decreased; Additionally, in contrast to the Low-phellopterin group, the Middle-phellopterin and High-phellopterin groups suggested an increase in peripheral blood CD4+ and CD8+ levels, and an obvious decrease in CD4+/CD8+; As against the Middle-phellopterin, the High-phellopterin exhibited an increase in peripheral blood CD4+ and CD8+ levels, and a clear decrease in CD4+/CD8+ ($P<0.05$).

Effect of phellopterin on inflammatory response in model mice

In Figure 5, it can be observed that the IL-6, IL-1β, and TNF-α were increased, while the IL-10 was lower in the peripheral blood of the Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups; In contrast, as against the Model group, the phellopterin groups indicated decreased IL-6, IL-1β, and TNF-α, and increased IL-10; The Middle-phellopterin and High-phellopterin groups had lower IL-6, IL-1β, and TNF-α, and higher IL-10 as against the Low-phellopterin ($P<0.05$).

Effect of phellopterin on M2-type polarization of colonic macrophages in model mice

Figure 6 shows that the expression levels of CD163, CD206, Arg-1, and Ym-1 in colonic macrophages were increased in the Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups; However, the expression levels of these markers were decreased in the Low-phellopterin, Middle-phellopterin, and High-phellopterin

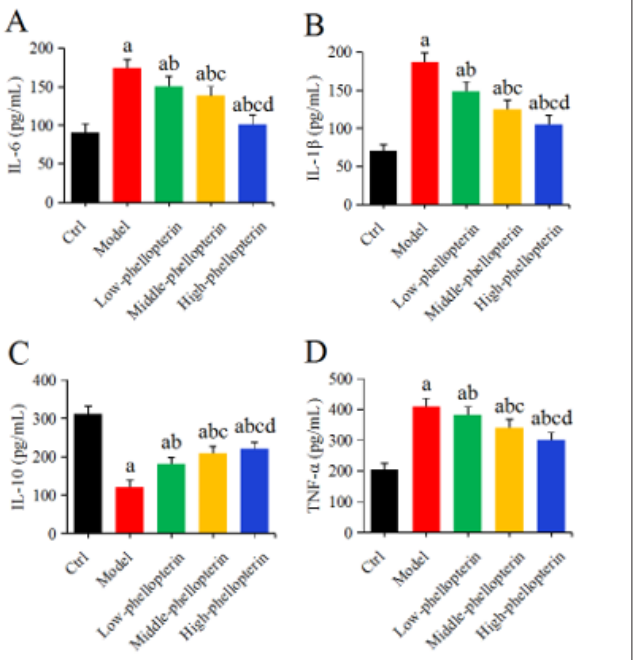

Figure 5. Differences in the levels of IL-6 (A), IL-1β (B), IL-10 (C), and TNF-α (D) in peripheral blood. Note: Distinct from Ctrl group, [a] $P<0.05$; Distinct from Model group, [b]$P<0.05$; Distinct from Low-phellopterin, [c] $P<0.05$; Distinct from Middle-phellopterin, [d]$P<0.05$.

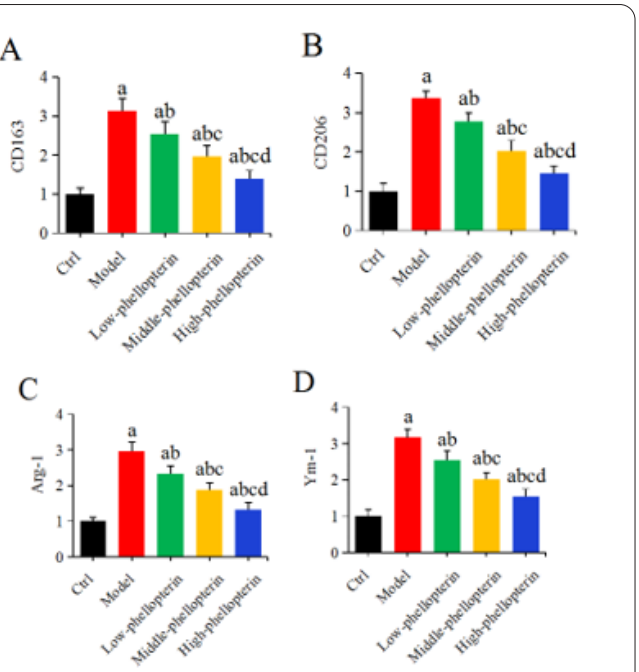

Figure 6. Differences in expression levels of CD163 (A), CD206 (B), Arg-1 (C), and Ym-1 (D) in mouse colonic macrophages. Note: Contrasting with Ctrl group, [a] $P<0.05$; Contrasting with Model group, [b]$P<0.05$; Contrasting with Low-phellopterin, [c]$P<0.05$; Contrasting with Middle-phellopterin, [d]$P<0.05$.

groups contrasting with the Model group; those were decreased in the Middle-phellopterin and High-phellopterin groups contrasting with the Low-phellopterin; Those had a clear decrease in the High-phellopterin group as against the Middle-phellopterin (P<0.05).

