MMP12 Knockout Prevent Weight and Muscle Loss Induced by Cancer Cachexia

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Abstract

Weight loss and muscle wasting can have devastating impacts on survival and quality of life of patients with cancer cachexia. Here, we have established a hybrid mouse of $Apc^{Min/+}$ mice and MMP12 knockout mice ($Apc^{Min/+}; MMP12^{-/-}$) and found that knockout MMP12 can suppress the weight and muscle loss of $Apc^{Min/+}$ mice. In detail, we found that interleukin 6 was highly upregulated in the serum of cancer patients and MMP12 was increased in muscle of tumor-bearing mice. Interestingly, the interleukin 6 secreted by tumor cells led to MMP12 overexpression in the macrophages, which further resulted in degradation of insulin and insulin-like growth factor 1 and interruption of glycolipid metabolism. Notably, depletion of MMP12 prevented weight loss of $Apc^{Min/+}$ mice. Our study uncovers the critical role of MMP12 in controlling weight and highlights the great potential of MMP12 in the treatment of cancer cachexia.

Keywords:

MMP12; Macrophage; IL-6; Weight and Muscle loss; Cancer cachexia; $Apc^{Min/+}$; $MMP12^{-/-}$

Running title:

Interleukin-6 Derived from Cancer Cells Increased MMP12 of Macrophage Aggravated Cancer Cachexia

1. Introduction

Many studies have shown that rapid skeletal muscle mass loss is a characteristic of cancer cachexia (CAC) in colorectal cancer (CRC) patients with advanced cancer stages, which cannot be completely reversed by conventional nutritional support or drugs therapy (Bonetto et al., 2016; Pettersen et al., 2017; Song et al., 2019; Yang et al., 2018; Yang et al., 2019; Yuan et al., 2015). Muscle loss caused by tumor development or growth would occur in various cancers, such as pancreatic cancer, esophageal cancer, gastric cancer, lung cancer, liver cancer and CRC. According to the statistics, nearly 80% of cancer patients have skeletal muscle loss as a late outcome, and the mortality rate is as high as 30% (Baracos et al., 2018; Herremans et al., 2019; Tisdale and Michael, 2002). 2018 estimates of global cancer data show that CRC is the third most common malignant tumor in the world, and its incidence and mortality are increasing year by year (Bray et al., 2018; Brody, 2015; Lin et al., 2016; Siegel et al., 2014), and cachexia muscle loss induced by CRC is an important cause of death. Although more and more attention has been paid to CAC in recent years and great progress has been made in the diagnosis and treatment of CRC, the mortality rate of CRC has not decreased and has been younger (Brody, 2015; Center et al., 2009). The key is that the mechanism of how the inflammatory environment of tumor causes muscle loss is still not clear, and CAC involves a variety of immune cells, a variety of cytokines, and metabolic disorders (Daou, 2020). The tumor-induced pro-inflammatory response plays an important role in the progression of CAC. This metabolic dysfunction is caused by changes in glucose, lipid and protein metabolism, and may lead to the loss of skeletal muscle and adipose tissue when maintained for a long time (Fonseca et al., 2020; Lobato et al., 2018; Patel and Patel, 2017). To date, although several drugs have had positive clinical effects in increasing lean body mass, their effects on body function are limited. There are no effective

64 medical interventions or approved drug therapies that can completely reverse muscle loss caused by CAC,
65 which brings difficulties to the treatment of chemotherapy drugs (Daou, 2020) (Fonseca et al., 2020).
66 MMP12 is a matrix protein metalloenzyme, also named macrophage metalloenzyme, from a family of
67 endoproteolytic enzymes whose activities depend on metal ions such as calcium and zinc and can degrade
68 extracellular matrix. It was discovered in a study of tadpole morphological changes during development
69 and is necessary for monocyte recruitment, and it is mainly secreted by inflammatory cells such as mon-
70 ocyte macrophages(Bauters et al., 2013). MMP12 is mainly secreted by M2 macrophages(Han et al.,
71 2018; Hotary et al.; Lee et al., 2016). Reports have demonstrated that MMP12 can decompose most
72 extracellular matrix and vascular components, and has obvious effects on elastic fiber-rich blood vessels,
73 lung, embryonic development, reproduction, and tissue remodeling(Atl1, 2017; Kraen et al., 2019;
74 Langlois et al.; Wagner et al., 2016; Wang et al., 2019; Wetzl et al.). MMP12 is associated not only with
75 smoking-induced emphysema(Kraen et al., 2019) but also with the typing of bone marrow cells and
76 myeloid derived suppressor cells(Qu et al., 2011). As early as 1981, studies reported that MMP12 can
77 specifically degrade insulin(Kettner et al.). In 2014, researchers of Washington University confirmed
78 that MMP12 regulates insulin sensitivity and is positively correlated with insulin resistance(Lee et al.,
79 2016). In 2016, MMP12 was identified as a target for insulin-related treatment of metabolic diseases,
80 and MMP12 promotes insulin resistance and prevents fat expansion under high-fat conditions(Amor and
81 Moreno-Viedma; Bauters et al., 2013).
82 In recent years, some inflammatory cytokines, such as Interleukin 6 (IL-6), monocyte chemoattractant
83 protein-1, tumor necrosis factor, zinc- α 2-glycoprotein and pancreatic enzymes, have been shown to be
84 related to muscle loss (Bing, 2011; Han et al., 2018; Pettersen et al., 2017; Talbert et al., 2018a; Yarla et

