Anticancer bioactive peptide combined with docetaxel and its mechanism in the treatment of breast cancer

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Abstract. Breast cancer remains a worldwide public-health issue. Novel drugs that increase the sensitivity and reduce the toxic side effects of chemotherapeutic agents are urgently required. The present study investigated the effect and mechanism of the short-term intermittent administration of an anticancer bioactive peptide (ACBP), docetaxel (DTX), ACBP combined with DTX (MIX) and ACBP combined with low dose DTX (L-MIX) to nude mice bearing human breast cancer tumors. The body weight, tumor length, tumor diameter, diet and water consumption of the tumor-bearing nude mice were calculated. The protein and mRNA expression levels of p53, p21 and Ki67 were detected via immunohistochemistry and reverse transcription-quantitative PCR, respectively. The results revealed that the activity level of each group of mice was consistent. However, the food and water consumption of the ACBP group was significantly increased compared with the NS group. Compared with the normal saline group, the tumor weights and volumes of the treatment groups were significantly decreased, indicating an inhibitory effect of the treatment. However, the MIX group exhibited lower tumor weights and volumes compared with the ACBP and DTX groups. Furthermore, no significant cell necrosis, edema or inflammatory cell infiltration was observed upon hematoxylin & eosin staining of the liver and spleen in all groups. The results also revealed that the p21, p53 and Ki67 protein and mRNA levels were decreased in the ACBP, DTX and MIX groups compared with the control group. Additionally, when compared with those in the MIX and L-MIX groups, the p21 and Ki67 protein, and p53 and Ki67 mRNA levels in the ACBP and DTX groups were significantly increased. The results suggested that the short-term intermittent use of ACBP alone had an inhibitory effect on tumor growth and improved the food and water consumption of tumor-bearing nude mice. Furthermore, the combination of ACBP and DTX reduced toxic side effects and the dosage requirement of drugs to achieve therapeutic effects on the tumor-bearing nude mice. Therefore, the antitumor effect of ACBP may be associated with the improvement of immune function in tumor-bearing nude mice and ACBP may serve an antitumor role via the p53-p21 signaling pathway in breast cancer.

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Introduction

The incidence of breast cancer is increasing and shifting to younger populations worldwide, becoming the main cause of cancer-associated death in females (1). In 2012, there were an estimated 1.66 million new breast cancer cases (25% of all cancer cases) and 521,000 breast cancer-associated deaths (2). In 2015, breast cancer accounted for ~15% of newly diagnosed cancer cases in China (3). In Western countries, breast cancer primarily occurs in 55-60-year-old women, but the trend of onset in China is significantly earlier, occurring primarily in 45-55-year-old middle-aged and young women (4). The occurrence of breast cancer in younger women has threatened the work, quality of life and health of those affected (5). Currently, the main treatment strategies for breast cancer are surgery, radiotherapy, chemotherapy, endocrine therapy and molecular-targeted therapy, which are all auxiliary (6). However, the side effects of chemotherapy in patients remain serious, with 39.7% of patients suffering from neutropenia and infection (7). The cardiotoxicity of chemotherapeutic drugs is a well-known major adverse reaction affecting the quality of life and mortality of patients with cancer (8). Anticancer drugs have been reported to cause various types of cardiac toxicity, including heart failure, bradycardia, prolonged QT intervals, myocardial ischemia and cardiomyopathy (9). Neurotoxicity is characterized by numbness and tingling of the fingers and toes, as well as myalgia and myasthenia (10). Due to the aforementioned toxic and adverse effects of chemotherapeutic drugs, a number of patients have to reduce their dosage and prolong the chemotherapy cycle (11). Furthermore, chemotherapy cessation leads to treatment failure (11). While prolonged survival remains the primary goal of chemotherapy, symptom relief and

quality of life are also important treatment considerations (12). Therefore, bioactive peptides are rapidly being developed as potential anticancer drugs, which may reduce the side effects and increase the sensitivity of traditional chemotherapeutic drugs without altering their anticancer effect (13).

