Baoyuan Jiedu Decoction Alleviates Cancer-Induced Myotube Atrophy by Regulating Mitochondrial Dynamics Through p38 MAPK/PGC-1α Signaling Pathway

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Cancer cachexia is a multifactorial syndrome characterized by continuous body wasting and loss of skeletal muscle. Impaired mitochondria function is closely associated with muscle atrophy in cancer cachexia. Our previous study confirmed the effectiveness of Baoyuan Jiedu decoction (BJD) in inhibiting cancer-induced muscle atrophy in an in vivo model. However, little is known about its mechanisms in regulating mitochondria dysfunction. In this study, we evaluated the therapeutic effect and action mechanisms of BJD against atrophy both in the Lewis-conditioned medium induced C2C12 myotube atrophy model and in a BALB/c mice xenograft model using mouse colon cancer C26 cells. The mitochondrial content was tested by 10-Non-ylacridine orange staining. Expressions of related proteins and mRNAs were detected by western blotting (WB) and qPCR, respectively. As a result, 18 major components were identified in BJD by ultra-high performance liquid chromatography-quadrupole (UHPLC-Q) Exactive analysis. As shown in the in vitro results, BJD treatment prevented prominent myotube atrophy and increased the myotube diameter of C2C12 cells. Besides, BJD treatment increased mitochondrial content and ATPase activity. Furthermore, the protein and mRNA expressions that were related to mitochondrial functions and generation such as cytochrome-c oxidase IV, Cytochrome C, nuclear respiratory factor 1, and mitochondrial transcription factor A were significantly increased in BJD treatment compared to the control group. The in vivo results showed that BJD treatment prevented body weight loss and improved the gastrocnemius index in cachexia mice. Moreover, the expressions of Atrogin-1 and muscle RING-finger protein-1 were decreased by BJD treatment. Mechanically, BJD increased the expression of peroxisome proliferator-activated receptor-gamma coactivator 1, and consistently, inhibited the expression of p38 MAPK and its phosphorylation both in vivo and in vitro. Taken together, this study identified that BJD effectively relieved cancer-induced myotube atrophy and provided a potential mechanism for BJD in regulating mitochondrial dynamics through p38 MAPK/PGC-1α signaling pathway.

Keywords: cancer cachexia, Baoyuan Jiedu decoction (BJD), traditional Chinese medicine, mitochondrial dynamics, p38 MAPK/PGC-1α pathway, in vitro, in vivo
INTRODUCTION

Cancer cachexia is a leading cause of morbidity and mortality, resulting in approximately 80% of cancer cases and 40% of cancer-related deaths (1). An ongoing loss of body weight including skeletal muscle mass is the characteristic of cancer cachexia, which lacks effective nutritional support (2, 3). However, there are no therapeutic drugs specific for it. Therefore, focusing on developing effective approaches to prevent cachexia is imperative (4).

The pathogenesis of cancer cachexia is a complex process that is involved in several systemic metabolic disorders, such as anemia, insulin resistance, chronic inflammation, and skeletal muscle protein degradation (5–7). Inflammatory cytokines, typically like TNF-α and IL-6, can increase protein degradation through activation of the NF-κB and the ubiquitin-proteasome pathway (UPP) pathway (8–10). Previous studies showed that muscle atrophy F-Box (MAFbx)/atrogin-1 and muscle RING-finger protein-1 (MuRF-1), two muscle-specific E3 ubiquitin ligases of UPP, were overexpressed in skeletal muscle under cancer-induced muscle atrophy (11). Importantly, UPP-mediated mitochondrial function plays an important role in cancer-induced skeletal muscle atrophy (12). Uncoupling proteins, such as uncoupling protein-2 (UCP2) and uncoupling protein-3 (UCP3), could disrupt the external and internal mitochondrial membrane so that reduce the mitochondrial ATP synthesis for muscle energy metabolism (13).

