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Study on the mechanism of action of effective monomeric, berberine of Xianglian Pill in inhibiting human colon cancer cells based on fatty acid synthase target

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ABSTRACT

Background and aim: Xianglian Wan (XLW) as a classic prescription of traditional Chinese medicine protects digestive function; however, few studies have investigated its anti-colorectal cancer effects. This study verified that the effective monomer berberine of XLW plays an antitumo r role by regulating the acetyl-CoA carboxylase (ACC)/fatty acid synthase (FASN) lipid metabolism-related signaling pathway.
Experimental procedure: The connection between XLW and FASN was identified through literature mining, bioinformatics and structural biology. In vivo experiments verified the rationality of the anti-tumor effect of berberine by regulating the ACC/FASN pathway, and in vitro experiments verified the regulatory relationship between berberine and FASN.
Results and conclusion: The most frequent Chinese medicine component in XLW was *Coptis chinensis*. Berberine, the active ingredient of XLW, has a FASN binding site. FASN expression is higher in tumor tissues than in normal tissues. FASN is related to colorectal adenocarcinoma occurrence and patient survival time. Experiments showed that XLW, berberine and orlistat (FASN inhibitor) can cooperate with palmitic acid (PA) to inhibit tumors in mice. Berberine can downregulate FASN and ACC expression in tumor tissues and inhibit the increase in acetyl-CoA, the intermediate product of exogenous PA intake. The mechanism by which berberine inhibits colon cancer cell proliferation by lowering lipids is related to its downregulation of FASN protein expression. The ACC/FASN signaling pathway is a critical pathway through which berberine, the effective monomer of XLW, plays an antitumor role in colon cancer.

1. Introduction

According to the global cancer statistics of the International Agency for Research on Cancer, the number of new cases of colorectal cancer in 2020 was approximately 1.9 million, and the number of deaths from colorectal cancer was approximately 935,000, ranking second in mortality.[1] A high-fat diet is recognized as a high-risk factor for colon cancer and can lead to obesity and abnormal lipid metabolism, thus promoting the occurrence and development of colon cancer.

Palmitic acid (PA) is a fatty acid produced during adipogenesis; palmitate has a negative effect on acetyl-CoA carboxylase (ACC), while fatty acid synthase (FASN) is responsible for the de novo synthesis of PA from acetyl coenzyme A (acetyl-CoA) and malonyl coenzyme A (malonyl-CoA). Studies have shown that PA can inhibit the development of liver cancer by regulating membrane fluidity and glucose metabolism.[2] Activation of the ACC/FASN signaling pathway can promote fat accumulation[3,4] and the synthesis of long-

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chain fatty acids. However, whether PA can affect colorectal cancer by regulating lipid metabolism has not been proven.

Xianglian Wan (XLW) is recorded in the Pharmacopoeia of the People's Republic of China (2020 Edition) as a prescription of traditional Chinese medicine, which is composed of 800 g of Huanglian (*Coptis chinensis.* Ranunculaceae.

黄连huáng lián) processed by Wuzhuyu (*Tetradium ruticarpum.* Rutaceae 吴茱萸wú zhū yú) and 200 g of Muxiang (*Aucklandia costus Falc.* Asteraceae 木香mù xiāng). Huanglian can be cultivated and is distributed in Sichuan, Guizhou, Hunan, Hubei, and southern Shaanxi province, especially montane forests or shady places in valleys between 500 and 2000 m. While Muxiang is distributed in Sichuan, Yunnan and Tibet in mainland China, mostly growing in alpine grasslands and thickets, and is a wild plant that has not been cultivated by artificial introduction. With these two herbs, XLW is prepared in the form of pills and is administered orally. This prescription has the effect of clearing heat, dryness and dampness and dispersing qi and stagnation. There is class III evidence of it protecting digestive function and improving digestive system syndrome.[5] Some experimental reports have proven that XLW can improve diarrhea caused by chemotherapy[6,7] and can protect the intestinal barrier.[8–10] Damage to the intestinal barrier is one of the pathological factors in the occurrence and development of ulcerative colitis.[11] Patients with ulcerative colitis are more likely to develop colon cancer.

Recent network pharmacological studies on XLW have mostly focused on the treatment of ulcerative colitis.[12–14] However, Wang[15] constructed the "traditional Chinese medicine-target-pathway" network and found that XLW was involved in 6 cancer-related signaling pathways and had the highest correlation with colorectal cancer, suggesting that XLW has research value in the treatment of colon cancer.

This study showed that *Coptis chinensis* is the core traditional Chinese medicine in XLW and that berberine is an effective monomer. Based on bioinformatics, FASN is one of the key target proteins in the occurrence and development of colon cancer, and berberine binds to FASN. Through in vivo and in vitro experiments, the study verified that XLW and berberine can downregulate the protein expression of FASN and ACC by inhibiting the fatty acid synthesis pathway to exert a lipid-lowering effect and inhibit the occurrence and development of colon cancer, which provides a theoretical basis for the new indications of XLW and berberine.

2. Methods

2.1. Data mining

2.1.1. Analysis of XLW

All the prescriptions named XLW were searched on the medical encyclopedia website, and the composition of XLW was input into the Traditional Chinese Medicine (TCM) inheritance assistant system V2.5 to analyze the traditional Chinese medicine that matched and appeared the most frequently. Th e number of supports was 6, and the confidence level was 0.6.

In the Encyclopedia of Traditional Chinese Medicine (ETCM) database, "XIANG LIAN WAN" was searched, the FDR values were sorted from small to large, and the Gene ontology (GO) terms with significant differences in the top 20 were selected. Four items were selected: GO term description, odds ratio, p value and target count, and an enrichment bubble diagram was made.

2.1.2. Analysis of FASN

In the Gene Expression Profiling Interactive Analysis (GEPIA) 2 database (http://gepia2.cancer-pku.cn/#index), the filtering conditions were set as follows: "Expression Analysis: Expression DIY"; "Box plot"; "Gene: FASN"; "log2FC | Cutoff: 2, p value Cutoff: 0.001"; "Cancer name: COAD"; and "Match TCGA Normal and GTEX Data". The filter conditions were set as follows: "Expression Analysis: General"; and Quick Search: FASN. In the TIMER2.0 database, the filter criteria were set as follows: Exploration; Gen_De; and Gene Expression: FASN.

2.1.3. Relationship between FASN, body mass index (BMI) and survival time

In the GEPIA2 database, the parameters were as follows: "Expression Analysis: Survival Analysis"; "Survival Analysis"; "Gene A: FASN"; "Methods: Overall Survival"; "Group Cutoff: Median"; "Cutoff-High: 50"; "Cutoff-Low: 50"; "Axis Units: Days"; and "Cancer name: COAD". The relationship between FASN and the total survival time of patients with colon adenocarcinoma was analyzed. The body weight and survival time data of people with FASN gene expression were obtained from the TCGA database (https://cancergenome.nih.gov/). According to the difference in body weight and FASN expression, the Kaplan–Meier survival curves of subjects in different groups were drawn by R programming language.

2.1.4. Relationship between berberine and FASN

In the PubChem database, "Berberine" was search, and the 3D structure was downloaded. In the protein structure database (PDB), "FASN" was searched, and "4Z49 Homo sapiens Fatty Acid Synthetase, Thioesterase Domain at 1.7 Å Resolution" was downloaded. Molecular docking was completed through the CB-DOCK website (http://clab.labshare.cn/cb-dock/php/dockingresult.php).