A wide range of bioactive peptides exist in natural resources, including specific small molecular protein fragments that, although inactive within the protein sequence, can be released during proteolysis or fermentation and are activated by the digestive, endocrine, cardiovascular, immune and nervous systems, which serve as important roles in human health (14). Several peptides released from animal proteins have been reported to have different health effects *in vitro* and *in vivo*, including antimicrobial properties, blood pressure reduction, cholesterol reduction, antithrombotic and antioxidant activity and opioid-like activity (14-16). These peptides have also been reported to enhance mineral absorption and bioavailability, exhibit cellular and immunomodulatory effects and exhibit antiobesity and antigenotoxic activities (14-16). Bioactive peptides have low immunogenicity, excellent tissue penetration, low production costs and are easy to modify to enhance their stability and biological activity within the body, making these molecules ideal candidates for cancer therapy (17). The anticancer bioactive peptide (ACBP) used in the present study is a low molecular weight bioactive substance extracted from goat spleen following induced immunization (relative molecular weight, <8000 Da; patent no. ZL961222236.0), which is a novel method of anticancer biological preparation. A previous study has reported that ACBP inhibits tumor angiogenesis, regulates protein degradation, interferes with DNA synthesis, regulates the cell cycle, induces apoptosis and influences further antitumor mechanisms (18). A large number of previous cell- and animal-based experiments reported that ACBP served an inhibitory effect on the BGC-823 and MGC-803 human gastric cancer cell lines, the MKN-45 leukemia cell line, the H-22 hepatoma cell line, the CNE nasopharyngeal carcinoma cell line and the GBC-SD gallbladder cancer cell line (19-21). Collectively, these aforementioned studies suggested that ACBP may be a potential tumor stem cell-targeted drug and when combined with chemotherapeutic drugs, ACBP may effectively improve their therapeutic efficacy and reduce their toxicity in patients (22).

Docetaxel (DTX) is an effective anticancer agent that is widely used and has demonstrated extensive anticancer activity against breast, lung, pancreatic, prostate, ovarian and head and neck cancer (23-26). DTX is one of the most commonly used chemotherapy drugs for breast cancer (23). DTX binds to the β-subunit of microtubule proteins, leading to stable and non-functional microtubule formation by promoting polymerization and inhibiting decomposition, ultimately resulting in mitosis arrest and apoptosis induction (10). Therefore, the present study investigated the effect of the intermittent short-term application of ACBP and ACBP combined with DTX, on the quality of life of nude mice bearing human breast cancer tumors. Furthermore, the expression of p53, p21 and Ki67 were assessed. The effect of ACBP on the human breast cancer cell line MDA-MB-231 in nude mice, as well as the toxicity-reducing and sensitivity-increasing mechanisms of ACBP were also studied.

Materials and methods

Cell lines and mice. All animal experiments were approved by the Ethics Committee for Animal Experiments of Inner Mongolia Medical College (approval no. YKD2016152). A total of 40 female Balb/c-Nu nude mice (age, 4-6 weeks; weight, 16±2 g) of specific pathogen-free grade were used. The animals were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. [license no. SCXK (Beijing) 2012-0001]. The human breast cancer cell line MDA-MB-231 was purchased from the China Infrastructure of Cell Line Resources, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

Establishment of an axillary-transplanted tumor model using the human breast cancer cell line MDA-MB-231 in nude mice. The human breast cancer cell line, MDA-MB-231, was cultured in DMEM (Thermo Fisher Scientific, Inc.) supplemented with 10% fetal calf serum (HyClone; GE Healthcare Life Sciences) and 1% penicillin-streptomycin solution (Thermo Fisher Scientific, Inc.) in a humidified atmosphere of 5% CO_2 at 37˚C. The cells in the logarithmic growth phase were selected for further experiments. A single-cell suspension of $5x10^7$/ml was used for injection.

Mice were maintained at 20-25˚C with 40-70% humidity and free access to food and drinking water. Animals were also housed under a 12 h light/dark cycle. Eating, feeding and operating procedures strictly followed aseptic principles. After 3 days of free access to food in nude mice, a 0.1 ml single-cell suspension was inoculated into the right axilla of the 40 nude mice. The activity and tumorigenesis of the nude mice were observed daily. At 6 days post-injection, rice-sized nodules were identified at the injection site of the nude mice and the tumor formation rate was 100%. Therefore, the breast cancer model was successfully established in nude mice.

Experimental grouping, administration and observation records. The 40 nude mice were randomly divided into five groups (n=8) as follows: Normal saline group (NS), anticancer bioactive peptide group (ACBP), docetaxel group (DTX), ACBP combined DTX treatment group (MIX) and ACBP combined with low dose DTX treatment group (L-MIX). The treatments were administered by tail vein injection twice a week for a total of 3 weeks. The NS group was administered 0.4 ml saline, the ACBP group was administered 0.4 ml ACBP (70 mg/ml; Prepared by The Clinical Medical Research Center, Affiliated Hospital of Inner Mongolia Medical University), the DTX group was administered 5 mg/kg DTX (0.5 ml/20 mg; Prepared by Jiangsu Hengrui Pharmaceutical Co., Ltd.; Chinese medicine standard no. H20020543), the MIX group was administered 0.4 ml ACBP combined with 5 mg/kg DTX, and the L-MIX was administered 0.4 ml ACBP combined with 2.5 mg/kg DTX. The activity status of the nude mice was observed daily. The weight and tumor boundaries were measured every other day to calculate the tumor volume (V) using the following formula: $V=ab^2/2$, where a is tumor length and b is the shortest tumor diameter. Food and water intake were measured every 2 days to calculate the quantity consumed. After 3 weeks, the retro-orbital blood of nude mice were collected following anesthesia and the nude mice were