The normal mitochondrial function is critical to the maintenance of skeletal muscle energy metabolism (14, 15). However, prior studies have noted that the mitochondrial DNA (mtDNA) related with ATP synthesis is significantly reduced and the expression of cytochrome-c oxidase IV (COXIV) is down-regulated in cancer cachetic mice (16–18). This pathogenesis is mainly triggered by increased expression of UCP-2 and UCP-3 (13, 19). Notably, peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1α) is a transcriptional coactivator that is responsible for the mitochondrial generation (20). Proliferator-activated receptor-gamma coactivator 1 promotes the expression of nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (TFAM), which regulates the transcription of mitochondrial genomes (20). Furthermore, PGC-1α also inhibits the induction of Atrogin-1 expression. However, PGC-1α activities can be impaired by many events, including p38 MAPK and AMPK pathway activations, acetylation by the longevity gene SIRT1, and methylation (21–24). p38 MAPK is most abundantly expressed in skeletal muscle and regulates skeletal muscle mass and myotube differentiation (25). It is indicated that p38 MAPK can induce the expression of E3 ligases, including Atrogin-1 and MuRF-1, by targeting the activation of UPP in gastrocnemius muscle (26). Furthermore, a major effect triggered by p38 MAPK during the process of mitochondrial dysfunction is the inhibition of PGC-1α activation (27). Therefore, to improve cancer cachexia treatment, the ways of regulating p38 MAPK/PGC-1α pathway should be figured out.

Traditional Chinese Medicine (TCM) has been widely used in clinical practice for thousands of years in China. Numerous studies have indicated that TCM had important anti-tumor effects (28, 29). Baoyuan Jiedu decoction (BJD), a classical traditional Chinese herbal formula, is used to treat cancer cachexia. Our previous study demonstrated that BJD ameliorated cancer-induced myotube atrophy in ApcMin/+ cachetic mice (30, 31). Furthermore, BJD has been proven to suppress the expression of mitochondria-related UCP2 and UCP3 via inhibiting UPP in vitro (32). Due to the close relationship between UPP and mitochondria function, it is proposed that BJD could regulate mitochondrial dynamics and the potential molecular mechanism may be related to regulating p38 MAPK/PGC-1α pathway.

In this study, UHPLC-Q Exactive analysis was applied to identify the main components of BJD. The in vitro and in vivo model were conducted to investigate the improvement of BJD in preventing cancer-induced myotube atrophy. Also, we unveiled the molecular mechanism of BJD in regulating mitochondria dysfunction via the inhibition of p38 MAPK/PGC-1α signaling pathway. These results indicate that BJD plays an important role in cancer-induced myotube atrophy and could become a potential therapeutic drug for cancer cachexia treatment.

MATERIALS AND METHODS

Cell Lines and Conditioned Medium

The lung adenocarcinoma cell lines Lewis cells and mouse C2C12 myoblast were purchased from Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences (Shanghai, China) and routinely cultured in DMEM/high glucose medium (Invitrogen, United States) supplemented with 10% fetal bovine serum (Invitrogen, United States), 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in 5%CO2. The conditioned medium was collected as previously described (32). Briefly, Lewis cells were cultured in DMEM/high glucose medium at 37°C in 5%CO2 for 2 days. Then the culture medium was collected and filtered through a 0.22 µm membrane. The conditioned medium was conducted by mixed with fresh culture medium (DMEM/high glucose medium supplemented with 2% horse serum (Invitrogen, United States), 100 U/mL penicillin and 100 µg/mL streptomycin) in a ratio of 1:2, which named Lewis-cell conditioned medium (LCM).