al., 2018). IL-6, a multi effect proinflammatory cytokine, is secreted by normal human monocytes, fibroblasts, endothelial cells, Th2 cells, vascular endothelial cells. And a variety of tumor cells also secrete IL-6 (Carson and Baltgalvis, 2010b; Han et al., 2018; Pettersen et al., 2017). It targets macrophages, hepatocytes, resting T cells, activated B cells and plasma cells (Han et al., 2018; Utsumi et al., 1990). The IL-6 level has been proposed to be high in patients with skeletal muscle loss (Peixoto da Silva et al., 2020a). IL-6 levels were increased in tumor tissue and involved in skeletal loss progression in cancer patient (Narsale et al., 2014). IL-6 can directly induce alternative macrophage activation (Ayaub et al.; Hopkins et al.). And systemic overexpression of IL-6 accelerates CAC muscle loss in *Apc^{Min/+}* mice (Baltgalvis et al., 2010). A high level of IL-6 in serum is considered to be an important contributor to the progression of muscular dystrophies, including muscle loss induced by CAC and duchenne muscular dystrophy. Blocking IL-6 receptor may inhibit dystrophic muscle loss and lipolysis by suppressing the downstream Janus kinase/signal transducer and transcription activator (JAK/STAT) pathway to promote muscle regeneration (Hu et al., 2019; Wada et al., 2017). Ville Wallenius et al found that centrally acting IL-6 exerts anti-obesity effects in rodents but does not increase energy expenditure (Franckhauser et al., 2008; Wallenius et al., 2002). IL-6 has been shown to induce insulin resistance (Liaqat et al., 2017). It is well known that insulin affects glucose uptake through the PI3K-AKT-mTOR pathway by binding to insulin receptors (Hopkins et al.). Skeletal muscle is the primary tissue involved in insulin-stimulated glucose uptake. IL-6 mediates glucose intolerance and promotes insulin resistance in skeletal muscle (Deshmukh et al.; Han et al., 2018; Nicholson et al., 2019). Moreover, insulin resistance can promote muscle wasting (Peixoto da Silva et al., 2020b). IL-6 suppresses insulin action through the Signal transducer and activator of transcription 3 (STAT3) pathway, which then may affect the insulin receptor by

suppressing insulin receptor substrate-1 and downstream targets(Puppa et al., 2012). In short, various studies have proven that IL-6 can induce insulin resistance, thereby indirectly exacerbating muscle loss in CAC. In our study, we found that IL-6 secreted by tumors would target muscle macrophages, and then MMP12 in these activated macrophages will be upregulated, degrading insulin and insulin-like growth factor 1. So, we thought one molecular crosstalk may exist in bearing mice. Increased expression of IL-6 derived from cancer cells up-regulates MMP12 in macrophages, which affects skeletal glycolipid metabolism over a long period of time, may resulting in loss of skeletal muscle for a long time. To date, for patients with muscle and weight loss induced by CAC, multimodal interventions including drugs, nutritional support and physical exercise may be a reasonable approach for future research to better understand and prevent loss of muscle (Fonseca et al., 2020). Although several drugs have had positive clinical effects in increasing lean body mass, their effects on body function are limited. There are no effective medical interventions or approved drug therapies that can completely reverse muscle loss caused by CAC, which brings difficulties to the treatment of chemotherapy drugs (Daou, 2020) (Fonseca et al., 2020). Taken together, the combination of MMP12 inhibitors and chemotherapy drugs may bring new challenges and ideas for the treatment of cancer cachexia to improve the quality of life.

2. Materials and Methods

2.1 Mice

B6.129X-MMP12tm1Sds/J macrophage metalloelastase-deficient (MMP12^{-/-}) mice (no. 004855), with a C57BL/6 background were purchased from the Jackson Laboratory, USA (<https://www.jax.org/strain/004855>). Apc^{Min/+} mice (no. T001457) were obtained from Gem Pharmatech, China (<http://www.gempharmatech.com/cn/index.php/searchinfo/59/17.html>). Wild-type (WT/ C57BL/6J) mice were purchased from Guangdong Medical Laboratory Animal Center (China). The production license number was SCXK (Guangdong) 2017-0125. Apc^{Min/+}; MMP12^{-/-} mice were obtained by crossing Apc^{Min/+} mice and MMP12^{-/-} mice ([Figure 1-figure Supplement 1A](#)). All mice were housed in specific pathogen-free conditions. All mice studies complied with the Guangdong Pharmaceutical University, and all protocol was approved by the animal experimental ethics committee of Guangdong Pharmaceutical University.