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Sequence (5'→3')	
	Forward	Reverse
p53	TCAACAAGATGTTTTGCCAACTG	ATGTGCTGTGACTGCTTGTAGATG
p21	AAACTTTGGAGTCCCCTCAC	AAAGGCTCAACACTGAGACG
Ki67	CTTGCCTCCTAATACGCCTCTC	CCTGACTCTTGTTTTCCTGATGGT
GAPDH	TCCACCACCCTGTTGCTGTA	ACCACAGTCCATGCCATCAC

subsequently euthanized by cervical dislocation. The tumor tissues, livers and spleens of the nude mice were isolated and weighed. The isolated tissues were divided into two parts: One was fixed in 4% paraformaldehyde for 24 h at 25˚C, and the other was stored at -80˚C until further analysis.

Determination of the liver and spleen coefficients. Following sacrifice, the body weights of the nude mice were measured. The livers and spleens were removed from the mice, dried with filter paper and were then weighed. The liver and spleen coefficients were calculated as follows: Liver coefficient=liver mass/nude mouse body mass and spleen coefficient=spleen weight/body mass of nude mice.

Paraffin-embedded tissue sections and hematoxylin & eosin staining. Tissues fixed in 4% paraformaldehyde were trimmed to a size of 5x5x3 mm^3, placed in an embedding box, labeled and slowly flushed with flowing water overnight at 25˚C. The tumor tissue was dehydrated with an ascending ethanol series in 30 min increments as follows: 50% ethanol, 60% ethanol, 70% ethanol, 80% ethanol, 95% ethanol I, 95% ethanol II, 95% ethanol I and anhydrous ethanol II at 25˚C. The liver tissue was dehydrated with an ascending ethanol series as follows: 50% ethanol for 15 min, 60% ethanol for 15 min, 70% ethanol for 15 min, 80% ethanol for 15 min, 95% ethanol I for 30 min, 95% ethanol II for 30 min, 95% ethanol I for 30 min and anhydrous ethanol II for 30 min at 25˚C. Furthermore, the spleen tissue was dehydrated with an ascending ethanol series as follows: 50% ethanol for 10 min, 60% ethanol for 10 min, 70% ethanol for 10 min, 80% ethanol for 10 min, 95% ethanol I for 30 min, 95% ethanol I for 30 min, anhydrous ethanol II for 30 min and anhydrous ethanol II again for 30 min at 25˚C. The tissue sections were deparaffinized using xylene I for 20 min and xylene II for 20 min at 37˚C. Subsequently, the wax block was cut into 4-μm-thick slices for hematoxylin & eosin staining for 2 h at 25˚C.

p53, p21 and Ki67 protein expression in transplanted tumor tissues as detected by immunohistochemistry. Streptavidin-peroxidase immunohistochemistry was performed to analyze the protein expression of p53, p21 and Ki67 in transplanted tumor tissues. For dewaxing (xylene I for 10 min and xylene II for 10 min), hydration and antigen repair (1,000 ml citrate buffer), the fragments were heated at 60˚C for 1 h until the wax melted. Paraffin embedded sections were then rehydrated using a descending alcohol series. Subsequently, the endogenous peroxidase/phosphatase activity was blocked by incubating sections with 3% H_2O_2 at room temperature for 9 min. Sections were then treated with the following primary antibodies, washed three times for 3 mins with PBS and placed in a wet box overnight at 4˚C: p53 (1:50; Abcam; cat. no. ab32049), p21 (1:50; Cell Signaling Technology, Inc.; cat. no. 2946) and Ki-67 (1:150; Abcam; cat. no. sc-9976). Subsequently, secondary antibodies (1:50; Abcam; cat. no. ab205718) were added to the slides for 30 min at room temperature. PBS was added to the sections for 3 min at 25˚C to wash away the DAB (Abcam; cat. no. ab64238) chromogenic agent and subsequently, the slides were counterstained with hematoxylin & eosin for 5 min at 25˚C. The sections were slowly rinsed with running water for 5 sec, and 1% ethanol hydrochloride was mixed 10 times for cell differentiation. The samples were then rinsed with tap water for 30 sec. Subsequently, the samples were observed under a light microscope (magnification, x100) for statistical analysis by a semiquantitative scoring system. The staining intensity was scored as follows: No staining, 0; light yellow, 1; brown, 2; or tan, 3. Four high power visual fields were randomly selected and the percentage of positive cells was determined as follows: No positive cells, 0; positive cells <25%, 1; positive cells <25-49%, 2; and positive cells >50%, 3. The final score was calculated as staining intensity x percentage of positive cells. Scores <3 were defined as negative and scores ≥3 were defined as positive (27).