Preparation of the Extracts for BJD

Baoyuan Jiedu decoction was composed of six crude herbs: Panax ginseng C.A.Mey., Aconitum carmichaelii Debx., Astragalus mongholicus Bunge., Angelica sinensis (Oliv.) Diels., Lonicera japonica Thunb., and Glycyrrhiza uralensis Fisch. ex DC.in a ratio of 9:9:8:15:12:6 (9.0, 9.0, 18, 15, 12, and 6.0 g). The information on the drug materials were given in Supplementary Table 1. All the herbs were purchased from the Clinic Department of Zhejiang Chinese Medical University (Zhejiang, China) and identified by the Department of Pharmacy, Clinic Department of Zhejiang Chinese Medical University (Zhejiang, China). Baoyuan Jiedu decoction was prepared as hot-water extracts from the six crude herbs. Briefly, the mixture of P. ginseng C.A.Mey. and A. carmichaelii Debx. was macerated for 1 h and decocted for 1.5 h with 552 mL deionized water (1:8, w/v), A. mongholicus Bunge., A. sinensis (Oliv.) Diels., L. japonica Thunb., G. uralensis Fisch. ex DC., and 414 mL deionized water (1:6, w/v) was added.
and decocted for 1 h. The filtrates were blended and concentrated to 1.15 g crude drug/mL by rotary evaporation, which was stored at 4°C and filtered through a 0.22 μm membrane prior use.

**Liquid Chromatography and Mass Spectrometry**

For UHPLC-Q Exactive analysis, the extracts obtained above was taken 200 μL, added 800 μL methanol and centrifuged at 20,000 rpm for 10 min at 4°C. The supernatant was filtered through a 0.22 μm membrane, then stored at 4°C. UHPLC-Q Exactive analysis was performed on a Thermo Scientific Dionex™ UltiMate™ 3000 RSLC system equipped with a binary pump, autosampler, online vacuum degasser, and automatic thermostatic column oven, coupled with a Thermo Scientific™ Q Exactive™ MS (Thermo Fisher Scientific, Bremen, Germany) equipped with ESI. The data were recorded by Xcalibur 3.0 (Thermo Fisher Scientific). Chromatographic separation was performed using a Thermo Hypersil GOLD column (2.1 mm × 100 mm, 1.9 μm) at a flow rate of 0.4 mL/min. Sample (5 μL) was injected into the system, and the column temperature was maintained at 45°C. The mobile phase consisted of water containing 0.1% (v/v) formic acid (A) and acetonitrile (B). Linear gradient elution was applied (0–5 min, 5-30% B; 5–10 min, 30–40% B; 10–20 min, 40–65% B; 20–25 min, 65–90% B). For MS detection, the operating parameters were as follows: HESI, spray voltage, 3.2 kV (Positive); capillary temp, 300°C; sheath gas pressure, 40 psi; auxiliary gas flow rate, 3 L/min; capillary temp, 300°C; scan mode: positive and negative ion switching scanning; scan range, m/z 100–1500, and the resolution (R) is 70,000.

**Reagents and Antibodies**

Antibodies against Atrogin-1 (Cat# ab74023), MuRF-1 (Cat# ab172479), NRF-1 (Cat# ab175932), TFAM (Cat# ab131607), Cytochrome C (Cyt C) (Cat# ab13573), p38 MAPK (Cat# ab197348), p-p38 MAPK (Cat# ab195049), PGC-1α (Cat# ab54481), and GAPDH (Cat# ab9484) were purchased from Abcam (Cambridge, United States). Antibody against COXIV (Cat# bs1533) was purchased from Bioss (Beijing, China). p38 MAPK inhibitor SB203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole) was purchased from Med Chem Express (NJ, United States). Megestrol acetate (Cat# B1377) was purchased from Ape × Bio (Houston, United States).

**In vitro Cancer-Induced Myotube Atrophy Model Construction**

C2C12 cells were seeded in a 96-well plate and cultured in DMEM/high glucose medium. When the cell fusion reached 80–90%, the C2C12 cells were randomly divided into four groups: normal group (normal, n = 3), model group (n = 3), BJD group (BJD, n = 3), and p38 MAPK inhibitor group (SB203580, n = 3). The normal group was cultured in DMEM/high glucose medium without LCM, the model group was cultured in LCM. And the BJD group was cultured in LCM with 125 mg/mL BJD treatment, according to our previous study, BJD at concentrations up to 125 mg/mL has prevented C2C12 cells from LCM-induced myotube atrophy (32). Therefore, we chose a concentration of BJD at 125 mg/mL as an optimal dose in subsequent experiments. The p38 MAPK inhibitor group was cultured in LCM with 50 ng/mL SB203580 treatment. All the groups were continually cultured for 96 h. Morphological performance and transverse diameters of myotubes in C2C12 cells were observed every 24 h. The picture data were processed by Image J software (CA, United States).