2.2. Cell line and cell culture

The LoVo cell line was provided by Zhonghua Wu of Shanghai University of Traditional Chinese Medicine. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 0.1% penicillin–streptomycin and incubated in a 37 °C, 5% CO_2 atmosphere-designated incubator.

2.3. Reagents

DMEM, albumin from bovine serum (BSA), FBS and phosphate buffer saline (PBS) were obtained from Gibco Life Technologies (NY, USA). PA was purchased from Sigma (St. Louis, United States). The berberine and Cell counting kit (CCK-8) were purchased from Noble Biotech (Shanghai, China), and orlistat was purchased from Zein Biotech (Chongqing, China). The electrophoresis solution, transfer solution, radio immunoprecipitation assay (RIPA), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and bicinchoninic acid (BCA) protein assay kit were purchased from Beyotime Biotech (Shanghai, China). An enhanced chemiluminescence (ECL) Kit was provided by Tanon Biotech (Shanghai, China). ACC (C83B10) rabbit mAb (#3676) and FASN (C20G5) rabbit mAb (#3180S) were purchased from Cell Signaling Technology (Danvers, USA).

2.4. Drug preparation

The granules of the XLW prescription for in vivo experiments were purchased from Jiangyin Tianjiang Pharmaceutical Co. Ltd. of China, supervised by Shanghai Hospital of Integrative Medicine and supported by the Scientific Research Program of Shanghai Science and Technology Commission (No. 19401971600). The contents of XLW include Muxiang, roots uesd as medicine, 2.1 g; Wuzhuyu, fruit used as medicine, 4.2 g and Huanglian, rootstock used as medicine, 8.4 g, which are divided as the taxonomy for each species

in *Chinese Materia Medica*. The equivalent dose of XLW was 3.82 g/kg according to the conversion of drug doses for experimental animals, the concentration of the drug was calculated as 380 mg/mL, and the dose was 200 μL for each mouse. The clinical oral dose of berberine was 200 mg/58 kg, and according to the conversion, the low dose was 15.69 mg/kg, the medium dose was 31.38 mg/kg, and the high dose was 47.07 mg/kg. In the orlistat group, a dose of 15.6 mg/kg was administered to mic. All drugs were fully dissolved in double-distilled water, stored in a refrigerator at 4 °C and heated in a 37 °C water bath before gavage.

2.5. Establishment of the orthotopic transplantation tumor model

Forty-eight male nude mice (6 weeks old, 20 ± 2 g) were purchased from Shanghai B&K Laboratory Animal Company (Shanghai, China), housed in the experimental animal center of Shanghai University of Traditional Chinese Medicine and maintained under specific-pathogen-free (SPF) conditions at 22 ± 2 °C, a relative humidity of 55 ± 5%, and a 12-h/12-h light/dark cycle. All animal experiments conformed to the animal welfare guidelines and were approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine (PZSHUTCM200628010).

To establish subcutaneous tumor-bearing mice, 5×10^6 LoVo cells suspended in 100 μL of PBS were injected into the right flank of the mice. After the mice were sacrificed, tumor tissues were harvested to establish the orthotopic transplantation tumor model. The mice were anesthetized, an incision was made in the middle of the abdomen, and a 2-mm incision was made in the colorectal plasma membrane layer facing the intestinal wall. Two pieces of 1-mm^3 subcutaneous tumor were fixed to the mucosal layer of the intestinal wall with tissue glue, and then the intestine was recovered and the incision was sutured.

Mice were randomly allocated to 8 different groups (6 mice per group) according to the weight of the mice. Each group of mice received the respective treatment for 28 days. The blank group and model group received equivalent intragastric volumes of saline daily and were fed a normal diet, and all of the treatment groups were fed a 5% paleo diet (5 g PA powder + 95 g common feed powder). The PA group was gavaged with 200 μL of saline daily; the XLW + PA group was fed 200 μL of XLW daily by gavage; the berberine (low) + PA group was fed 200 μL of low-dose berberine suspension by gavage daily; the berberine (middle) + PA group was fed 200 μL of middle-dose berberine suspension by gavage daily; the berberine (high) + PA group was fed 200 μL of high-dose berberine suspension by gavage daily; and the orlistat + PA group was fed 200 μL of orlistat suspension by gavage daily. According to the ethical requirements of experimental animals, mice were humanely killed by CO_2 suffocation after 4 weeks. The weight of all mice was recorded, and tumors were isolated, weighed and photographed.

2.6. CCK-8 assay

Log-phase LoVo cells were collected and incubated in a 96-well plate, and five wells were set for each group (5×10^3 cells per well). After 24 h and 48 h of culture, 10 μL of CCK-8 reagent was added to each well, followed by cell incubation for 2 h at 37 °C. Then, the optical density (OD) value was detected at a wavelength of 450 nm with a microplate reader.

2.7. Western blotting

The tumor tissues and LoVo cells were fully lysed with protein lysis buffer, and the supernatants were collected for quantitative protein analysis by the BCA method. The protein samples were then used for SDS–PAGE and transferred to polyvinylidene fluoride (PVDF) membranes through wet transfer. The PVDF membranes were blocked with 5% skim milk for 2 h at room temperature. Then, incubation with the related primary antibody was performed overnight at 4 °C. After washing, the secondary antibody was added and incubated at room temperature for 1.5 h. Finally, the protein expression was detected by using an ECL detection kit and visualized by using the imaging system. The quantification of the protein bands was carried out with ImageJ software by densitometry.

2.8. Immunohistochemistry (IHC)

IHC was performed to investigate the levels of FASN and ACC in intestinal tissues and tumors. The tissue was fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned. After dewaxing and rehydration, the sections were boiled in citric acid antigen extract for 10 min. Next, the sections were incubated with primary antibodies at 4 °C overnight and then with secondary antibodies at room temperature for 1 h. After incubation of the sections with 3,3-diaminobenzidine-tetrachloride (DAB) for 3 min and counterstaining with hematoxylin, images were recorded using an Imagery Management System. The positive area of immunohistochemical staining was observed under three visual fields, and the visual field was selected according to the random nonoverlapping principle after scanning.

2.9. Biochemical detection

Biochemical detection of blood lipids and lipid indicators in mice, including blood triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), was performed using a BECKMAN COULTER AU680 automatic biochemical analyzer (Beckman, USA).

2.10. Enzyme-linked immunosorbent assay (ELISA)

Acetyl-CoA, malonyl-CoA and nicotinamide adenine dinucleotide phosphate (NADPH) were quantified using ELISA kits following the manufacturer's instructions.

2.11. Statistical analysis

SPSS 26.0 for Windows (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 9.0 (GraphPad Software Inc., California, USA) were adopted for performing statistical analyses and plotting. All data for the continuous variables are presented as the mean ± standard deviation (SD). Data conforming to a normal distribution were analyzed using either Student's *t*-test for two groups or one-way ANOVA for three or more groups, and the LSD test was used for two-way multiple comparisons. In the case of nonconformity with a normal distribution, the Kruskal–Wallis H statistical analysis method was used for the rank sum test. The counting data are presented as the rate (%) and were analyzed with the chi-square test. All statistical tests were two-tailed, and a P value of less than 0.05 was considered statistically significant.