Detection of p53, p21 and Ki67 mRNA expression in transplanted tumor tissues by reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the tumor tissues using TRIzol® reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Pectrophotometric quantification was utilized to determine RNA purity using an OD ratio of 260 nm/280 nm and a BeekmanDU 800 UV spectrophotometer. A total of 1 mg total RNA was reverse transcribed into first-strand cDNA using the Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. qPCR was performed using the Taq-Man™ Gene Expression assay (Applied Biosystems; Thermo Fisher Scientific, Inc.) on an Mx3000P real-time PCR system (Agilent Technologies, Inc.) according to the manufacturer's instructions. The primer pairs used in the present study were designed and synthesized by Sangon Biotech Co., Ltd. (Table I). The following thermocycling conditions were used: Initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 10 sec, annealing at 60˚C for 30 sec, elongation at 60˚C for 30 sec and a final extension step at 60˚C for 40 sec. mRNA levels were normalized to the

internal reference gene GAPDH. $^{\Delta\Delta}$CT values of each group were calculated separately=[(CT experimental target gene - CT internal reference target gene)-(CT control target gene-CT internal reference control gene)]. mRNA relative expression was calculated using $2^{-\Delta\Delta Cq}$ values (28).

Statistical analysis. Statistical analyses were performed using SPSS software (version 19.0; IBM Corp.). Data are presented as the mean ± standard deviation. Data containing two samples were analyzed using a Student's t-test. Comparisons in data-sets containing >3 groups were evaluated by one-way ANOVA followed by Bonferroni post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Food intake, water consumption and body weight of tumor-bearing nude mice. The levels of food intake and water consumption of the nude mice in each group exhibited significant differences. The ACBP group exhibited no significant dietary intake (P>0.05) and decreased drinking water consumption (P<0.01) compared with the NS group (Fig. 1A and B). In addition, the dietary intake and drinking water consumption were significantly reduced in the DTX and MIX groups compared with the NS group (P<0.05; Fig. 1A and B). Furthermore, there was a statistically significant decrease in water consumption in the L-MIX group compared with the NS group (P<0.05). Each treatment group exhibited no significant difference in body weight compared with the NS group (Fig. 1C).

Tumor growth in nude mice. The treatment groups exhibited significantly decreased tumor weights and volumes compared with the NS group, indicating that the treatments had an inhibitory effect (Fig. 2A and B). The tumor weight of the NS, ACBP, DTX, MIX and L-MIX groups were 1.10±0.36, 0.78±0.16, 0.71±0.17, 0.47±0.20 and 0.66±0.24 g, respectively (Fig. 2A). The tumor weights were significantly decreased in the DTX, MIX, L-MIX (P<0.05) and ACBP groups (P<0.01) compared with the NS group (Fig. 2A). Furthermore, the tumor volumes of the NS, ACBP, DTX, MIX and L-MIX groups were 1.82±0.70, 0.77±0.24, 0.65±0.29, 0.42±0.24 and 0.61±0.26 mm^3, respectively (Fig. 2B). The tumor volumes in the ACBP and DTX groups were significantly reduced compared with the NS group (P<0.05; Fig. 2B). Additionally, the tumor weights and volumes were lower in the MIX group, although not significantly different, compared with the ACBP and DTX groups, which suggested that ACBP not only exerted an inhibitory effect on the tumors alone, but also acted synergistically with DTX (Fig. 2A). The maximum tumor diameter observed was ~10 mm and the maximum tumor volume observed was ~2.5 mm^3, both in the NS group (Fig. 2C). Therefore, the results suggested that ACBP inhibited tumor growth in breast cancer.

Liver and spleen tissue structure and coefficients in nude mice. The liver coefficients of the treatment groups exhibited no significant differences compared with the NS group (Fig. 3A). However, the ACBP group displayed a significantly higher spleen coefficient compared with the NS group (P=0.041; Fig. 3B). Furthermore, the spleen coefficients of the DTX, MIX and L-MIX groups were not significantly different compared with the NS group (Fig. 3B). Hematoxylin & eosin staining of the liver and spleen revealed no marked cell necrosis, edema or inflammatory cell infiltration in all groups (Fig. 3C and D).