**Mitochondrial Contents and ATPase Activity Assays**

Mitochondrial contents in C2C12 cells were detected by 10-Nonyl-acridine orange (NAO) stained. At the end of the experiment, the cells were washed with pre-cooled PBS and fixed with 0.2% glutaraldehyde at 4°C for 10 min. Then the NAO solution (10 μmol/L) was added in the dark for 10 min. The imaging was observed by a fluorescence microscope at 488 nm. The fluorescence intensity was proportional to the mitochondria contents. The level of mtDNA production was calculated by a TIANGEN Genomic DNA Kit (Cat# DP304, TIANGEN, China) and ATPase activity was detected by an ATP Colorimetric/Fluorometric Assay Kit (Cat# MAK190-1, Sigma, United States) according to the manufacturer's instructions.

**In vivo Xenograft Cachexia Experiment**

C26 tumor-bearing mice (20 ± 2) g and male BALB/c mice (20 ± 2) g were purchased from the Beijing Huakang Biotechnology Company (Beijing, China) and housed in an SPF (specific pathogen-free) and temperature-controlled (25 ± 2) °C environment with a 12 h light/dark cycle in Zhejiang Chinese Medical University Laboratory Animal Research Center (Hangzhou, China). The experiment began after mice adapted to the new environment for 1 week. The xenograft cachexia model was established as previously described (31). Briefly, C26 colorectal cancer cells were obtained from the C26 tumor-bearing mice after sacrificed and commercially countered to plate 2 × 10⁶/mL in flasks. And the BALB/c mice were injected 0.2 mL C26 cells subcutaneously through the right axillary skin. Then the mice were randomly divided equally (n = 10) into five groups: normal group (normal), model group (model), BJD group (BJD), megestrol acetate group (MA), and p38 MAPK inhibitor group (SB203580). In the BJD group, the dosage of BJD was 23 g/kg·d according to our previous study (30, 31). The dose of megestrol acetate was 24 mg/kg·d in the MA group, and the dose of SB203580 was 10 mg/kg in the p38 MAPK inhibitor group, while the normal and model group were given equal doses of normal saline. The MA group and SB203580 group were treated by intraperitoneal injection, while the normal group, model group, and BJD group were treated by gavage. The body weights were evaluated and recorded every day.

**Gastrocnemius Tissues Collection and H&E Stained**

Mice were euthanized at the end of the experiment and the gastrocnemius tissues were collected for weighed and fixed in 4% phosphate-buffered paraformaldehyde, embedded
in paraffin, and placed on microscope slides for routinely H&E histopathologic examination. The gastrocnemius index was calculated by the formula as the gastrocnemius weight (mg)/the body weight (g).

**Western Blotting**
Tissues and cells were washed twice in 1× PBS and lysed in radioimmunoprecipitation assay (RIPA) buffer on ice for 30 min. The lysate was centrifuged at 9000 rpm for 10 min at 4°C and the supernatant was collected. The protein concentration was quantified by a BCA Assay Kit (Thermo Fisher Scientific, United States) according to the manufacturer’s instructions. Samples were loaded and electrophoresed in 8–10% SDS-PAGE and transferred to PVDF membranes (Millipore, United States). Then the membranes were blocked in 5% non-fat milk powder diluted in TBS-T with enhanced chemiluminescence. The intensity of blots was analyzed by Image J. Each value was normalized by the respective value for GAPDH as an internal control.