3. Result

3.1. Coptis chinensis is the core drug of XLW

According to the statistics of the frequency of each traditional Chinese medicine in the prescriptions whose names include XLW, it was found that the frequency of *Coptis chinensis* was the highest. Five core traditional Chinese medicines were summarized: *Coptis chinensis*, *Aucklandia costus*, *Myristica fragrans*, *Terminalia chebular*

and *Paeonia lactiflora*. The frequency of specific traditional Chinese medicines and compatibility patterns from high to low are shown in Table 1 and Supplementary Table 2.

3.2. Gene ontology enrichment analysis of XLW

It was found that there were 348 pathways in the gene ontological enrichment of XLW, of which the lipid metabolism regulation pathway ranked 16th. It was suggested that XLW plays a certain role in the regulation of lipid metabolism (Fig. 1A).

3.3. Differences in FASN expression between pancancerous tissues and normal tissues

In the GEPIA2 database, FASN was significantly higher in bladder urothelial carcinoma (BLCA), colon adenocarcinoma (COAD), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD) and uterine corpus endometrial carcinoma (UCEC) than in the same type of normal tissues ($p < 0.001$). The expression of FASN in cervical squamous cell carcinoma and adenocarcinoma (CESC), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC) and kidney renal papillary cell carcinoma (KIRP) was significantly higher than that in normal tissues of the same type. The expression of FASN in tumor tissues of patients with HNSC and human papilloma virus (HPV) infection was lower than that of uninfected patients. FASN expression in kidney renal clear cell carcinoma (KIRC) tissues was higher than that in normal tissues of the same type. The expression of FASN in glioblastoma multiforme (GBM), lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) was significantly lower than that in normal tissues ($p < 0.001$) (Fig. 1B).

In the TIMER 2.0 database, the expression of FASN in BLCA, CESC, COAD, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), LIHC, ovarian serous cystadenocarcinoma (OV), PRAD, READ, testicular germ cell tumors (TGCT), thymic adenocarcinoma (THYM), UCEC, and uterine carcinosarcoma (UCS) was higher than that in normal tissues. However, the expression in acute myeloid leukemia (LAML) and thyroid carcinoma (THCA) was lower than that in normal tissues (Fig. 1C).

By taking the intersection of the results of two databases, it can be found that the expression of FASN in tumor tissues of 7 kinds of cancer (namely, BLCA, COAD, CESC, LIHC, PRAD, READ, and UCEC) is higher than that in normal tissues, including COAD (Fig. 1D).

3.4. Differences in FASN expression between human colonic adenocarcinoma and normal intestinal tissue

A total of 275 patients with COAD and 349 healthy people were included. The results showed that the expression ratio of FASN-log2 (TPM+1) (Fig. 1E) in human COAD tissue and normal intestinal tissue was 6.89 and 5.39, respectively ($p < 0.05$) (Fig. 1F). The expression of FASN in tumors was higher than that in normal tissues, and FASN was related to the carcinogenesis of COAD.

3.5. Relationship between FASN and the survival time of patients with COAD

Of the 643 patients with COAD, 129 had complete follow-up data. According to the order of FASN values from high to low, the first 100 cases were classified as the high FASN expression group, and the last 29 cases were classified as the low FASN expression group. The survival time of the group with high FASN expression was longer than that of the group with low FASN expression ($p < 0.05$) (Fig. 2A).

The expression of FASN in COAD patients with BMI< 25 was higher than that in obese colon cancer patients (BMI $\geq$25) ($p < 0.05$) (Fig. 2B).

As for obese COAD patients (BMI $\geq$25), patients with high FASN expression showed higher short-term survival rate. When it came to the long-term survival rate, the patients with low FASN expression showed better survival probability ($p < 0.05$) (Fig. 2C).

3.6. Active pocket position of berberine and FASN

The figure shows the overall molecular docking diagram of berberine binding to FASN (Fig. 3A). There were five active pockets where berberine had the highest affinity with FASN (Fig. 3B–F). Supplementary Table 3 shows the Vina score, cavity size, center coordinates of the docking pocket, and size of the docking pocket along the x-, y-, and z-axes.

3.7. Effects of XLW and berberine on the body weight and tumor weight of colon cancer mice

Compared with the model group, the body weight of nude mice in the PA group increased, indicating that the PA diet can lead to lipid metabolism disorder and can promote fat accumulation. Compared with that of the PA group, the body weight of mice in each treatment group was lighter, which showed that XLW, berberine and orlistat could reduce the body weight and fat accumulation of mice, but there were no significant differences ($p > 0.05$) (Fig. 4A).

Compared with the PA group, the tumor weight of the XLW, berberine and orlistat groups decreased, and the positive drug orlistat had the best antitumor effect, but there was no statistical significance among all the groups ($p > 0.05$) (Fig. 4B). Among all berberine groups, low-dose berberine had the best antitumor effect (Fig. 4C).

3.8. Effects of XLW and berberine on fat metabolic intermediates in mice

Acetyl-CoA, NADPH and malonyl-CoA are the intermediates of the FASN-catalyzed formation of long-chain fatty acids. Compared with the model group, the feedback inhibition of acetyl-CoA after high intake of exogenous PA was statistically significant ($p < 0.05$). Compared with the PA group, high-dose berberine inhibited the level of PA in mice, resulting in the accumulation of the intermediate acetyl-CoA ($p < 0.05$). Except for the XLW group, the content

Table 1
Traditional Chinese medicine with a frequency of more than 5 times in the prescriptions of XLW (n = 31).

Herb	Family	Frequency	Compound
Coptis chinensis Franch. (Huang-Lian)	Ranunculaceae	29	Berberine, Coptisine, Palmatine, Epiberberine, Jatrorrhizine
Aucklandia costus (Falc.) Lipech. (Mu-Xiang)	Asteraceae	26	Dehydrocostus lactone, Saussurea costus
Paeonia lactiflora Pall.(Bai-Shao)	Ranunculaceae	8	Paeoniflorin, Albiflorin, Oxypaeoniflora
Terminalia chebular Retz. (He-Zi)	Combretaceae	7	Gallic acid, Chebulinic acid, Arjugenin
Myristica fragrans Houtt. Rou-Dou-Kou)	Myristicaceae	7	Myristic acid, Myristicin