Effect of phellopterin on TLR4/NF-κB pathway in the colon of model mice

The results in Figure 7 indicate that the protein expression levels of TLR4 and NF-κB p65 were increased in the colon of the Model, Low-phellopterin, Middle-phellopterin, and High-phellopterin groups. Those were decreased in the colon of the phellopterin groups as opposed to the Model group. Those were decreased in the Middle-phellopterin and High-phellopterin groups as opposed to the Low-phellopterin. Those were decreased in the High-phellopterin group as opposed to the Middle-phellopterin (P<0.05).

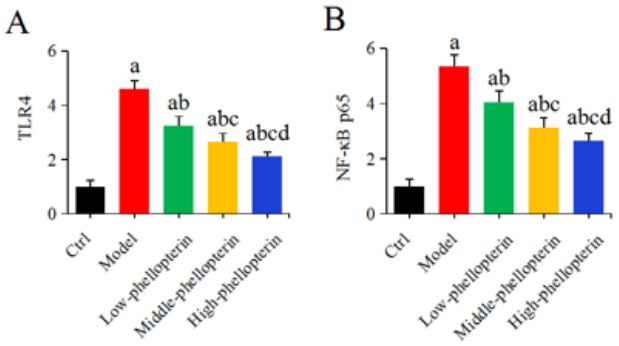

Figure 7. Differences in protein expression levels of TLR4 (A) and NF-κB p65 (B) in mouse colon. Note: As opposed to the Ctrl group, [a] P< 0.05; As opposed to the Model group, [b]P<0.05; As opposed to Low-phellopterin, [c]P<0.05; As opposed to Middle-phellopterin, [d]P<0.05.

Discussion

There is a close relationship between inflammatory bowel disease and inflammation-related CC. The risk of progression to inflammation-related CC gradually increases in patients with IBD 8-10 after diagnosis, and with the prolongation of the duration of inflammation, the extent of colonic damage in patients expands and the degree of inflammation deepens (12,13). This article used the AOM/DSS method to establish a mouse model of CC associated with inflammation. As the increase of modeling time, the body weight of the model mice gradually decreased, and the hematochezia rate and mortality gradually increased, the colon length decreased, and the spleen index increased. The spleen is an important peripheral immune organ in the body, which is rich in lymphocytes and macrophages and participates in humoral immunity (14). In this article, a mouse model of inflammation-related CC was successfully prepared using the AOM/DSS method, which laid a foundation for subsequent research. According to TCM, the weakness of the spleen and stomach caused by diet fatigue, stagnation of liver qi, and damage to the spleen and stomach, will cause the injury of large intestine mucosa and induce the occurrence of inflammation-related CC (15). The method of invigorating qi and invigorating the spleen is commonly used in the treatment of CC. Decoction of Seven Ingredients Including White Peony Root is a TCM compound, that has been used to treat ulcerative colitis in clinical practice (16). As the main component of Decoction of Seven Ingredients Including White Peony Root, common aucklandia root has attracted much attention in anti-tumor, anti-inflammatory, action on the digestive system, and anti-bacterium (17). In addition, phellopterin was found to be one of the effective components of common aucklandia root by TCMSP database screening (OB >= 30, DL >= 0.18). Phellopterin has good anti-tumor activity (18). Therefore, this article analyzes the effect and mechanism of phellopterin in the treatment of inflammation-related CC.