2.2 Genotype identification

We established crossbred mice and genotyped the 3-week-old mice. The polymerase chain reaction products were subjected to gel electrophoresis (1.2%), and a gel imaging system (GboxGyngene system, UK) was used to obtain electrophoresis images ([Figure 1-figure Supplement 1B](#)). The details of genotype identification can be found on the website of the Jackson Laboratory <https://www.jax.org/strain/004855>.

2.3 Mice experiments and Tissue collection

Mice were anesthetized by inhalation of carbon dioxide anesthesia, marrow, blood, epididymal white fat, gastrocnemius and soleus muscle and brown back fat were collected. For immunohistochemistry Mice were anesthetized by inhalation of carbon dioxide anesthesia, marrow, blood, epididymal white fat,

gastrocnemius and soleus muscle and brown back fat were collected. For immunohistochemistry staining analysis, we obtained muscle tissues wax tissue blocks from clinical surgery patients. For serum detection, all fresh clinical blood samples were obtained from the Sun Yat-Sen University Cancer Center, Guangzhou, China. all fresh clinical blood samples were taken individuals undergoing a clinical health individuals and colorectal cancer patients (30-60 years, excluding individuals with diabetes and hyperthyroidism) and frozen at -80°C until the experiments were performed.

2. 4 Antibodies and Reagents:

An anti-F4/80 anti-body (Cat: 14-4801-81) was purchased from eBioscience and anti-MMP12 (MA5-24851) were purchased from Thermo Fisher (Thermo Fisher Scientific, Cambridge, Massachusetts, USA). An anti-GAPDH (5174P) and anti- β -actin (4970S) were purchased from Cell Signaling Technology Inc (CST). Recombinant mouse MMP-12 protein (3467-MPB-020) was purchased from R&D Systems, Inc. The MMP12 inhibitor MMP408 (444291) was purchased from Merck Millipore Company. Alexa Fluor-488 donkey antibody (P/N SA11055S) was purchased from Invitrogen (Thermo Fisher Scientific, Cambridge, Massachusetts, USA).

2. 5 Total RNA extraction and Real-time polymerase chain reaction

All tissues from mice were quick-frozen in liquid nitrogen and stored at -80°C until they were dissolved with Trizol (TaKaRa, Guangzhou, China, A161050A). RNA extraction was performed according to the manufacturer's instruction, and the total extracted RNA was reverse-transcribed into cDNA for polymerase chain reaction amplification using the real-time polymerase chain reaction SYBR Green kit (TaKaRa, Guangzhou, China). The reverse transcription steps were as follows: denaturation at 94°C for 5 minutes; 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at

72°C for 30 seconds; 72°C for 5 minutes. The mRNA samples were quantified in triplicate. The house-keeping gene GAPDH was used as an internal control to normalize the real-time polymerase chain reaction data for each sample of mRNA. All real-time polymerase chain reaction primers were synthesized by Shanghai Sangon Biotechnology Inc., China, and the primer sequences are listed in S [Table 1](#).

2. 6 Histological analysis and Hematoxylin and eosin staining

Formalin-fixed (10% neutral buffered formalin), gradient dehydration and paraffin-embedded to obtain 3-µm tissue sections from mice were subjected to perform the experiments of hematoxylin and eosin staining, immunohistochemical staining and immunofluorescence staining in accordance with the protocols. To assess the cross-sectional area of muscle, image J software was used after hematoxylin and eosin staining

2. 7 Immunohistochemistry

Tissue sections were dewaxed and incubated with 30% hydrogen peroxide in methanol and blocked with 10% bovine serum albumin diluted with phosphate buffered saline (PBS). The sections were incubated with primary antibodies against MMP12 (1:100) at 4°C overnight. Finally, the primary antibody-treated sections were incubated with secondary antibody (1:100) horseradish peroxides (HRP) (goat anti-rabbit IgG) conjugated with HRP at 37°C for 1hour, stained with 3,3-diaminobenzidine, and counterstained with hematoxylin.

2. 8 Double immunofluorescence staining

Tissue sections were dewaxed and blocked with 10% bovine serum albumin diluted with PBS. The sections were incubated with a mixture of primary antibodies against MMP12(1:100) and F4/80 (1:100) overnight at 4°C. The next day, the primary antibody-treated sections were incubated with mixtures of

secondary antibodies conjugated Alexa Fluor 488 (1:100) and Alexa Fluor 555 (1:100) for 1 hour at room temperature. Immunostaining signals and DAPI-stained nuclei were visualized under a confocal microscope.