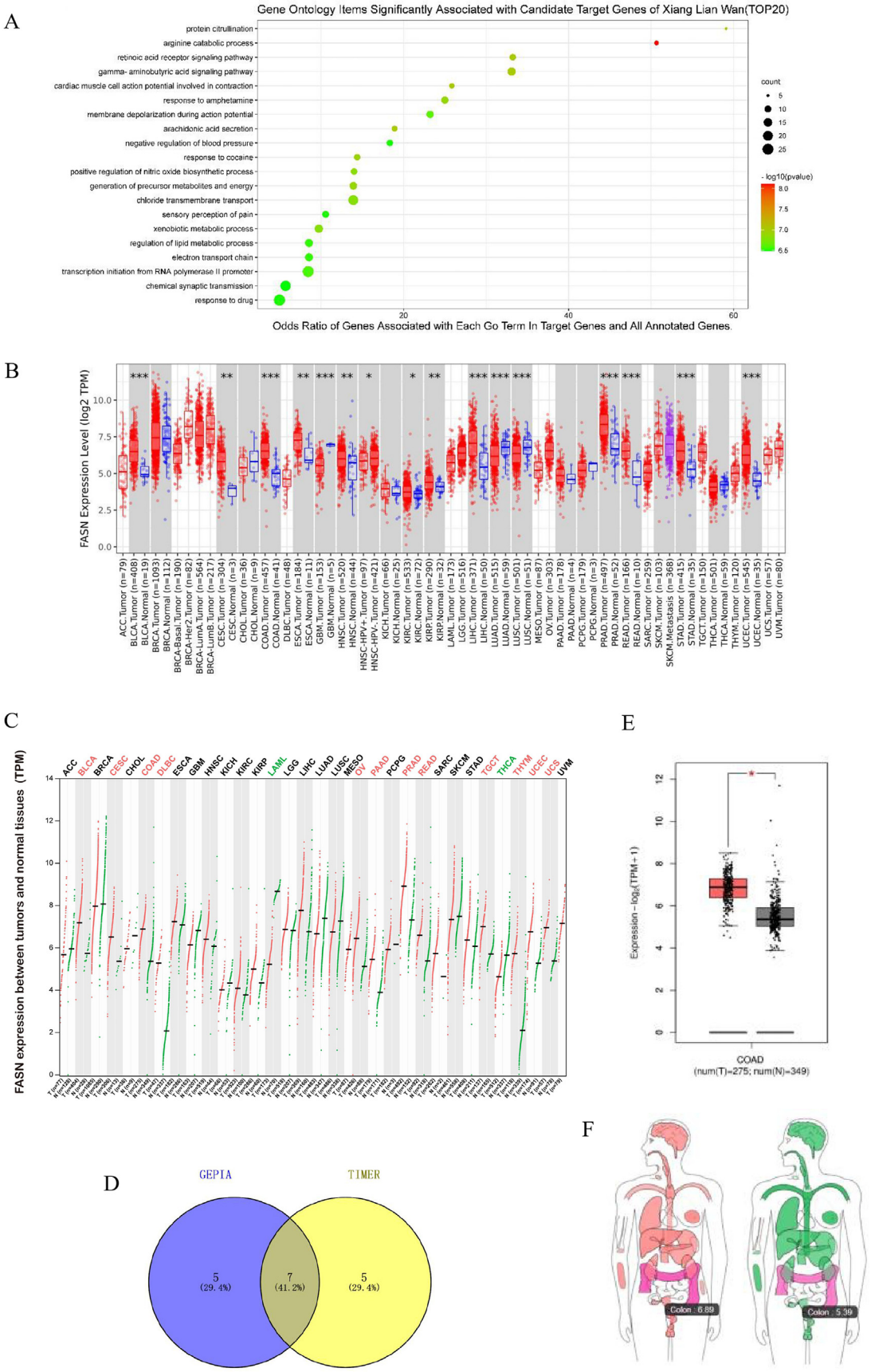


Fig. 1. (A) Gene ontology enrichment analysis of XLW. (B) Difference of FASN expression between pan-cancerous tissues and normal tissues, *p < 0.05; **P < 0.01; ***P < 0.001. (C) Gene expression profiles of all tumor samples and matched normal tissues. (D) The Wayne Diagram of GEPIA and TIMER. (E–F) Difference of FASN expression between human colonic adenocarcinoma and normal intestinal tissue (6.89:5.39). The red module represents the human COAD tissue, the gray module represents the normal intestinal tissue. The red body picture showed the tumor population, the green body picture shouwed the healthy people, *p < 0.05.

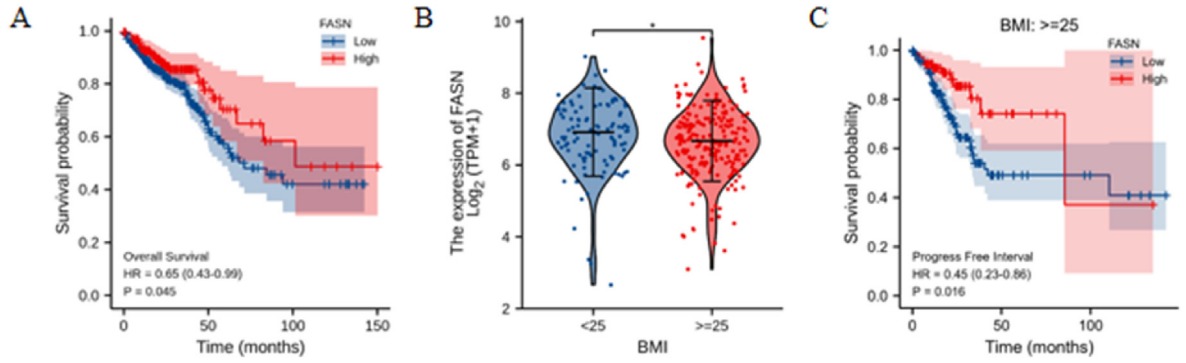


Fig. 2. (A) Survival curve of human COAD patients. Red line represents high FASN expression group, blue line represents low FASN expression group. (B) Relationship between BMI index and FASN expression, *p < 0.05. (C) Relationship between FASN expression and survival time in obese patients.

of the intermediate malonyl-CoA in the other treatment groups was lower than that in the model group ($p > 0.05$). The result indicated that it could inhibit the increase in the intermediate acetyl-CoA caused by exogenous PA intake (Fig. 4D).

3.9. Effects of XLW and berberine on blood lipids in mice with colon cancer

After inoculating colon tumors in mice, the TC decreased significantly ($p < 0.05$). Compared with the model group, PA upregulated the contents of TG, TC and LDL-C in the serum of colon cancer mice ($p < 0.01$). Compared with PA, XLW inhibited the increase in LDL-C induced by PA ($p < 0.01$), and middle-dose berberine decreased TC and LDL-C ($p < 0.01$, $p < 0.05$). High-dose berberine increased TC and decreased TG ($p < 0.01$, $p < 0.05$). Orlistat could only reduce LDL-C ($p < 0.05$), indicating that FASN was related to LDL-C (Fig. 5).

3.10. Effects of XLW and berberine on the expression of FASN and ACC in mice

Compared with the blank group, the expression of FASN in the intestinal tissue of the model group increased ($p < 0.05$), indicating that colon cancer was related to the upregulation of FASN expression in intestinal tissue (Fig. 6A). Compared with levels in the tumor group, the FASN protein in intestinal tissue and tumors decreased (Fig. 6C), the ACC protein in intestinal tissue increased and the ACC protein decreased in tumors after exogenous PA intake ($p < 0.01$, $p < 0.001$, $p < 0.001$, and $p < 0.001$). After drug intervention, under the premise of exogenous PA intake, the low, middle and high doses of berberine and orlistat reduced the expression of FASN protein in intestinal tissue ($p < 0.01$, $p < 0.01$, $p < 0.05$, and $p < 0.001$). Both low-dose berberine and orlistat reduced the expression of ACC protein in intestinal tissue ($p < 0.05$), and both low-dose berberine and orlistat reduced the expression of FASN protein in tumors ($p < 0.01$ and $p < 0.05$) (Fig. 6B). XLW; low-, medium- and high-dose berberine; and orlistat reduced the expression of ACC protein in tumors ($p < 0.01$, $p < 0.05$, $p < 0.001$, $p < 0.001$, and $p < 0.001$). Compared with levels in the XLW group, ACC protein in tumors was upregulated, and FASN protein in intestinal tissue was downregulated in the middle-dose berberine group ($p < 0.01$) (Fig. 6D).