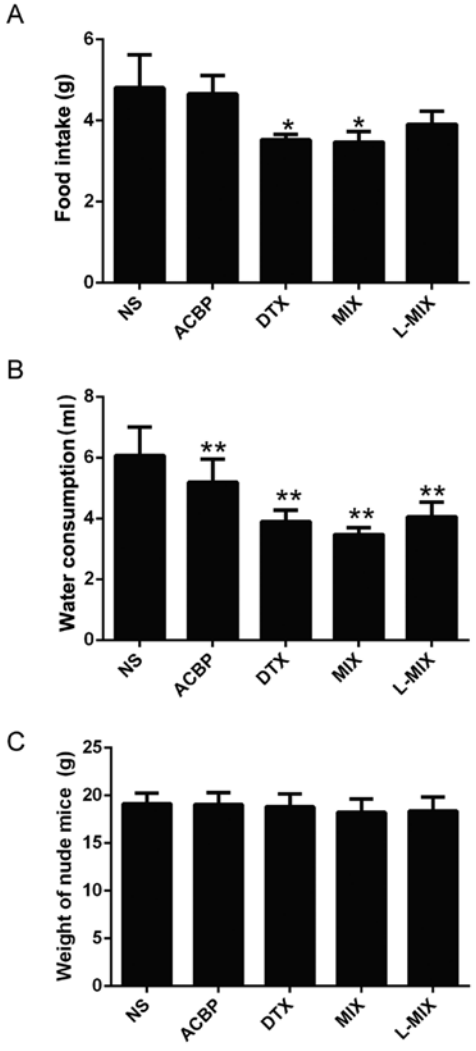

Figure 1. Behavioral characteristics and weight of tumor-bearing nude mice. The (A) food intake, (B) drinking water consumption and (C) weight of nude mice were calculated. *P<0.05 and **P<0.01 vs. the NS group. NS, normal saline; ACBP, anticancer bioactive peptide; DTX, docetaxel; MIX, ACBP combined with DTX; L-MIX, ACBP combined with low dose DTX.

p21, p53 and Ki67 immunohistochemical scores in tumor tissues from nude mice. Immunohistochemical staining displayed lower Ki67 protein expression in the ACBP, DTX, MIX and L-MIX groups compared with the NS group (Fig. 4A). p21 immunohistochemical scores were significantly lower in the MIX and L-MIX groups compared with the NS group (P<0.01) (Fig. 4B). p21 immunohistochemical scores were also decreased in the ACBP and DTX groups, but the decrease was not significantly different compared with the NS group (Fig. 4B). The p21 immunohistochemical score was significantly reduced in the MIX and L-MIX groups compared with the NS and ACBP groups, but no significant difference was identified between the MIX and L-MIX groups (Fig. 4B).

The p53 immunohistochemical score was significantly decreased in the L-MIX, DTX and MIX groups compared

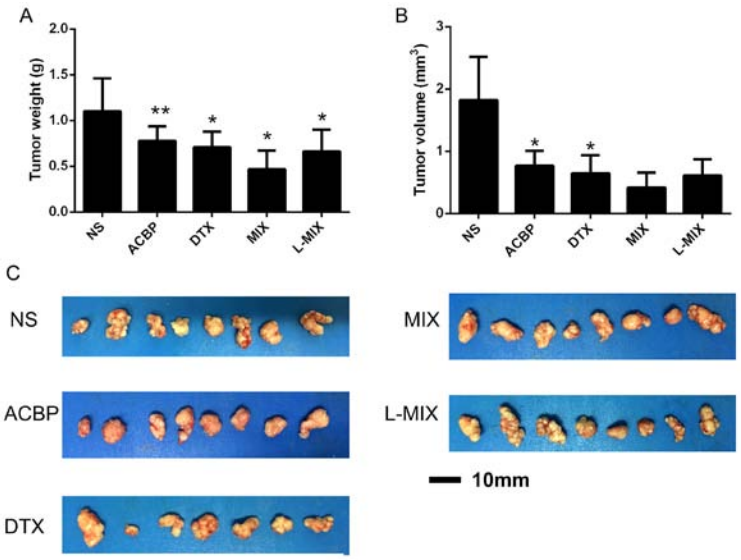

Figure 2. Measurements of tumor size in tumor-bearing mice. Tumor (A) weight, (B) volume and (C) morphology in tumor-bearing nude mice are presented. *P<0.05 and **P<0.01 vs. the NS group. NS, normal saline; ACBP, anticancer bioactive peptide; DTX, docetaxel; MIX, ACBP combined with DTX; L-MIX, ACBP combined with low dose DTX.