2. 9 Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) was performed with kits with serum samples from patients and mice according to the manufacturer's protocol. The human-IL6 kit (EHC007), mouse JE/MCP1/CCL2 (EMC113) kit, mouse IL-6 ELISA kit (EMC004), and mouse KC/IL-8/CXCL1 ELISA kit (EMC104) were purchased from NeoBioscience Technology Company (ShenZhen, China). The rat/mouse insulin kit (EZRMI-13K) was purchased from EMD Millipore Corporation. A mouse MMP12 ELISA kit (ARG81803) was purchased from Arigobiolaboratories company. The human CXCL1/KC kit (EK-196) was purchased from Multi Science Company. The data of ELISA was analyzed by using Curve Expert1.4 software.

2. 10 Western blotting

Tissue samples (50-80 mg) or cells were homogenized and lysed with radio immunoprecipitation assay buffer (Thermo Scientific, 89900) containing with protease and phosphatase inhibitors, and then the supernatants were clarified by centrifugation. Quantitative analysis based on the bicinchoninic acid (BCA) protein assay was used to detect the protein concentration. Denatured proteins in the supernatant were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (Millipore Corporation, Billerica, MA, USA) membranes, blocked with 5% nonfat milk powder at room temperature, and then incubated with the primary antibodies (1:1000) overnight at 4°C. The next day, the protein strips were further incubated with HRP-conjugated anti-rabbit secondary

antibodies (1:5000) and the bands were visualized after exposure to film after incubated with enhanced chemiluminescence detection reagents. These bands were visualized after exposure to film. We used imageJ software to analyze the optical density of the protein band. All experiments were repeated three times.

2. 11 Cytokine antibody assay

Qualitative assessment of 38 cytokines in the supernatants from media for culture (+MC38) or non-culture with MC38 (-MC38) cells was performed with the Ray Bio Mouse Cytokine Antibody Array 5 (AAM-INF-1-2, Ray Biotech) according to the provided manufacturer's protocol. The detection procedure was as follows: The membranes were blocked by incubation with the blocking buffer. Diluted biotin-conjugated anti-cytokine antibodies and HRP-conjugated streptavidin were detected to immunocomplexes. The visualized X-ray film was exposed to chemiluminescence for quantification with ECL chemiluminescence. Semiquantitative data analysis was performed for signal intensity ImageJ, and positive controls were used to normalize the results. Every cytokine to positive control ratio (cytokine density/positive control density) is used to represent the relative content of every cytokine. The cytokines and their abbreviations are shown in ([Figure 3-figure Supplement 7](#)).

2. 12 Oral glucose tolerance test, Insulin tolerance test and Blood glucose level measurement

Mice were fasted for 8 hours and then housed overnight. And then, they were given either oral glucose (2 g/kg body weight or an intraperitoneal insulin injection (0.75 IU/kg). The tail vein blood glucose level of mice was measured for tail blood glucose at 0, 15, 30, 45, 60, 90, and 120 minutes after treatment. Blood samples were collected at 0, 15, 30 and 60, 90, and 120 minutes for glucose measurement in tail

231 vein blood with a blood glucose meter (Johnson & Johnson) at the specified time points. All blood glu-

232 cose levels were performed using the glucose meters.

233 2. 13 Serum lipid composition assay

234 The levels of total cholesterol (TC), total triglycerides (TG), high density lipoprotein cholesterol (HDL-

235 C) and low density lipoprotein cholesterol (LDL-C) were determined according to the manufacturer's

236 protocols. The assay kits all were purchased from Jiancheng (Nanjing, China).

237 2. 14 Cell culture

238 The RAW264.7 cell line, MC38 cell line and CT26 cell line were purchased from the American Typical

239 Culture Collection (ATCC) and cultured according to international standard protocols. All cell lines were

240 maintained in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Scientific HyClone, Beijing,

241 China) +10% fetal bovine serum (FBS, HyClone) + 1% penicillin (HyClone)/streptomycin (HyClone).

242 The RAW 264.7 cell line, MC 38 cell line and CT26 cell line were cultured in DMEM. All cell lines in

243 the experiments were incubated with a mixture of 95% air and 5% CO₂.

244 2. 15 Co-culture experiment

245 All cells were grown in DMEM+10% FBS+10% penicillin-streptomycin. The co-culture of RAW264.7

246 and MC38/CT26 cells was seeded into performed using a chamber with filter inserts (pore size, 0.4 μm)

247 in 6-well dishes (Corning, NY, USA). All cell lines could not pass to the filter because the pore size of

248 the filter was smaller than the diameter of cell lines. RAW264.7 cells non co-cultured with MC38/CT26

249 cell lines (-MC38/CT26) were used as the negative controls. MC38/CT26 cell lines (control, 1x10⁴, 3x10⁴,

250 5x10⁴) were seed in upper chamber, while RAW264.7 cells (1-2x10⁵) were seed in the lower chamber.

251 We can separate physically RAW264.7 cells or MC38/CT26 cell lines to obtain RAW264.7

(+MC38/CT26) cells in lower chamber. The RAW264.7 cells were homogenized and lysed with radio immunoprecipitation assay buffer to quantitative analysis and then subjected to western blotting.