In terms of the morphology of intestinal tissue, the intestinal barrier of the model group was destroyed, the order was disordered, and the morphology of villi was destroyed. A large number of FASN deposits could be seen at the edge of intestinal villi with PA. In the orlistat group, the deposition disappeared, the intestinal villi were neat, and the intestinal mucosal barrier was intact. In the middle-dose berberine group, the intestinal villi recovered neatly, and the positive expression decreased, but there were still brown particles on the edge of the villi. The morphology of the other drug groups was improved to some extent (Fig. 6E).

3.11. Time-dose-effect relationship of the inhibitory effect of berberine on LoVo cells

After 24 h and 48 h of berberine intervention, it was found that when berberine was ≥100 μM, the OD value began to decrease, and IR began to increase as the intervention time increased, indicating that a high concentration of berberine had a certain inhibitory effect on LoVo cells (Fig. 7A and B).

3.12. Berberine antagonizes lipid deposition in LoVo cells by inhibiting the expression of FASN protein

Compared with the blank group, low-, middle- and high-dose berberine downregulated the expression of FASN protein in LoVo cells ($p < 0.05$) (Fig. 7D).

Compared with the blank group, all the experimental groups showed reduced expression of FASN protein, and compared with the PA group, the 50 μM orlistat + PA group and 200 μM berberine + PA group showed reduced expression of FASN protein ($p < 0.05$) (Fig. 7D).

4. Discussion

XLW plays an important role in digestive system diseases, and it mainly treats intestinal damp-heat syndrome, which has a curative effect on colorectal cancer. Early perioperative colorectal cancer patients showed mainly damp-heat syndrome,[16] so there is theoretical support for XLW in the treatment of colon cancer. XLW has undergone thousands of years of evolution, its prescription is exquisite, and the curative effect is clear. This study found that the drug pairing with the highest frequency of compatibility with traditional Chinese medicine was consistent with the composition of XLW in the Pharmacopoeia. This showed that the prescription development of XLW had inheritance and continuity. The toxicological experiment on the effective components of XLW showed that XLW in the normal dose range is safe to use.[17] Experimental pharmacological studies have confirmed that the effective and main component of XLW is berberine.[18,19] In this study, the gene ontology functional enrichment analysis of XLW showed that XLW could regulate the lipid metabolism pathway. A meta-analysis showed that berberine is effective and safe in the treatment of

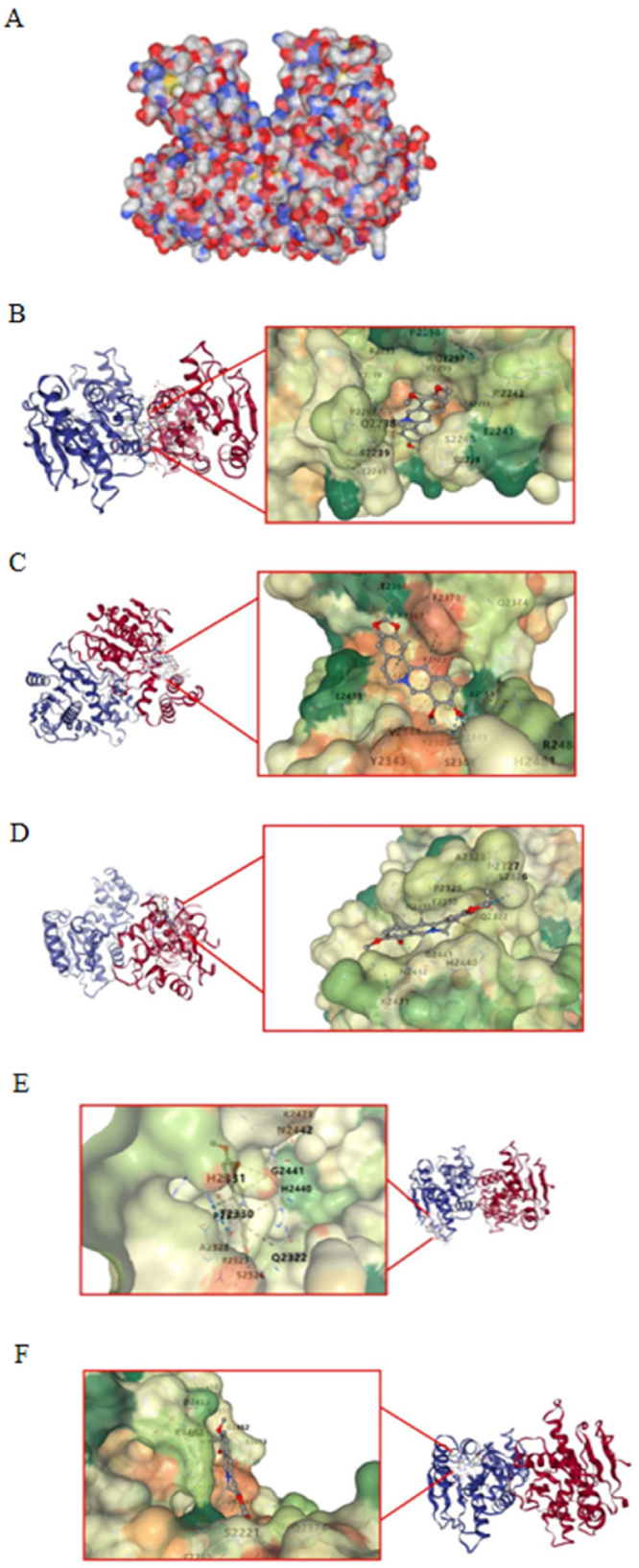


Fig. 3. (A) Overall diagram of molecular docking. (B–F) Specific docking of berberine with FASN.

dyslipidemia.[20] In summary, berberine, the key component of XLW, is closely related to the regulation of lipid metabolism.

FASN is the only enzyme in the human genome that can synthesize palmitate ab initio using acetyl-CoA, malonyl-CoA and NADPH. It is the key enzyme in the synthesis of fatty acids. The results of this study showed that the expression of FASN in many kinds of cancer tissues was higher than that in normal tissues, and the difference was also significant in COAD. It is suggested that the high expression of FASN is an unfavorable factor, which is consistent with the conclusion of abnormally high expression in tumor tissues. While the expression of FASN in COAD patients with BMI< 25 was higher than that in obese colon cancer patients (BMI≥25). A large cohort study of 647 patients showed that non-obese COAD patients' over-expression of FASN in tumors led to their increasing survival time. While the moderately overweight or obese patients (BMI≥27.5 kg/m^2), the over-expression of FASN might predict worse outcomes.[21] Obesity is an unfavorable factor in the incidence of tumors, but it may be beneficial in the treatment and development of tumors. It has been found epidemiologically that about 10%–20% of deaths in cancer patients are attributed to malnutrition or malignancy rather than to the tumor itself.[22,23] The expression of FASN in obese COAD patients is relatively low compared with the normal weight patients, which may be due to the inhibition of catalytic enzyme synthesis by negative feedback after the substantial accumulation of lipids. As the high expression of FASN promotes lipid accumulation, patients with higher body weight may be less likely to develop cachexia caused by treatment and the tumor itself. Chan[24] used chemical-shift-encoded magnetic resonance imaging (CSE-MRI)[25] to study the effects of lipid components around breast cancer on the proliferation and differentiation of tumor cells. The results showed that the distribution of lipids was related to the differentiation and proliferation of tumor cells. There has not been a definitive statement on the study of the relation between lipid metabolism and the tumor microenvironment. Being of normal weight may be beneficial for COAD patients in the short term but disadvantageous in the long term because they might meet the problems such as nutritional deficiencies. A certain level of fat may help patients achieve a better quality of life, which was showed in the results above. In summary, FASN may be an important target in the pathogenesis of colon cancer, which has significant value for research. In addition, the structural bioinformatics molecular docking results suggested that berberine had an active pocket for docking with FASN. A new randomized, double-blind, placebo-controlled clinical trial published in the Lancet in 2020 showed that berberine can prevent the recurrence of adenomas in colorectal precancerous diseases and reduce the risk of tumor recurrence by 23%, with good safety and few adverse reactions.[26] In conclusion, this study provides a basis for the feasibility of berberine in the treatment of colorectal cancer by regulating FASN.