The immune status of the body can regulate the occurrence and development of tumors. After the occurrence of tumors, the body can generate specific immune responses to tumor antigens, and cellular and humoral immunity coordinate with each other to regulate the tumor process (19). T lymphocytes mediate cellular immunity in the process of tumor immunity. T lymphocytes can be divided into CD4+ and CD8+ according to the molecular phenotype of surface cluster differentiation antigens. CD4+T lymphocytes belong to helper T lymphocytes, which can be stimulated by tumors to produce cytokines. While directly acting on tumors, they can also induce anti-tumor inflammatory responses (20). After being stimulated by tumor antigens, CD8+T lymphocytes can differentiate and directly kill tumor cells (21). This article found that peripheral blood CD4+ and CD8+ levels were decreased in mice with inflammation-related CC models, whereas phellopterin intervention increased peripheral blood CD4+ and CD8+ levels. At present, it is generally believed that the pathogenesis of inflammation-related CC follows the "inflammation-dysplasia-canceration" pathway, in which inflammatory factors, immune microenvironment, and TLR4/NF-κB related pathway play an important role in the pathogenesis of inflammation-related CC (22). IL-6 is a kind of pro-inflammatory factor, which is involved in mediating cellular immune response and is closely related to tumor size and metastasis (23). TNF-α, mainly produced in the initiation of inflammation, is a pro-inflammatory factor (24). IL-1β is a typical multifunctional cytokine and a kind of pro-inflammatory cytokine, which is mainly produced by lymphocytes, macrophages, and monocytes (25). IL-10 can negatively regulate the secretion of IL-6 and other factors, and inhibit inflammatory response (26). This article examined the changes in the levels of peripheral blood inflammatory factors IL-6, IL-1β, IL-10, and TNF-α, and revealed that the IL-6, IL-1β, and TNF-α in the peripheral blood of the model mice were raised, and the IL-10 was decreased. Phellopterin could inhibit the expression of pro-inflammatory factors (IL-6, IL-1β, TNF-α) and increase the content of anti-inflammatory factors (IL-10). Phellopterin can reduce the levels of pro-inflammatory factors, increase the levels of anti-inflammatory factors, inhibit the inflammatory response, and prevent the occurrence of inflammation-related CC.

Tumor-associated macrophages are a prominent type of immune cell infiltrating the tumor microenvironment of colorectal cancer and have an obvious role in its progression (27). Intestinal macrophages are key players in maintaining immune homeostasis and regulating inflammation in the intestine. In response to various environmental stimuli, they can adopt a pro-inflammatory M1 phenotype or an anti-inflammatory M2 phenotype (28,29). Normally,

intestinal macrophages protect the gut from inflammatory damage. However, an imbalance in the polarization of intestinal M1/M2 macrophages, resulting from genetic and environmental factors, can disrupt the regulation of intestinal inflammation and turn a physiological inflammatory response into pathological intestinal damage (30). Studies have confirmed that inhibiting the M2 polarization of macrophages can effectively prevent the occurrence of colorectal cancer (31). CD163 and CD206 are markers of M2-type macrophages that promote tumor progression (32). In addition, when macrophages are polarized to the M2 phenotype, the expression of Arg-1 and Ym-1 is also up-regulated (33). It found that the expression of CD163, CD206, Arg-1, and Ym-1 in colonic macrophages of model mice was up-regulated, and phellopterin could inhibit the expression of CD163, CD206, Arg-1, and Ym-1 in model mice. Phellopterin could inhibit M2 polarization of macrophages, slow down the process of inflammatory reaction, and play a role in anti-inflammatory cytokines leading to inflammatory injury.

TLR is an important pattern recognition receptor in innate immunity, which is mainly expressed in immune cells. TLR can specifically recognize pathogenic molecular patterns, induce T cells to differentiate into immunocompetent cells with different functions, secrete cytokines with different functions, and initiate the innate immune response of the intestinal mucosa (34). Previous studies have confirmed that the expression of TLR4 is up-regulated in inflammatory bowel disease and CC tissues, which is involved in the formation of colitis-related tumors (35). Studies have shown that the activation of the LPS/TLR4/NF-κB signaling pathway releases many inflammatory factors and inflammatory mediators, which can promote the occurrence and metastasis of colorectal cancer (36). TLR4 and NF-κB p65 protein expression was up-regulated in the colon tissues of model mice. NF-κB activation can lead to autonomous genetic changes in tumor cells, regulate the secretion of inflammatory factors, and recycle the growth and invasion of tumor cells (37). In addition, NF-κB activation can lead to the expansion and deepening of inflammatory response, leading to tumor deterioration. Continuous activation of NF-κB can also promote cell proliferation, and improve the survival rate, growth rate, and inflammation-cancer transition of cancerous cells (38). Subsequently, these results suggest that phellopterin may play an important role in inhibiting inflammation-cancer transformation.