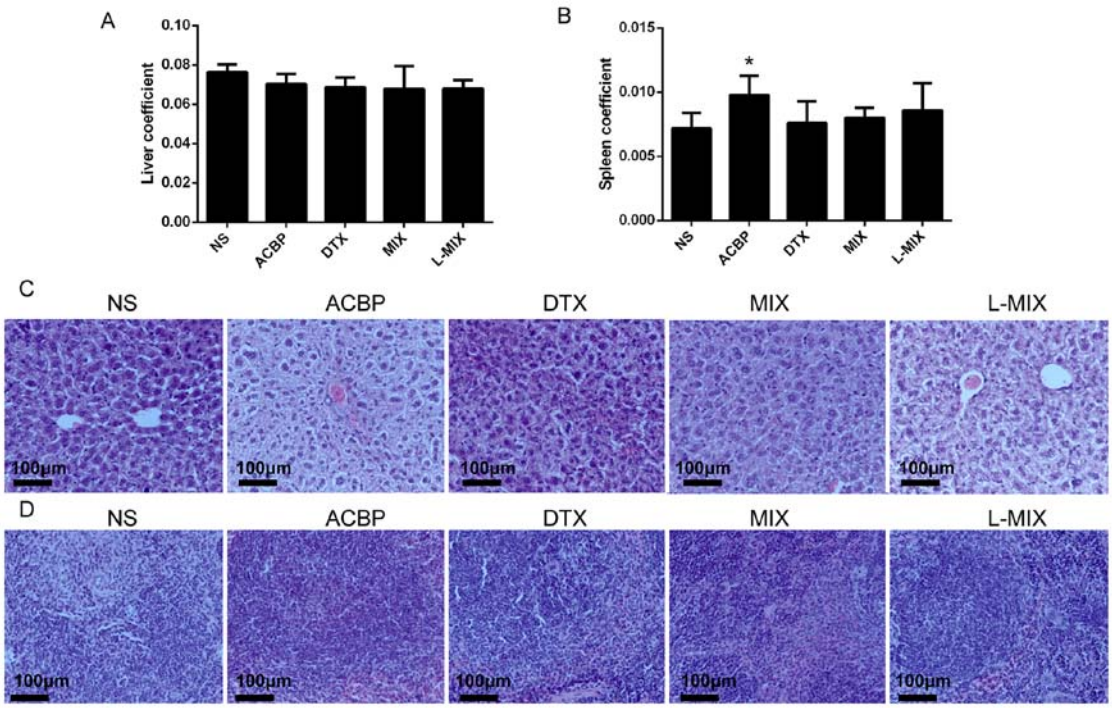

Figure 3. Effect of treatment on the liver and spleen of tumor-bearing mice. The (A) liver coefficient and (B) spleen coefficient. (C) Hematoxylin & eosin staining of the liver and (D) spleen are presented (magnification, x100). *P<0.05 vs. the NS group. NS, normal saline; ACBP, anticancer bioactive peptide; DTX, docetaxel; MIX, ACBP combined with DTX; L-MIX, ACBP combined with low dose DTX.

with the NS group (P<0.01; Fig. 4B), but the ACBP group displayed a score that was not significantly different compared with the NS group (P<0.05; Fig. 4B). The MIX group exhibited a significantly decreased p53 immunohistochemical score compared with the ACBP (P<0.01; Fig. 4B). Furthermore, the L-MIX group displayed a significantly decreased p53 immunohistochemical score compared with the ACBP group (P<0.01; Fig. 4B). The L-MIX group also exhibited significantly reduced p53 immunohistochemical scores compared with the ACBP, and DTX (P<0.05).

Ki67 immunohistochemical scores were significantly lower in the four treatment groups compared with the NS group (P<0.01; Fig. 4B). The MIX and L-MIX groups exhibited significantly decreased Ki67 immunohistochemical scores compared with, ACBP and DTX groups (P<0.01), but there was no significant differences between the MIX and L-MIX groups (Fig. 4B).

Expression of the tumor-associated genes p21, p53 and Ki67. Using GAPDH as an internal reference gene, the relative mRNA levels of p21, p53 and Ki67 were detected by RT-qPCR.