2. 16 Interleukin 6 treatment of macrophages

The Interleukin 6 (IL-6) freeze-dried powder was purchased from Pepertech (216-16) and was dissolved in trehalose-bovine serum albumin aqueous solution. RAW264.7 cells ($1-2 \times 10^5$) were seeded into 6-well plates and treated with increasing different doses of IL-6 (+IL-6, 0, 2, 5 10, 30 ng/ml) for 72 hours. Next, RAW264.7 cells ($1-2 \times 10^5$) were treated continuously with IL-6 (+IL-6, 30 ng/ml) for 0, 3, 6, and 9 hours. Cells incubated with fresh media were used as the untreated (-IL-6) negative controls. Finally, western blotting was used to quantify MMP12 in RAW264.7 cells under different conditions.

2. 18 Isolation of primary peritoneal macrophages

24-week-old WT and *Apc^{Min/+}* mice were sterilized with 75% ethanol after cervical dislocation. The mouse abdomen was opened from the peritoneum, and 5 mL fetal bovine serum was injected with a syringe, which was allowed to remain inside the abdomen for 5 minutes, with gentle massaging for 30 s. The peritoneal fluid was collected, and this fluid was transferred to a 15 mL sterile tube to obtain peritoneal macrophages. After centrifugation (4°C, 1000 rpm) for 10 minutes, the supernatant was removed, and the collected cells were resuspended in Dulbecco's Modified Eagle's Medium. The resuspended cells were cultured in a petri dish for 2 hours (37°C), and the primary peritoneal macrophages were prepared from the remaining adherent cells after the medium was removed.

2.19 MMP12 and Peptide experiments:

Recombinant mouse MMP-12 protein (3467-MPB-020) was purchased from R&D Systems, Inc. According to the instructions, dissolve MMP12 in a buffer containing 50mM Tris, 10 mM CaCl₂, 150 mM

NaCl, 0.05% (w/v) Brij-35, 5 μ M ZnCl₂, pH 7.5 at a concentration of 250ug/ml. Insulin polypeptide

and insulin-like factor polypeptide were synthesized by ChinaPeptides Co., Ltd.

The fluorescent peptide sequence:

(1) Insulin:5-FAM-NQHLCGSHLVEALYLVCGERGFFYTPK(Dabcyl);

(2) insulin-like growth factor 1: 5-FAM-GPETLCGAELVDALQFVCGDRGFYFNK(Dabcyl). Ac-

cording to the instructions, the peptide freeze-dried powder was dissolved in 25% ACN and 75%

ddH₂O solvent at a concentration of 1 mg/ml.

The two experiments are as follows: (1) Fluorescence intensity: After mixed incubation of MMP12 and

peptide (37°C, 2hours), the fluorescence intensity was measured with a fluorescence microplate reader.

(2) MS Analysis Report: After the MMP12 and peptide were mixed and incubated, the lower liquid af-

ter being filtered by a 34KD filter is subjected to Electrospray Ionization Mass Spectrometry (IMS) to

detect its characteristic peaks. The experimental conditions are: Ion Source: ESI, Capillary (KV): $\pm$

(2500~3000), Desolvation(L/hr):800, Desolvation Temp:450°C, Cone(V): 30~50, Run Time: 1min.

2. 20 Mice administration

All 17-week-old Apc^{Min/+} mice were randomly divided into three groups (five mice in each group). A

group of Apc^{Min/+} mice were administered intragastric with MMP12 inhibitor (MMP408) at a dose of

5mg/kg, and the other group was intraperitoneally administered with 5-FU (30mg/kg) combined with a

dose of intragastric administration of MMP408. At the same time, Apc^{Min/+} mice injected with normal

saline intraperitoneally served as a control group. The body weight was weighed by the administration

every 2 days and after this continued for 10 days, the final weight change from the initial body weight

was calculated.

294 2. 21 Data calculation and Statistical analysis

295 All mouse organ ratios represented the percentage of the organs/tissues weight compared to the body
 296 weight. The skeletal muscle to weight ratio was (Gastrocnemius +Soleus muscle)/body (%). All data
 297 were processed with GraphPad Prism 8.0 software and are presented as the means $\pm$ standard deviation
 298 (SD). A two-tailed test was used. Statistically significant differences were set at $*P < 0.05$, $**P < 0.01$,
 299 $***P < 0.001$, $****P < 0.0001$. All schematic images were created with BioRender.com.