Phellopterin can inhibit the activation of the TLR4/NF-κB signaling pathway and M2 polarization of macrophages, reduce the inflammatory response, and prevent the occurrence of inflammation-related CC. In this article, only an animal model of inflammation-related CC was prepared to analyze the therapeutic mechanism of phellopterin. It is also necessary to prepare an in vitro model to analyze the mechanism of phellopterin blocking the inflammation-cancer transformation chain. In conclusion, it can provide reference materials for the development of new therapeutic drugs for inflammation-related CC, as well as the prevention and treatment of inflammation-related CC.

References

1. Huang L, Zhao Z, Li C. Lupeol inhibits stemness in colon cancer-stem like cells and promotes chemosensitivity via degrading β-catenin. J Biol Regulat Homeost Agent 2023; 37(3): 1293-1302. https://doi.org/10.23812/j.biol.regul.homeost.agents.20233703.130
2. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Farkas L, Garrido-Laguna I, Grem JL, Gunn A, Hecht JR, Hoffe S, Hubbard J, Hunt S, Johung KL, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Nurkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Saltz L, Schneider C, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Gregory KM, Gurski LA. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2021; 19(3): 329-359. https://doi.org/10.6004/jnccn.2021.0012
3. Bocchetti M, Ferraro MG, Ricciardiello F, Ottaiano A, Luce A, Cossu AM, Scrima M, Leung WY, Abate M, Stiuso P, Caraglia M, Zappavigna S, Yau TO. The Role of microRNAs in Development of Colitis-Associated Colorectal Cancer. Int J Mol Sci 2021; 22(8): 3967. https://doi.org/10.3390/ijms22083967
4. Zhou G, Wu H, Lin J, Lin R, Feng B, Liu Z. TRIM21 Is Decreased in Colitis-associated Cancer and Negatively Regulates Epithelial Carcinogenesis. Inflamm Bowel Dis 2021; 27(4): 458-468. https://doi.org/10.1093/ibd/izaa229
5. Gupta R, Bhatt LK, Johnston TP, Prabhavalkar KS. Colon cancer stem cells: Potential target for the treatment of colorectal cancer. Cancer Biol Ther 2019; 20(8): 1068-1082. https://doi.org/10.1080/15384047.2019.1599660
6. Xiang Y, Guo Z, Zhu P, Chen J, Huang Y. Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. Cancer Med 2019; 8(5): 1958-1975. https://doi.org/.1002/cam4.2108
7. Zhong Y, Zheng XB, Ye H, Guo M, Wu Q, Zou Y, Chi HG, Zhu YZ. [Effect of Shaoyao Tang on ulcerative colitis in rats via regulation of TLR4/NF-κB signal pathway]. Zhongguo Zhong Yao Za Zhi 2019; 44(7): 1450-1456. Chinese. https://doi.org/10.19540/j.cnki.cjcmm.20181213.007
8. Xu X, Zhang L, Liu Z, Pan Y, Chen D, Yang Z, Deng Q, Cao X, Sun YU, Yang Z, Hoffman RM, Yuan H. Therapeutic Efficacy of the Traditional Chinese Medicine Baishaoqiwu on TNBS-induced Colitis is Associated with Down-regulation of the TLR4/MyD88/NF-κB Signaling Pathway. In Vivo 2016; 30(3): 181-186.
9. Xu X, Liu H, Pan Y, Lui Z, Chen D, Zhang L, Yuang H. Albiflorin attenuates inflammation and apoptosis by upregulating AMPK-mediated expression of CDX2 in a mouse model of ulcerative colitis. Trop J Pharm Res 2020; 19(5): 995-999. https://doi.org/10.4314/tjpr.v19i5.13
10. Sumiyoshi M, Sakanaka M, Taniguchi M, Baba K, Kimura Y. Anti-tumor effects of various furocoumarins isolated from the roots, seeds and fruits of Angelica and Cnidium species under ultraviolet A irradiation. J Nat Med 2014; 68(1): 83-94. https://doi.org/10.1007/s11418-013-0774-z
11. Li D, Wu L. Coumarins from the roots of *Angelica dahurica* cause anti-allergic inflammation. Exp Ther Med 2017; 14(1): 874-880. https://doi.org/10.3892/etm.2017.4569
12. Tong YL, Yu XF, Yu Y, Mi L, Zhang ZY, Bao ZJ, Yu XF. Small intestinal bacterial overgrowth and low-grade systemic inflammation in patients with inflammatory bowel disease. Acta Med Mediterr 2021; 5: 2941. https://doi.org/10.19193/0393-6384_2021_5_454
13. Seyedian SS, Nokhostin F, Malamir MD. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. J Med Life 2019; 12(2): 113-122. https://doi.org/10.25122/jml-2018-0075
14. Lewis SM, Williams A, Eisenbarth SC. Structure and function