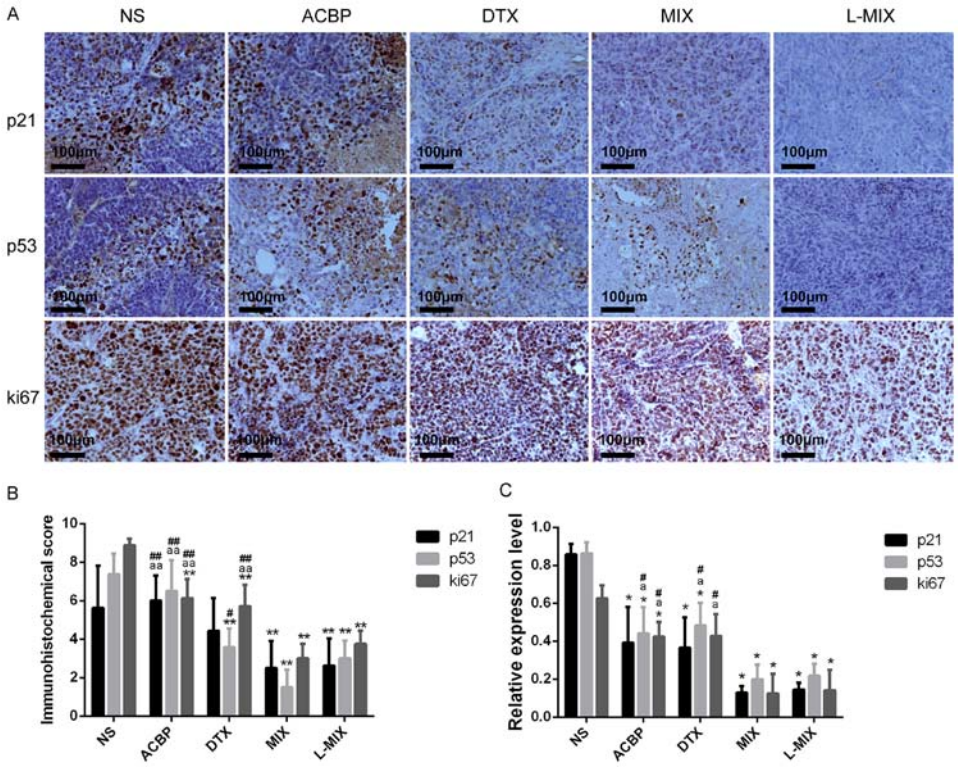

Figure 4. Expression of p21, p53 and Ki67 in tumor tissues. Expression of p21, p53 and Ki67 in tumor tissues by (A) immunohistochemical staining (magnification, x100) and (B) immunohistochemical scoring. (C) Relative expression of p21, p53 and Ki67 in tumor tissues, measured by reverse transcription-quantitative PCR. *P<0.05 and **P<0.01 vs. the NS group. ^{a}P<0.05 and ^{aa}P<0.01 vs. the MIX group. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the L-MIX group. NS, normal saline; ACBP, anticancer bioactive peptide; DTX, docetaxel; MIX, ACBP combined with DTX; L-MIX, ACBP combined with low dose DTX.

The relative mRNA expression of p21 and p53 was significantly lower in the four treatment groups compared with the NS group (P<0.05). Regarding p21, the four treatment groups displayed decreased expression levels compared with the NS group (P<0.05), but there was no significant difference between the ACBP and DTX groups and the MIX, L-MIX groups. The expression of p53 was significantly reduced in the four treatment groups compared with the NS group (P<0.05; Fig. 4C). Furthermore, p53 expression levels were significantly reduced in the MIX and L-MIX groups compared with the ACBP and DTX groups (P<0.05; Fig. 4C), but there was no significant difference between the MIX and L-MIX groups. The ACBP, MIX and L-MIX groups exhibited significantly reduced Ki67 gene expression compared with the NS group (P<0.05; Fig. 4C). However, there was no significant difference in the expression of Ki67 between the DTX and NS groups (Fig. 4C). The MIX and L-MIX groups displayed significantly decreased Ki67 levels compared with the ACBP and DTX groups (P<0.05; Fig. 4C). However, there was no significant difference in Ki67 expression between the MIX and L-MIX groups (Fig. 4C).

Discussion

Neoadjuvant and adjuvant chemotherapy have become routine treatment strategies for breast cancer. Approximately 81.4% of patients with invasive breast cancer are treated with chemotherapy (29). At present, chemotherapy is not effective and is often limited as the majority of tumors have pre-existing resistance mediators (30). Furthermore, treatment prior to chemotherapy is often ineffective and initial chemotherapeutic drugs decrease in effectiveness over the course of the treatment, eventually leading to disease progression and tumor recurrence (30). Common complications of chemotherapeutic drugs are nausea, vomiting, phlebitis, alopecia, oral mucositis and bone marrow suppression (31). A large number of studies have reported ovarian failure (mainly menstrual atresia) and cognitive impairment caused by breast cancer chemotherapy, resulting in physical and mental complications in young and middle-aged female patients (31-33). Therefore, the present study aimed to identify a novel drug that enhanced the sensitivity and reduced the associated toxic and adverse effects of chemotherapeutic drugs.

In addition to the more commonly used methods of clinical surgery, radiotherapy, chemotherapy and endocrine therapy, the use of biotherapy as a cancer treatment is increasing (34). It has been reported that ACBP treatment can effectively inhibit the human breast cancer cell line MDA-MB-231, inhibit tumor cell proliferation, induce apoptosis and block cell cycle progression (35). Furthermore, ACBP can activate the immune system to improve the immune function of the human body and achieve an antitumor effect (35). In addition, numerous studies have suggested that ACBP regulates energy consumption and alleviates digestive tract toxicity (18-20,22). Therefore, ACBP treatment may be potentially decrease the toxic side effects of DTX.