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3 Results

3.1 Knockout MMP12 can suppress weight and muscle loss in $Apc^{Min/+}$ mice

To investigate the weight dynamics of the mice, we determined the mouse body weight by retroactive examination of the body weight from 5-week to 24-weeks old. The current weight curves have shown that compared with the weight gain in wild type (WT) mice over time, the weight of $Apc^{Min/+}$ mice reached its maximum peak at 12-week-old and then declined until to die at approximately 24-week-old ([Figure 1A](#)). Surprisingly, in comparison to the $Apc^{Min/+}$ mice control, the body weight of $Apc^{Min/+}$; $MMP12^{-/-}$ mice increased by approximately 70% at the same age ([Figure 1B](#)). While, there were no significant differences between WT mice and $MMP12^{-/-}$ mice in body weight ([Figure 1C](#)). As well known, the $Apc^{Min/+}$ mouse is a model of muscle loss with cancer cachexia (CAC) and intestinal tumor burden. Because weight loss induced by CAC may be related to the wasting of skeletal muscle weight and fat weight (Peixoto da Silva et al., 2020a), here, we verified whether the weight gain of $Apc^{Min/+}$ mice caused by MMP12 knockout was due to reduction of fat and skeletal muscle loss at 24-week-old CAC stage in $Apc^{Min/+}$ mice. We assessed the histologic white adipose tissue (WAT), compared with that in WT mice, the WAT-to-body weight ratio in $Apc^{Min/+}$ mice decreased and the weight ratio of $Apc^{Min/+}$; $MMP12^{-/-}$ mice tended to increase compared with that of $Apc^{Min/+}$ mice but the increase was not statistically significant ([Figure 1D](#)). However, a significant increase of approximately 4.5% in the muscle-to-body weight ratio was observed in $Apc^{Min/+}$; $MMP12^{-/-}$ mice compared with $Apc^{Min/+}$ mice ([Figure 1E](#)). To further confirm the histological changes of WAT ([Figure 1F](#)) and muscle area ([Figure 1H](#)) in the four mice group at 24-week-old, we performed hematoxylin and eosin staining to assess the histological area by the ImageJ software ([Figure 1F, H](#)). We observed the fat area is larger in $MMP12^{-/-}$ mice

compared with WT mice, but there has no difference between $Apc^{Min/+}$ mice and $Apc^{Min/+}; MMP12^{-/-}$ mice ([Figure 1G](#)). The H&E staining of muscle to show that the area of $Apc^{Min/+}; MMP12^{-/-}$ mice is estimated to be approximately 1-2-fold larger than $Apc^{Min/+}$ mice ([Figure 1I](#)). Meanwhile, no difference in food intake was observed between $Apc^{Min/+}$ and $Apc^{Min/+}; MMP12^{-/-}$ mice ([Figure 1-figure Supplement 3A](#)). Taken together, knocking out MMP12 prevents muscle from wasting in tumor-burden ($Apc^{Min/+}$) mice at CAC stage, but not in WT mice.

3. 2 MMP12 is upregulated in muscle tissue and peritoneal macrophages of $Apc^{Min/+}$ mice

To confirm whether MMP12 is expressed in muscle tissue, we use immunohistochemical staining, immunofluorescence staining and western immunoblotting to detect the expression of MMP12 in muscle. The immunohistochemistry results proved that MMP12 positive staining was expressed not only in skeletal muscles from clinical individuals ([Figure 2A](#), [Figure 2-figure Supplement 3B](#)), but also in WT mice ([Figure 2B](#)). In order to investigate why the reduction in muscle loss caused by knocking out MMP12 only occurred after tumor-bearing mice and not in WT mice, furthermore, we used immunohistochemistry methods to detect MMP12 expression in muscle from 24-week-old WT mice and $Apc^{Min/+}$ mice ([Figure 2C](#)). The results of immunohistochemical staining showed that MMP12-positive staining was increased in the muscle of $Apc^{Min/+}$ mice compared with that in WT mice at 24-weeks old by image J ([Figure 2D](#)). Because MMP12 is mainly secreted by macrophages (Lee et al., 2014a; Lee et al., 2014b), next, we performed double immunofluorescence (IF) to detect the expression of macrophages and MMP12 in muscle and found that the F4/80 and MMP12 markers were colocalized in mice ([Figure 2E](#)). Quantitative PCR (qPCR) revealed that a tendency towards higher MMP12 mRNA levels in peritoneal macrophages (as described in the Materials) was seen in $Apc^{Min/+}$ mice ([Figure 2F](#)), which

was consistent with immunohistochemistry results. We detected the dynamic circulating serum MMP12 level by enzyme-linked immunosorbent assay and found that the MMP12 levels in 9-, 15-, and 24-week-old WT mice and Apc^{Min/+} mice did not differ ([Figure 2G](#)). Taken together, MMP12 is expressed in muscle tissue and co-localized with macrophage. In comparison to the WT mice control, MMP12 is increased in skeletal muscle tissue and peritoneal macrophages of Apc^{Min/+} mice, but no difference in serum was witnessed between the two groups.