of the immune system in the spleen. Sci Immunol 2019; 4(33): eaau6085. https://doi.org/10.1126/sciimmunol.aau6085
15. Zhang Y, Pu W, Bousquenaud M, Cattin S, Zaric J, Sun LK, Rüegg C. Emodin Inhibits Inflammation, Carcinogenesis, and Cancer Progression in the AOM/DSS Model of Colitis-Associated Intestinal Tumorigenesis. Front Oncol 2021; 10: 564674. https://doi.org/10.3389/fonc.2020.564674
16. Yu H, Sun H, Wang K, Liang X, Ding Y, Chang X, Guo J, Peng D, Gui SY. Study of the therapeutic effects of Painong powder on ulcerative colitis and the role of Platycodonis Radix in the prescription based on pharmacodynamic, pharmacokinetic, and tissue distribution analyses. J Ethnopharmacol 2022; 285: 114872. https://doi.org/10.1016/j.jep.2021.114872
17. Zhou Q, Zhang WX, He ZQ, Wu BS, Shen ZF, Shang HT, Chen T, Wang Q, Chen YG, Han ST. The Possible Anti-Inflammatory Effect of Dehydrocostus Lactone on DSS-Induced Colitis in Mice. Evid Based Complement Alternat Med 2020; 2020: 5659738. https://doi.org/10.1155/2020/5659738
18. Chen X, Zhang Y, Pei J, Zeng X, Yang Y, Zhang Y, Li F, Deng Y. Phellopterin alleviates atopic dermatitis-like inflammation and suppresses IL-4-induced STAT3 activation in keratinocytes. Int Immunopharmacol 2022; 112: 109270. https://doi.org/10.1016/j.intimp.2022.109270
19. Locy H, de Mey S, de Mey W, De Ridder M, Thielemans K, Maenhout SK. Immunomodulation of the tumor microenvironment: turn foe into friend. Front Immunol 2018; 9: 2909. https://doi.org/10.3389/fimmu.2018.02909
20. Hao S, Wang X, Wang J. Determination of Breast Milk Cell Immune Function and Maternal Health Education. Cell Mol Biol (Noisy-le-grand) 2022; 68(8): 156-162. https://doi.org/10.14715/cmb/2022.68.8.28
21. Farwell MD, Gamache RF, Babazada H, Hellmann MD, Harding JJ, Korn R, Mascioni A, Le W, Wilson I, Gordon MS, Wu AM, Ulaner GA, Wolchok JD, Postow MA, Pandit-Taskar N. CD8-Targeted PET Imaging of Tumor-Infiltrating T Cells in Patients with Cancer: A Phase I First-in-Humans Study of ^{89}Zr-Df-IAB22M2C, a Radiolabeled Anti-CD8 Minibody. J Nucl Med 2022; 63(5): 720-726. https://doi.org/10.2967/jnumed.121.262485
22. Yao D, Dong M, Dai C, Wu S. Inflammation and Inflammatory Cytokine Contribute to the Initiation and Development of Ulcerative Colitis and Its Associated Cancer. Inflamm Bowel Dis 2019; 25(10): 1595-1602. https://doi.org/10.1093/ibd/izz149
23. Zhou Z, Yang H, Wang X, Yi L. Blocking *CCR10* Expression Activates m6A Methylation and Alleviates Vascular Endothelial Cell Injury. Discov Med 2023; 35(174): 36-44. https://doi.org/10.24976/Discov.Med.202335174.5
24. Cruceriu D, Baldasici O, Balacescu O, Berindan-Neagoe I. The dual role of tumor necrosis factor-alpha (TNF-α) in breast cancer: molecular insights and therapeutic approaches. Cell Oncol (Dordr) 2020; 43(1): 1-18. https://doi.org/10.1007/s13402-019-00489-1
25. Tulotta C, Ottewell P. The role of IL-1B in breast cancer bone metastasis. Endocr Relat Cancer 2018; 25(7): R421-R434. https://doi.org/10.1530/ERC-17-0309
26. Saiki P, Nakajima Y, Van Griensven LJLD, Miyazaki K. Real-time monitoring of IL-6 and IL-10 reporter expression for anti-inflammation activity in live RAW 264.7 cells. Biochem Biophys Res Commun 2018; 505(3): 885-890. https://doi.org/10.1016/j.bbrc.2018.09.173