**Real-Time PCR (qPCR) Analysis**
Total RNAs were extracted from cells and gastrocnemius samples using Trizol reagent (Invitrogen, United States) according to the manufacturer’s instructions. Real-time PCR analyses were performed using SYBR Green (TOYOBO, Japan) and results were calculated based on the comparative cycle threshold method (2^ΔΔCT). β-actin was used to normalize the gene expression of other mRNAs. As for the analysis of the expression of mtDNA, β-globin was used as an internal gene. All of the primers were synthesized by Sangon Biotech (Shanghai, China). Primers sequences were as follows: Atrogin-1 forward 5′-GAGAACAGTATGCGGTTCA-3′, reverse 5′-TAATAAGTCCTTGGGGTTG-3′; MuRF-1 forward 5′-GCCACCTTCCCTTGGAGT-3′, reverse 5′-CTTTGTTCGTC TCCCC-3′; NRF-1 forward 5′-GCACAGAAGGCAAAG-3′, reverse 5′-CGAAAGCATAAGACAAGG-3′; TFAM forward 5′-GTGGGCGCTGCTAAAGAC-3′, reverse 5′-GCTGACAGGCG GAGGGTAT-3′; Cyto C forward 5′-ACCCCTGATGGAGATTTG-3′, reverse 5′-GCTATTAGCTGTGCTTTC-3′; COXIV forward 5′-TGGATGACAAAGGCAGAGCA-3′, reverse 5′-CA CCCAGTCAGATCAAAGG-3′; p38 MAPK forward 5′-GGT GTGTCGTCCTTTTGA-3′, reverse 5′-TGTAGGCTCTTTTG CGT-3′; PGC-1α forward 5′-TTGGTGAAATTGAGGAATG-3′, reverse 5′-CACAGTGTAACCGTGTTGG-3′; ATP synthase-F subunit β peptide forward 5′-TCTTTGCGGGTGTTGG-3′, reverse 5′-GGGGCGCAGGGCGTGT-3′; β-actin forward 5′-TCAGCAATGCCGTGGTAT-3′, reverse 5′-ATCAGATTT GGCAACAGGCG-3′; β-globin forward 5′-GATGCTGAGA AAAGGCGTCT-3′, reverse 5′-CCTCTGGTCAAGGGTAGT-3′.

**Statistical Analysis**
Statistical analyses were performed using SPSS 26.0 (SPSS Inc., United States) and data were presented as means ± SD. Differences between multiple comparisons were assessed using one-way analysis of variance (ANOVA) with Tukey’s multiple comparison test whereas two groups were analyzed using an unpaired Student’s t-test. P < 0.05 was considered to be statistically significant.

**RESULTS**

**Identification of the Main Components in the BJD Extract**
Eighteen chemical components of BJD were identified by UHPLC-Q Exactive analysis. Besides, some chemical markers, such as chlorogenic acid, ferulic acid, were
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FIGURE 1 | UHPLC chromatogram profiles of BJD. (A) Total ion current chromatograms of BJD. Secondary mass spectrum of the 18 main components of BJD: (B) Cytidine. (C) Guanine. (D) Indole-3-acrylic acid. (E) Chlorogenic acid. (F) 2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid. (G) Isoliquiritigenin. (H) Quercetin-3p-D-glucoside. (I) Cynaroside. (J) Ferulic acid. (K) Hesperidin. (L) Formononetin. (M)α-Lactose. (N) 9S,13R-12-Oxophytodienoic acid. (O) 18-β-Glycyrrhetinic acid. (P) Bis(4-ethylbenzylidene)sorbitol. (Q) α-Linolenoyl ethanolamide. (R) Hexadecanamide. (S) Stearamide.
BJD Ameliorated LCM-Induced C2C12 Myotube Atrophy

The C2C12 myoblast is a classical cell for studying cancer-induced myotube atrophy. To examine whether BJD could

used for quality control of BJD. The total ion current diagram and the secondary mass spectrum results of the chemical components were shown in Table 1 and Figure 1.