3. 3 Tumor cells can secrete IL-6

Previous studies have shown that interleukin 6 (IL-6) is one of the cytokines predictive muscle loss induced by CAC(Bonetto et al., 2012; Kim et al., 2013; Mahadik and Sujata, 2013; Mauer et al., 2014), it can accelerate muscle loss induced by CAC. And tumor cells are an important source of IL-6(Carson and Baltgalvis, 2010b; Han et al., 2018; Pettersen et al., 2017). The clinical literature data also suggested that among many CAC-muscle loss patients who lost weight and were close to death, IL-6 was almost the only increased cytokine among many factors. Therefore, we mainly focus on whether IL-6, which is related to muscle loss, is caused by tumors (Carson and Baltgalvis, 2010b). We observed that the clinical colorectal cancer patients had significantly higher serum IL-6 levels than the normal healthy group ([Figure 3A](#)). In vivo, a similar trend was found in Apc^{Min/+} mice, and serum IL-6 levels in Apc^{Min/+} mice were significantly increased compared with WT mice at 15-24-week-old ([Figure 3B](#)). We demonstrated that the IL-6 mRNA levels were higher in intestinal tumors of Apc^{Min/+} mice than in normal intestinal epithelium of WT mice by qPCR ([Figure 3C](#)). Previous study reported MC38 cells and CT26 cells all can secrete IL-6(Li et al., 2018). Therefore, tumor cells can be the source of IL-6. In vitro, we used protein microarrays to detect inflammatory factors in the supernatant of mouse colorectal carcinoma MC38 cell lines

and the results showed that IL-6 expression was higher in the supernatant after cultured with MC-38 cells (Figure 3D, E). So, tumor cells can secrete IL-6.

3. 4 Tumor-derived IL-6 can upregulate MMP12 in macrophage

Because some previous studies reported that IL-6 may not directly lead to muscle loss in CAC(Carson and Baltgalvis, 2010a; Franckhauser et al., 2008). IL-6 in the tumor microenvironment may be an important determinant of alternative macrophage activation and induce macrophage M2 polarization and M2 macrophages can produce MMP12(Suzuki et al., 2017; Wang et al., 2018). Taken together, we speculated whether tumors regulate macrophage MMP12 by secreting IL-6 to affect muscle loss. To uncover the underlying mechanism communication between tumor-derived IL-6 and macrophages, we performed cell experiments in vitro (as described in the Materials). Mouse macrophage RAW264.7 cells co-cultured with mouse colorectal cancer MC38 cells (CT 26 cells) for 72hours to detect macrophage MMP12 by western blotting (Figure 4A) and the results confirmed that RAW264.7 cells exhibited increased MMP12 expression as the number of MC38 cells increased, with RAW264.7 cells cultured alone as the negative control group (Figure 4B, C). Similar trends were observed in CT26 cells (Figure 4D, E). We further treated RAW264.7 cells with IL-6 in different methods. RAW264.7 cells were seeded into 6-well plates and treated with increasing doses of IL-6 (0, 2, 5 10,30ng/ mL) for 72h. Next, RAW264.7 cells were treated continuously with IL-6 (30 ng/ml) for 0, 3, 6, and 9hours. Cells incubated with fresh media were used as the untreated negative controls (Figure 4F). We found that within a certain concentration range (<30ng/ml), as the IL-6 dose increased, the expression of MMP 12 in RAW264.7 cells also increased when treated with IL-6 (Figure 4G, H). Next, the expression of MMP12 in RAW264.7 increased as the stimulation time prolonged when the RAW264.7 cells treated with IL-6(30ng/ml) (Figure 4I, J).

Meanwhile, immune gene data proved that IL-6 receptor (IL-6R) is highly expressed on myeloid cells, including F480⁺ macrophages ([Figure 4K](#)). The previous studies proved that IL-6 can be derived from MC38 and CT26 tumor cells (Li et al., 2018). Taken together, these findings suggest that tumor-derived IL-6 can stimulate macrophages and up-regulate MMP12 in macrophages.

3. 5 MMP12 can degrade insulin and insulin-like growth factor-1

The present results have proved that tumor-derived IL-6 can up-regulate MMP12 in macrophages. Knockout of MMP12 can reduce muscle loss in Apc^{Min/+} mice. Recently, it has been demonstrated that insulin and insulin-like growth factor 1(IGF-1) have complex anabolic effects and are important regulators of muscle remodeling that can mediate muscle atrophy(Baker Rogers et al., 2020; Dev et al., 2019; Han et al., 2019; Masi and Patel, 2020; Takayama, 2019). Moreover, Jung-Ting Lee proposed that MMP12 expression significantly promoted insulin resistance and that insulin may be regulated by resident macrophages(Lee et al., 2014a). To understand the molecular mechanism underlying muscle loss by macrophage MMP12, we further examined the relationship between MMP12 and insulin (IGF-1) which affects muscle loss. Firstly, after the labeled insulin polypeptide is incubated with serum, the absorbance increases ($\lambda=488\text{nm}$) ([Figure 5A](#)). Because IGF-1 is similar in structure to insulin, furthermore, we verified the relationship between fluorescently labeled insulin (IGF-1) and MMP12, and measured its fluorescence intensity and characteristic peak changes ([Figure 5B](#)). We found that when the dose of insulin fluorescent peptide is constant, the more MMP12 protein, the stronger the fluorescence intensity. Similar Tendencies were observed in the IGF-1([Figure 5C](#)). The qualitative results of electrospray ionization mass spectrometry showed that the characteristic peak of insulin fluorescent peptide ($m/z = 436.99$) disappeared after incubation with MMP12 protein ([Figure 5-figure Supplement](#)

[2A](#)). When the IGF-1 polypeptide was incubated with MMP12, its characteristic peak ($m/z = 436.68$) disappeared ([Figure 5-figure Supplement 2B](#)). Taken together, MMP12 can indeed degrade insulin and IGF-1. It seems that the degradation of MMP 12 to IGF-1 is stronger than that of insulin.