27. Binnewies M, Pollack JL, Rudolph J, Dash S, Abushawish M, Lee T, Jahchan NS, Canaday P, Lu E, Norng M, Mankikar S, Liu VM, Du X, Chen A, Mehta R, Palmer R, Juric V, Liang L, Baker KP, Reyno L, Krummel MF, Streuli M, Sriram V. Targeting TREM2 on tumor-associated macrophages enhances immunotherapy. Cell Rep 2021; 37(3): 109844. https://doi.org/10.1016/j.celrep.2021.109844
28. Boutilier AJ, Elsawa SF. Macrophage Polarization States in the Tumor Microenvironment. Int J Mol Sci 2021; 22(13): 6995. https://doi.org/10.3390/ijms22136995
29. Huang C, Wang J, Liu H, Huang R, Yan X, Song M, Tan G, Zhi F. Ketone body β-hydroxybutyrate ameliorates colitis by promoting M2 macrophage polarization through the STAT6-dependent signaling pathway. BMC Med 2022; 20(1): 148. https://doi.org/10.1186/s12916-022-02352-x
30. Zhou X, Li W, Wang S, Zhang P, Wang Q, Xiao J, Zhang C, Zheng X, Xu X, Xue S, Hui L, Ji H, Wei B, Wang H. YAP Aggravates Inflammatory Bowel Disease by Regulating M1/M2 Macrophage Polarization and Gut Microbial Homeostasis. Cell Rep 2019; 27(4): 1176-1189. e5. https://doi.org/10.1016/j.celrep.2019.03.028
31. Lian G, Chen S, Ouyang M, Li F, Chen L, Yang J. Colon Cancer Cell Secretes EGF to Promote M2 Polarization of TAM Through EGFR/PI3K/AKT/mTOR Pathway. Technol Cancer Res Treat 2019; 18: 1533033819849068. https://doi.org/10.1177/1533033819849068
32. Nielsen MC, Hvidbjerg Gantzel R, Clària J, Trebicka J, Møller HJ, Grønbæk H. Macrophage Activation Markers, CD163 and CD206, in Acute-on-Chronic Liver Failure. Cells 2020; 9(5): 1175. https://doi.org/10.3390/cells9051175
33. Zhou D, Ji L, Chen Y. TSPO Modulates IL-4-Induced Microglia/Macrophage M2 Polarization via PPAR-γ Pathway. J Mol Neurosci 2020; 70(4): 542-549. https://doi.org/10.1007/s12031-019-01454-1
34. Irizarry-Caro RA, McDaniel MM, Overcast GR, Jain VG, Troutman TD, Pasare C. TLR signaling adapter BCAP regulates inflammatory to reparatory macrophage transition by promoting histone lactylation. Proc Natl Acad Sci U S A 2020; 117(48): 30628-30638. https://doi.org/10.1073/pnas.2009778117
35. Eisa NH, Said E, Khodir AE, Sabry D, Ebrahim HA, Elsherbini DMA, Altemani R, Alnasser DM, Elsherbiny NM, El-Sherbiny M. Effect of Diacerein on HOTAIR/IL-6/STAT3, Wnt/β-Catenin and TLR-4/NF-κB/TNF-α axes in colon carcinogenesis. Environ Toxicol Pharmacol 2022; 95: 103943. https://doi.org/10.1016/j.etap.2022.103943
36. Liu L, Li YH, Niu YB, Sun Y, Guo ZJ, Li Q, Li C, Feng J, Cao SS, Mei QB. An apple oligogalactan prevents against inflammation and carcinogenesis by targeting LPS/TLR4/NF-κB pathway in a mouse model of colitis-associated colon cancer. Carcinogenesis 2010; 31(10): 1822-1832. https://doi.org/10.1093/carcin/bgq070
37. Korbecki J, Simińska D, Gąssowska-Dobrowolska M, Listos J, Gutowska I, Chlubek D, Baranowska-Bosiacka I. Chronic and Cycling Hypoxia: Drivers of Cancer Chronic Inflammation through HIF-1 and NF-κB Activation: A Review of the Molecular Mechanisms. Int J Mol Sci 2021; 22(19): 10701. https://doi.org/10.3390/ijms221910701
38. Pflug KM, Sitcheran R. Targeting NF-κB-Inducing Kinase (NIK) in Immunity, Inflammation, and Cancer. Int J Mol Sci 2020; 21(22): 8470. https://doi.org/10.3390/ijms21228470