A mouse model of orthotopic colon cancer was established and treated with a PA diet. After intragastric administration of XLW and low, middle and high doses of berberine in mice with colon cancer, symptoms of constipation and abdominal distension were observed, which was related to the antidiarrheal effect of XLW and confirmed that berberine was the effective component of XLW. A modern pharmacological study revealed that berberine reduces blood lipids at the gene transcription level by stabilizing the 3′UTR of low-density lipoprotein receptor mRNA.[27] The experimental results showed that both XLW and berberine had antitumor effects and could effectively LDL-C and increase HDL-C, but contrary to expectations, the TGs of the XLW group and high-dose berberine group and the TCs of berberine groups and XLW group increased. From the view of traditional Chinese medicine, the Muxiang in XLW invigorates the spleen and eliminates food and can enhance the appetite of patients. Besides, XLW showed great effect on

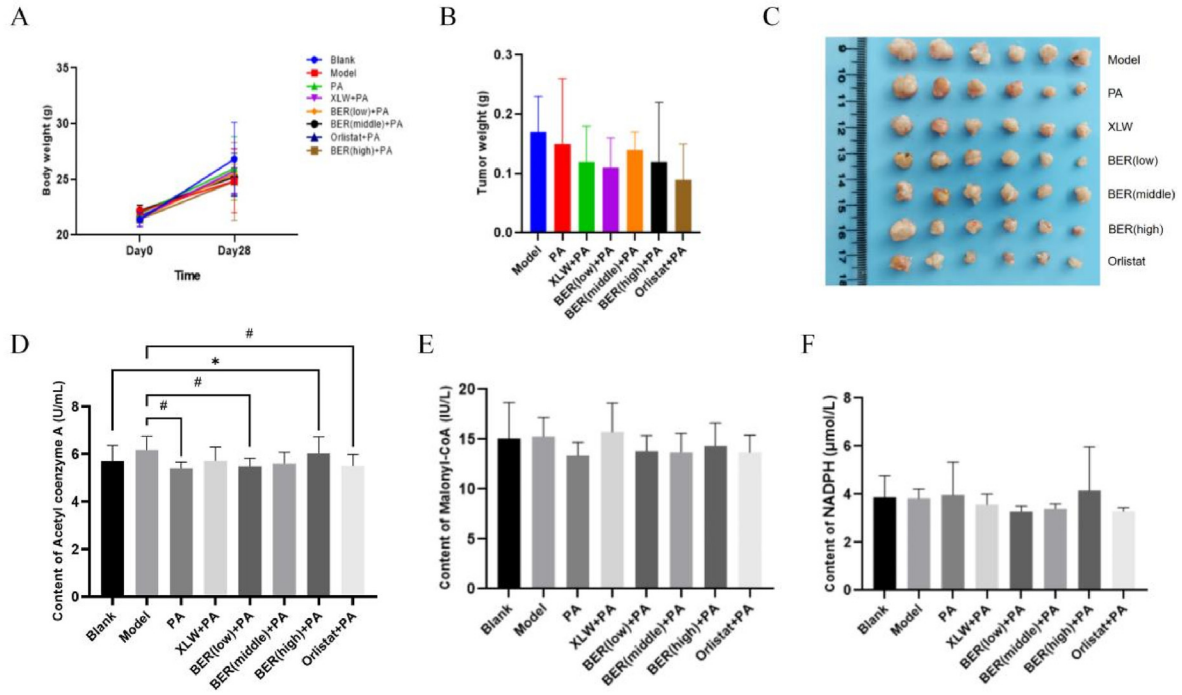


Fig. 4. (A) Effects of XLW and berberine on body weight of mice (n = 6). (B) Effects of XLW and berberine on tumor quality of mice (n = 6). (C) The tumor of each group of mice. (D–F) Effects of XLW and berberine on adipose metabolic intermediates in mice (n = 6). Compared with the PA group, *p < 0.05; compared with the model group, #p < 0.05.

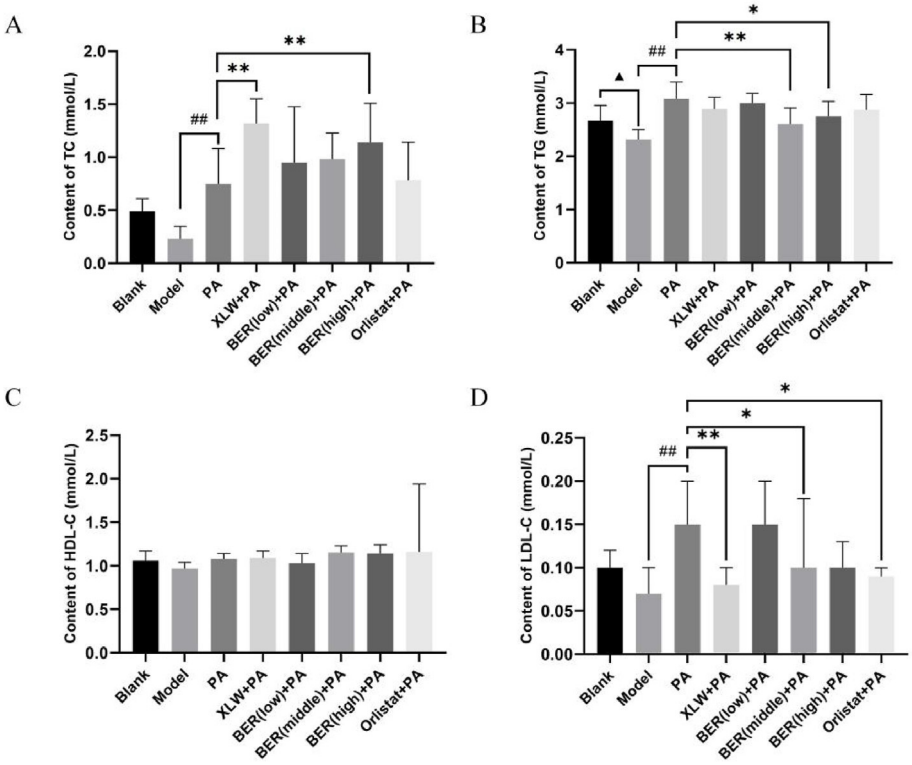
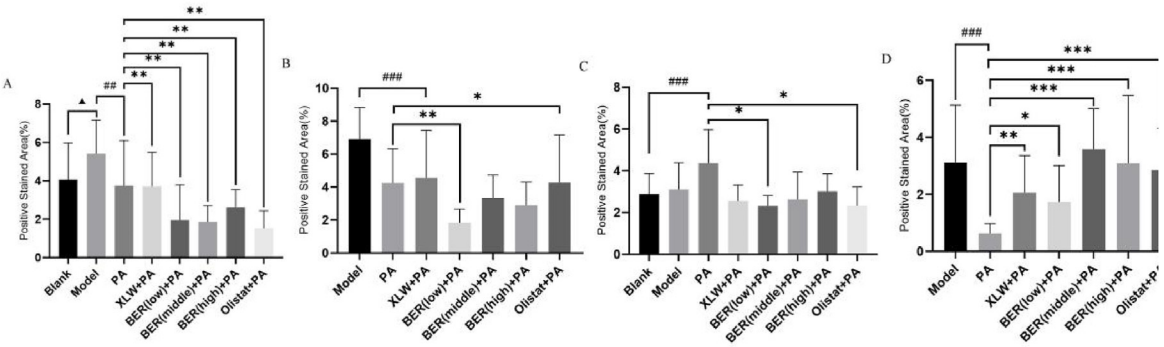