FIGURE 2 | BJD prevented LCM-induced myotube atrophy in C2C12 cells. (A) The morphological changes of myotubes in C2C12 cells (×200 magnification; scale bar: 50 µm). (B,C) The transverse diameters of myotubes in C2C12 cells (µm, n = 3). (D) The relative expression of Atrogin-1 and MuRF-1 was detected by RT-qPCR, with β-actin was used as an internal gene. (E) The expression of Atrogin-1 and MuRF-1 was detected by Western blotting, GAPDH was used as a loading control. The data are presented as the mean ± SD. **P < 0.01, ***P < 0.001.
prevent myotubes atrophy, C2C12 cells were induced by LCM and incubated with BJD for 72 h. As a result, LCM postponed the differentiation of myotubes in C2C12 cells while BJD promoted the differentiation of myotubes as compared with the normal group (Figure 2A). Measurements of the myotube transverse diameter showed that BJD treatment and SB203580 significantly increased the myotube thickness compared with the model group (Figures 2B,C).

BJD Decreased the Expression of Atrogin-1 and MuRF-1 in C2C12 Cells
The high expressions of muscle-specific atrophy marker proteins, such as Atrogin-1 and MuRF-1, caused the degradation of myotubes. Therefore, to further investigate whether the effect of BJD on improving the myotubes atrophy was achieved through modulating these proteins expressions, we used Western blotting and qPCR to determine their protein and mRNA levels. BJD treatment significantly decreased the expressions of Atrogin-1 and MuRF-1 both in protein and mRNA compared with the model group as predicted (Figures 2D,E). Besides, SB203580 had a similar impact with BJD but not significant.

BJD Promoted the Generation of Mitochondria
Mitochondria are almost enriched skeletal muscle and responsible for the metabolic function and physiological or pathological responses. Therefore, to investigate the effect of BJD on the generation of mitochondria, C2C12 cells were collected and mitochondrial contents were detected by NAO staining. As a result, BJD treatment increased the fluorescence intensity, which is similar with the result of SB203580, whereas fluorescence intensity was slight in the model group (Figure 3). Mitochondrial DNA is an important indicator for mitochondrial biosynthesis, therefore, to determine whether BJD could increase mtDNA synthesis, we detected the expression of mtDNA by qPCR. As a result, BJD increased the expression of mtDNA compared with the model group, as similar in SB203580 treatment (Figure 4A).

BJD Inhibited p38 MAPK/PGC-1α Signaling Pathway
The p38 MAPK/PGC-1α signaling pathway regulates the mitochondrial biosynthesis and energy metabolism and causes muscular atrophy. Therefore, to detect whether BJD could regulate p38 MAPK/PGC-1α signaling pathway to enhance...
FIGURE 4 | BJD improved mtDNA and increased the expression of ATPase, NRF-1, TFAM, COXIV, and CytC. (A) The relative expression of mtDNA, with β-globin was used as an internal gene. (B–F) The relative expression of ATPase, NRF-1, TFAM, COXIV, and CytC, with β-actin was used as an internal gene. (G) The expression of NRF-1, TFAM, COXIV, and CytC was detected by Western blotting, GAPDH was used as a loading control. The data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.
FIGURE 5 | BJD treatment inhibited p38 MAPK/PGC-1α signaling pathway. (A,B) The relative expression of p38 MAPK and PGC-1α, with β-actin was used as an internal gene. (C) The expression of p-p38 MAPK, p38 MAPK, and PGC-1α was detected by Western blotting. GAPDH was used as a loading control. The data are presented as the mean ± SD. ***P < 0.001.

mitochondrial function, their protein and mRNA levels were measured by Western blot and qPCR, respectively. Treatment with BJD significantly decreased the expression of p38 MAPK and p38 MAPK phosphorylation (Figures 5A,C) whereas increased the expression of PGC-1α (Figures 5B,C), compared with the model group. Similar to BJD, SB203580 treatment also promoted the expression of PGC-1α, but did not affect the expression of p38 MAPK.