According to the hematoxylin & eosin staining in the present study, the hepatocytes in each treatment group were neatly arranged and even in size and there was no obvious inflammatory cell infiltration, fibrosis or necrosis in the hepatocytes. However this may be due to the short period of intermittent drug administration. Additionally, there were no significant differences in

the liver coefficients between the groups of tumor-bearing nude mice, which also indicated that the short-term intermittent use of ACBP did not have a toxic effect on the liver. The spleen is the largest immune organ and is primarily involved in humoral immunity (36). The spleen coefficient can reflect the immune status of the individual; the higher the spleen coefficient is, the stronger the immunity of the individual in the absence of other diseases (36). The spleen coefficient was significantly higher in the ACBP group compared with control groups ($P<0.05$). The results indicated that ACBP may enhance the immunity of tumor-bearing nude mice.

Breast cancer is a highly heterogeneous disease, which can be divided into four types according to the expression of the estrogen receptor, progesterone receptor, human epidermal growth factor receptor-2 (HER2) and Ki-67, detected by immunohistochemistry. The four types are luminal A, luminal B, HER2-positive and triple-negative breast cancer (TNBC) (37). These classifications provide reliable guidance for the individualized treatment of breast cancer and the prediction of prognosis at the molecular level (37). The p53 gene is the most widely studied tumor suppressor gene (38). The p21 gene is a product of the Ras proto-oncogene and its upregulation is closely associated with the development of colorectal cancer (39). However, the relationship between p21 and breast cancer remains unclear. Ki67 is recognized as a breast cancer proliferation factor as Ki67 expression and breast cancer cell proliferation, malignancy degree, invasiveness and distant metastasis are positively correlated (40). The MDM2-p53-p21 signaling pathway is one of the most important signaling pathways involving the p53 gene. Alterations in the function or structure of any gene in this signaling pathway can result in tumor formation (41). p21 is located downstream of p53 and depends primarily on p53 activity during cell senescence, cell cycle regulation and apoptosis (41). The human breast cancer cell line MDA-MB-231 used in the present study is a TNBC with a high tumor-formation rate, high degree of malignancy, strong invasiveness and poor prognosis (42).

In the present study, p53 and p21 levels were not consistent at the level of protein and mRNA expression. The mRNA expression of p53 and p21 was significantly lower in tumor-bearing nude mice treated with ACBP and DTX compared with NS treated mice. From the results, it may be speculated that the anticancer effect of ACBP may occur via the regulation of p53 and p21 gene expression. Therefore, ACBP may increase the rate of apoptosis induced by tumor cells and regulate cell cycle progression in breast cancer cells. High expressions of p53, p21 and Ki67 has been associated with poor prognosis in a number of studies (40,43,44). The expression of the three genes in nude mice treated with ACBP was significantly decreased compared with the NS group. While p53 and Ki67 was significantly lower in the MIX group compared with the ACBP and DTX groups. As a result, ACBP alone had an antitumor effect, but when combined with DTX, ACBP enhanced DTX sensitivity. Regarding quality of life parameters, although the activity levels and weights of the nude mice were not significantly different between the groups, the water consumption in the ACBP group was decreased compared with the NS group ($P<0.01$). The tumor weights of the MIX and L-MIX groups were significantly lower compared with the NS groups ($P<0.05$), which also suggested that ACBP had an antitumor effect and enhanced DTX sensitivity. The present study identified a novel combined therapy for breast cancer.

To conclude, ACBP effectively inhibited the growth of the human breast cancer cell line MDA-MB-231 in tumor-bearing mice, which not only improved the quality of life of animals but also increased DTX sensitivity. Short-term use of ACBP did not elicit toxic and adverse effects associated with hepatocyte injury, and the increase in the spleen coefficient suggested that the antitumor effect of ACBP might be associated with improvements in immunity of nude mice. The expression of p53, p21 and Ki67 further suggested an antitumor effect for ACBP and indicated that ACBP combined with chemotherapy to reduce DTX toxicity. Therefore, the p53-p21 signaling pathway might serve a key role in the treatment of human breast cancer. The present study suggested a novel approach for the clinical treatment of breast cancer. Further investigation into the mechanisms of action of the antitumor and chemotherapy sensitization effects of ACBP are required.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XS conceived and designed the present study. BG and XL acquired, analyzed and interpreted the data. XL drafted the manuscript. XS agrees to be accountable for the work in ensuring that questions related to the integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee for Animal Experiments of Inner Mongolia Medical College (approval no. YKD2016152).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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