Fig. 5. Effects of XLW and berberine on blood lipids in mice (n = 6), compared with the blank group, ▲ p < 0.05; compared with the model group, ## p < 0.01; compared with the PA group, *p < 0.05, **p < 0.01.

improving the function of digestive system[28] and berberine can also reduce the inflammatory reaction caused by the gut. Compared with the other groups, the mice in the XLW group showed an increase in food intake during the experiment, and the increase in TGs and TCs might be related to excessive consumption of the PA diet. Some studies have shown that PA can induce the accumulation


A
B
C
D
Positive Stained Area(%)
Blank
Model
PA
XLW+PA
BER(low)+PA
BER(middle)+PA
BER(high)+PA
Olistat+PA

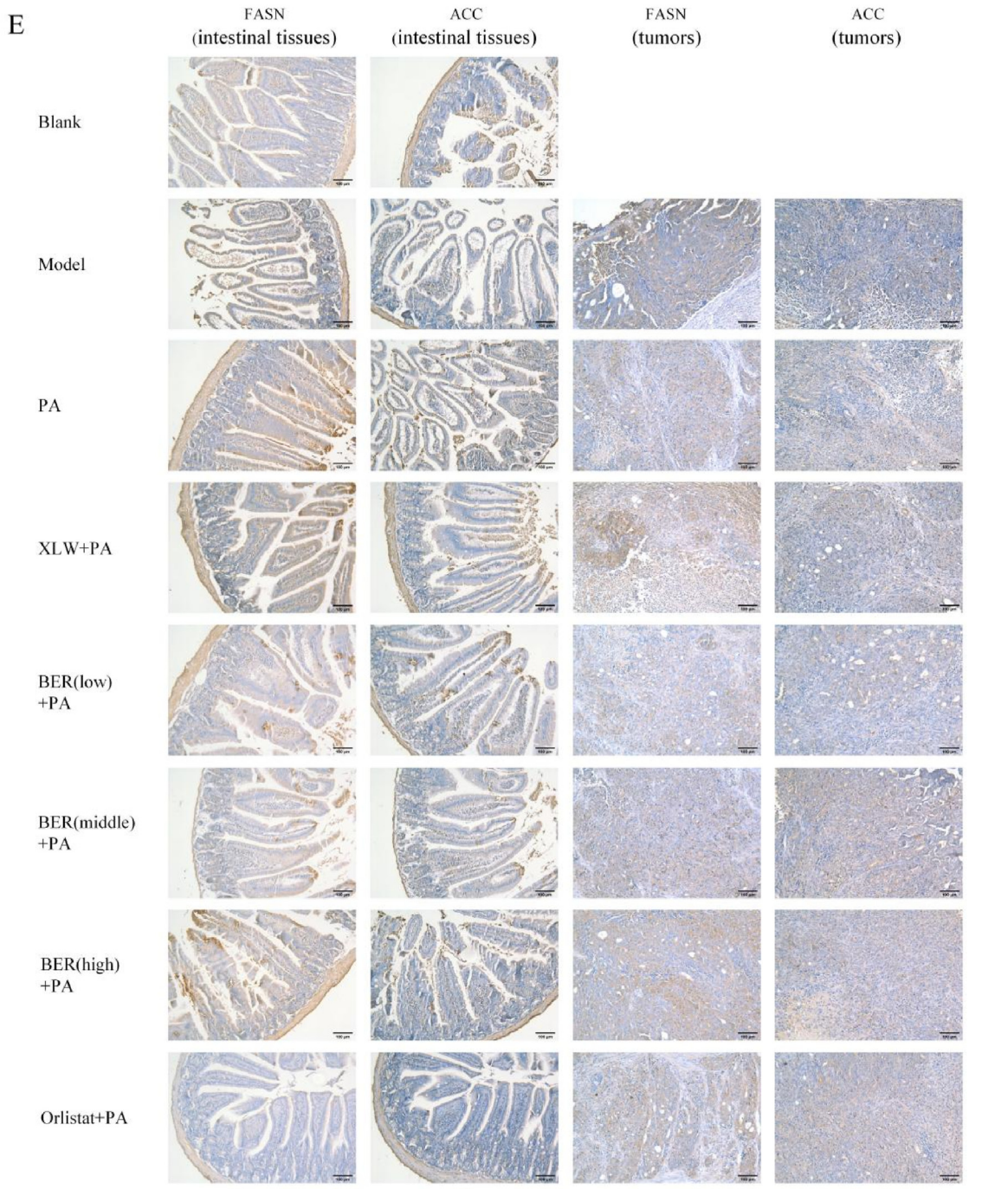

E
FASN (intestinal tissues)
ACC (intestinal tissues)
FASN (tumors)
ACC (tumors)
Blank
Model
PA
XLW+PA
BER(low) +PA
BER(middle) +PA
BER(high) +PA
Orlistat+PA

Fig. 6. Effects of XLW and berberine on the expression of FASN and ACC protein in tumor and intestinal tissues of mice. (A) Expression of FASN in intestinal tissue. (B) Expression of FASN in tumors. (C) Expression of ACC in intestinal tissues. (D) Expression of ACC in tumors. Compared with the blank group, ▲ $p < 0.05$; compared with the model group, ## $p < 0.01$; compared with the PA group, *$p < 0.05$,**$p < 0.01$,***$p < 0.001$. (E) Distribution of FASN and ACC proteins in intestinal and tumor tissues of mice (immunohistochemical staining, × 200).

of TGs in liver cancer cells, which might explain the increase in TGs in the XLW group.[29] The increase in TGs in the high-dose berberine group might be due to the compensatory increase in TGs caused by tumors. A study[30] assessed the composition of fatty acids in cells exposed to hypoxia by gas chromatography and it was found that the content of palmitic acid increased significantly during hypoxia. This reflects the increase of exogenous SFA uptake and storage in tumor tissue induced by hypoxia microenvironment.And a report[31] proved that berberine and magnolol up-regulate *ABCA1* mRNA expression and promote intracellular cholesterol efflux, indicating the increase of TCs.