BJD Alleviated the Gastrocnemius Atrophy in Xenograft Cachexia Mice

To verify the effect of BJD on cancer cachexia model in vivo, mice were treated with or without BJD. And the results showed that the ongoing body weight loss was prevented by BJD treatment (Figures 6A,B). Similarly, megestrol acetate and SB203580 also exhibited protection on body weight loss. Moreover, BJD treatment improved the gastrocnemius index compared with the model group (Figure 6C), the same as the megestrol acetate and SB203580 treatment. Additionally, BJD treatment improved the diameters of the gastrocnemius muscle, whereas megestrol acetate and SB203580 treatment had a similar effect (Figure 6D). Furthermore, BJD treatment significantly decreased the expression of Atrogin-1 and MuRF-1 protein and mRNA levels, and megestrol acetate had a similar effect (Figures 7A,B,E). Besides, BJD treatment inhibited the expression of p38 MAPK and p38 MAPK phosphorylation whereas increased the expression of PGC-1α (Figures 7C,D,F). Interestingly, SB203580 did not affect MuRF-1 expression but slightly decreased the expression of p38 MAPK (Figures 7B,E,F).

DISCUSSION

Cancer cachexia, considered as a multifactorial syndrome, frequently occurs in advanced cancer patients (1, 4, 34). The loss of body weight, especially the loss of whole-body skeletal muscle mass with or without fat consumption, is the most prominent clinical characteristic of cachexia (35, 36). The progressive weight loss not only causes the intolerance of patient to radiotherapy and chemotherapy but also has extensive impact on the quality of life (37). However, the therapeutic approaches or agents for cancer cachexia are currently limited.

Traditional Chinese Medicine is widely used in relieving cachexia symptoms (38, 39). According to the theory of TCM, cancer cachexia belongs to a kind of consumptive disease, and the Yang-qi deficiency along with toxin stagnating is considered as its main pathogenesis. BJD, a Chinese formula consisted of 6 herbals including *P. ginseng* C.A.Mey., *A. carmichaelii* Debx., *A. mongholicus* Bunge., *A. sinensis* (Oliv.) Diels., *L. japonica*
Thunb., and G. uralensis Fisch. ex DC., has the function of benefiting Yang-qi, resolving toxin and strengthening muscles. In this study, we identified the main components of BJD through UHPLC-Q Exactive analysis and some chemical markers that provided the basis for the pharmacology. Some of these components, such as luteolin, ferulic acid, have anti-inflammatory, immunomodulatory, and anti-tumor effects (40, 41). Chlorogenic acid and ferulic acid showed obvious anti-cancer effect (42, 43). Notably, ginsenoside Rb1 is reported to contribute to recover the cancer cachexia state (44). Ginsenoside Rb3, the main constituent of ginseng, has the function of upregulating myotube formation and mitochondrial function (45). Therefore, these results provide a material basis of BJD for cachexia treatment.
The degradation of muscle-related proteins induced by systemic inflammation, UPP, and mitochondrial dysfunction, is closely associated with skeletal muscle atrophy in cachexia (46). TNF-α, a strong inflammatory cytokine, can activate the NF-κB-mediated transcription of MuRF-1, thereby leading to increased protein degradation (8, 9). Besides, the NF-κB

![FIGURE 7 | BJD inhibited p38 MAPK/PGC-1α singling pathway in mice. (A–D) The relative expression of Atrogin-1, MuRF-1, p38 MAPK, and PGC-1α was detected by RT-qPCR, with β-actin was used as an internal gene. (E–F) The expression of Atrogin-1, MuRF-1, p-p38 MAPK, p38 MAPK, and PGC-1α was detected by Western blotting, GAPDH was used as a loading control. The data are presented as the mean ± SD. ***P < 0.001.](image-url)
signaling pathway inhibits the expression of mitochondrial genes so that reduces mitochondrial biosynthesis and reduces its oxidative phosphorylation capacity and ATP production (22, 47). Therefore, activated cytokines can up-regulate the expression of E3 ubiquitin ligase by activating UPP and accelerate muscle protein degradation (46). Previously, our study revealed that BJD prevented the loss of boy weight and induced C2C12 myotube differentiation. BJD also reduced TNF-α level and inhibited the expression of MuRF-1 by inhibiting the UPP-mediated muscle protein degradation (30–32). In this study, we used a xenograft cachexia mice model and found that BJD prevented body weight loss and improved the pathological process of muscle atrophy. These results indicate that BJD has a potential therapeutic effect on cancer cachexia.

Mitochondria play important roles in maintaining the generation and function of skeletal muscle. Recently, it has been reported that the capability of mitochondria generation, oxidative phosphorylation function, and ATP synthesis is impaired in cancer-induced cachexia (1, 17, 18, 48). Therefore, improving mitochondria function may become a new therapeutic target in cancer cachexia (49). We found that BJD can promote C2C12 cells differentiation, and the transverse diameter of the myotube gradually increases along with the intervention time. Besides, after BJD treatment, the mitochondrial fluorescence intensity and the expression of mtDNA increased significantly in C2C12 cells. As NRF-1 and TFAM are key factors in mitochondrial biosynthesis, BJD treatment can increase their expression levels, and COXIV and Cyt C, which are two major oxidative phosphorylation markers, were up-regulated by BJD treatment. These results indicate that BJD can improve mitochondrial biosynthesis and increase oxidative phosphorylation function.

The p38 MAPK/PGC-1α signaling pathway mainly regulates mitochondrial functions from several aspects, including biosynthesis and oxidative phosphorylation. In the cancer cachexia condition, inflammatory factors, such as TNF-α, IL-1, and IL-6, can activate p38 MAPK signaling, thereby inhibiting PGC-1α transcription and reducing energy production (22, 50, 51). Proliferator-activated receptor-gamma coactivator 1, a key factor for mitochondrial synthesis, mainly distributes in skeletal muscle, heart, and other tissues that require high energy (33). Emerging evidence has reported that PGC-1α activation induces the expression of mitochondrial biogenesis factors, such as mtDNA, NRF-1, TFAM, and Cyt C (20, 50). Mice that knocked out PGC-1α showed a decreased mitochondrial capacity in skeletal muscle. On the contrary, increasing the expression of PGC-1α can promote the expression of mitochondrial protein and restore mitochondrial function (52). We found that BJD significantly decreased the expression of p38 MAPK and its phosphorylation, and increased the expression of PGC-1α in both in vitro and in vivo experiments. Besides, we found that SB203580 did not inhibit the expression of p38 MAPK in C2C12 cells, but slightly up-regulated PGC-1α. The reason is that SB203580 can inhibit the activity of p38 kinase on its substrates including p38 itself and its downstream substrate. Proliferator-activated receptor-gamma coactivator 1, as one of the downstream substrates of p38 MAPK, was showed up-regulation of protein and mRNA expression after SB203580 treatment, which indicated that SB203580 inhibited the process of p38 kinase on PGC-1α activity. This result is consistent with the previous report (53). However, SB203580 does not affect the upstream activation events of p38 MAPK, such as TNF-α or IL-6 induced p38 MAPK activation progress (54, 55). Therefore, the expression of p38 MAPK in the SB203580 group showed no significant difference compared with the model group. This may be due to the activation of p38 MAPK by upstream events. Our previous studies reveal that BJD can reduce the content of TNF-α and IL-6 (32). Therefore, it explains the phenomenon that BJD reduced the expression of p38 MAPK while SB203580 did not affect its expression in vitro.

In conclusion, the present findings reveal that BJD can improve mitochondrial function by regulating the p38 MAPK/PGC-1α signaling pathway, thereby preventing the cancer-induced myotube atrophy. Although our data confirm that BJD may be a potential drug for the treatment of cancer cachexia, there are some necessary investigations, such as conducting the toxicology experiments, screening and verifying effective small molecules of BJD for cachexia treatment, which are desirable for future work.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/Supplementary Material.

ETHICS STATEMENT

The studies involving animal study were reviewed and approved by Animal Ethical and Welfare Committee of ZCMU, Zhejiang Chinese Medical University.

AUTHOR CONTRIBUTIONS

XJ, HZ, and DW contributed to the study design, data analysis, and manuscript drafting. DW, XZ, and WC contributed to the animal experiment. QB and WC carried out the in vitro experiment. QB, JR, XY, and JW contributed to the data acquisition and analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.523577/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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