A long-term high-fat diet can lead to the occurrence and development of colorectal cancer, which is related to significant changes in lipid composition and metabolism of cancer cells.[32,33] Some authors have reported overexpression of FASN.[21,34] This study found that XLW and berberine could downregulate the protein expression of FASN in tumors. The paradox was that XLW and berberine downregulated the expression of ACC protein in intestinal tissue but upregulated the expression of ACC protein in tumors. This opposite trend may be related to the fact that XLW and berberine can increase the expression of ACC protein in tumors while maintaining their original function in normal tissues. Knockout of FASN is associated with inhibition of colorectal cancer cell proliferation.[35] In some tumor types, including COAD, FASN expression increases with tumor progression and is associated with disease invasion and metastasis and decreased survival and response to classic chemotherapeutic drugs.[36,37]

The synthesis of PA is a complex process involving a repeated addition reaction mediated by acetyl-CoA and malonyl-CoA under the catalysis of FASN. The original idea of this study was that PA could upregulate the expression of FASN, while berberine and orlistat could antagonize it. However, PA downregulated the expression of FASN. There were studies[38–40] demonstrated that PA could inhibit hepatic cell carcinoma progression through restraining glucose uptake and energy metabolism and it could also inhibit proliferation through autophagy mechanism in cancer cells. A

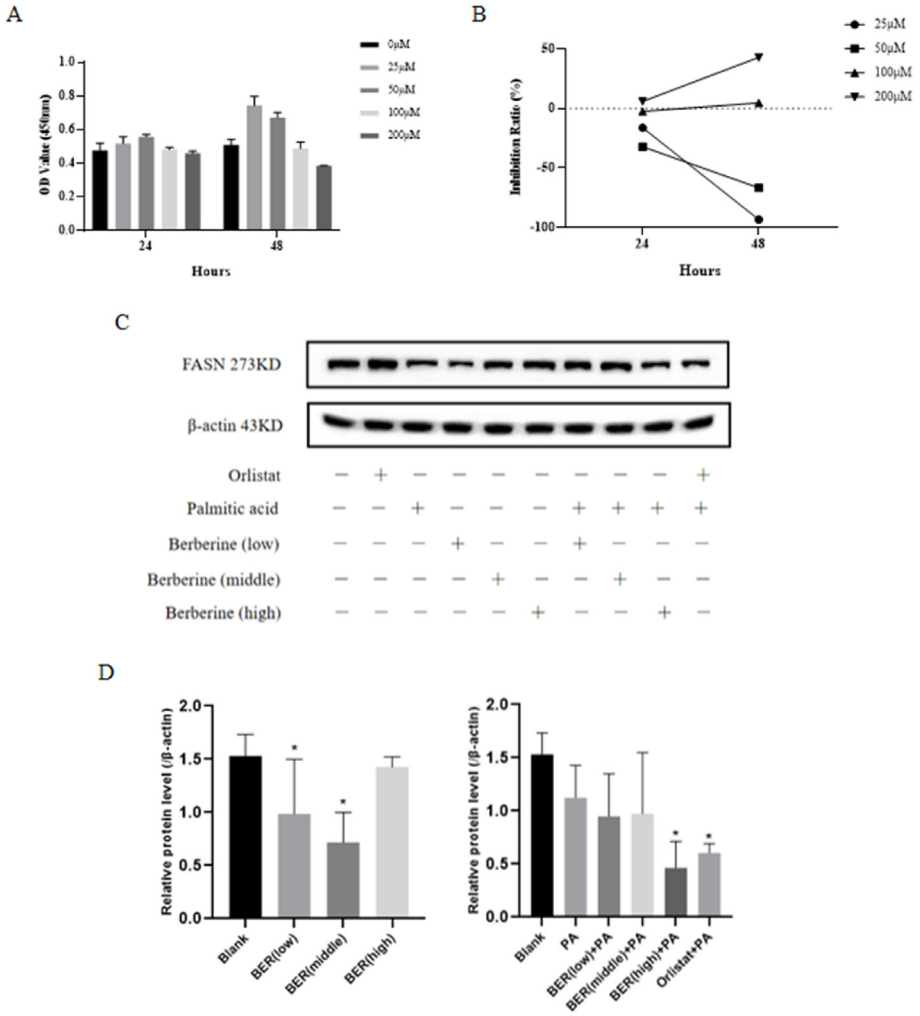


Fig. 7. (A) Effects of different dose berberine on the viability of LoVo cells. (B) Inhibitory effect of different dose berberine on LoVo cells at different time. (C) Expression of FASN protein in LoVo cells. (D) Effects of PA, berberine and orlistat on FASN protein expression in LoVo cells; PA = 62.5 μM; berberine (low) = 100 μM; berberine (middle) = 150 μM; berberine (middle) = 200 μM; orlistat = 50 μM, compared with blank group, *$P < 0.05$.

report also proved that PA could inhibit tumor growth in HT29 cell and SW-620 cell model mice. All these studies showed the potential anti-tumor effect of PA. Peciuliene[41] found that malnutrition hypoxia enhances variable splicing of fatty acid synthase (FASN) pre-m RNA and reduces total FASN m RNA and protein levels. This further reduces the de novo synthesis of fatty acids. In this study, we speculate that the excessive intake of exogenous PA may destroy the function of cell regulation of fat intake and consumption, leading to the disorder of FASN, which might explain why PA can downregulate FASN expression. Berberine can further inhibit the expression of FASN protein on the basis of PA, which may be the reason for its inhibition of LoVo cell proliferation. On the basis of animal experiments, cell experiments further verified that berberine, an effective monomer of XLW, inhibited the proliferation of LoVo cells by downregulating the expression of FASN.

5. Conclusion

This study proved that activating the ACC/FASN signaling pathway can promote the occurrence and development of colon cancer. XLW and berberine exert an antitumor effect by regulating the ACC/FASN lipid metabolism-related signaling pathway, which has certain clinical significance. However, further studies are needed to understand the synergistic effect between berberine and PA.

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Declaration of competing interest

All the listed authors have read and approved the submitted manuscript. The authors declare that there is no conflict of interest.

List of Abbreviations

Abbreviation	*Full Name*
XLW	Xianglian Wan
ACC	Acetyl CoA carboxylase
Acetyl-CoA	Acetyl coenzyme A
BSA	albumin from bovine serum
PBS	phosphate buffer saline
BLCA	Bladder urothelial carcinoma
BMI	Body mass index
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
COAD	Colon adenocarcinoma
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	Esophageal carcinoma
FASN	Fatty acid synthase
GBM	Glioblastoma multiforme
HDL-C	High density lipoprotein-cholesterol
HNSC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
IR	Inhibition rate
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LDL-C	Low density lipoprotein-cholesterol
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
Malonyl-CoA	Malonyl coenzyme A
NADPH	Nicotinamide adenine dinucleotide phosphate
OV	Ovarian serous cystadenocarcinoma
PA	Palmitic acid
PDB	Protein data bank
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
STAD	Stomach adenocarcinoma
TC	Total cholesterol
TCGA	The cancer genome atlas
TG	Triglyceride
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.05.008.

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