3.6 MMP12 inhibitor can rescue weight loss of $Apc^{Min/+}$ mice

It is reported that insulin and insulin-like growth factor 1 can indeed affect the muscle loss caused by cachexia and exacerbate weight loss (Baker Rogers et al., 2020; Dev et al., 2019; Han et al., 2019; Masi and Patel, 2020; Takayama, 2019). Therefore, we are concerned about whether the inhibition of MMP12 that degrades insulin and insulin-like growth factor 1 affects the weight change of cachexia mice. To investigate the effect of inhibiting MMP12 on colorectal cancer $Apc^{Min/+}$ mice, we combined the MMP12 inhibitor (MMP408) and a classic clinical anti-colon cancer drug (5-FU) ([Figure 6A](#)). After 2 weeks of administration in $Apc^{Min/+}$ mice, at 17-week-old, the results showed that the weight loss in the MMP12 inhibitor group (+MMP408) only accounted for 5% of the basal body weight and was only one third of that of the control normal saline group (+Control). There was a significant difference between the two groups. In the MMP12 inhibitor and anticancer drug combination group (+MMP408+5-FU), the weight change was decreased by approximately 8% of the basal body weight and was half that of the normal saline group (+Control). Unfortunately, there were no changes in body weight in the anticancer drug combination group (+MMP408+5-FU), when compared with the MMP12 inhibitor group alone (+MMP408) ([Figure 6B](#)). In summary, the above experiments proved that specifically inhibiting MMP12 at the CAC stage of weight loss in $Apc^{Min/+}$ mice can reduce weight loss.

4 Discussion

Researches on weight loss have received more and more attention in many fields, such as diabetes, abnormal thyroid metabolism, weight control, etc, among which, muscle loss caused by malnutrition similar to cancer cachexia (CAC) is more worrying. More than four-fifths of patients with CAC die from extreme loss of body weight and skeletal muscle. Our study suggested that MMP12 plays a new role in controlling weight and muscle loss and inhibiting MMP12 can reverse the body weight reduction with CAC. In details, the major findings of our study are as follows: (1) MMP12 promotes weight loss and accelerates the deterioration of CAC. The loss of weight and muscle induced by CAC was reduced in *Apc^{Min/+}* by MMP12 knockout. (2) In vivo, MMP12-positive immunostaining was found in muscle of human and mice. MMP12 was co-labeled with macrophages in the muscle tissue in situ. Importantly, MMP12 positive staining was substantially increased in the muscle and peritoneal macrophages from *Apc^{Min/+}* mice compared with those from wild type (WT) mice. (3) Clinically, serum interleukin 6 (IL-6) increased in cancer patients. A similar increasing trend was found in serum and tumor tissues of *Apc^{Min/+}* mice compared to those from WT mice. Crucially, IL-6 has been shown to be directly secreted by MC38 tumor cells. (4) In vitro, we proved that tumor cells have a positive relationship with MMP12 secreted by macrophages. At the cellular level, we found for the first time that macrophages can be stimulated and regulated by IL-6, and the level of MMP12 in macrophages was up-regulated. (5) Underlying mechanism, MMP12 can degrade insulin and insulin-like growth factor 1. In our study, the degradation effect of MMP12 on insulin-like growth factor 1 was proved for the first time, and it was found that the degradation effect of MMP12 was stronger than that of insulin. (6) Inhibiting MMP12 prevent weight loss in *Apc^{Min/+}* mice at CAC stage. In summary, the present study uncovered a novel

mechanism that MMP12 promotes weight and muscle loss. The crosstalk between tumor cells and macrophages is that MMP12 is upregulated by tumor cell-derived IL-6 and MMP12 can degrade insulin and insulin-like growth factor 1, affecting glycolipid metabolism, resulting in weight loss ([Figure 7](#)). Weight and muscle loss induced by CAC is main cause of death in cancer patients worldwide. The clinical definition of CAC by Fearon criteria includes the following characteristics: weight loss > 5% or weight loss > 2% and a BMI < 20 kg/m² or sarcopenia(van der Werf et al., 2018). In our study, 15-24-weeks old *Apc*^{Min/+} mice with weight loss > 15%, as well as muscle (gastrocnemius and soleus) loss, and certain other symptoms such as anemia, were considered as the characteristics of CAC. Our data exhibited that muscle weight and muscle cross-sectional area increased in *Apc*^{Min/+}, *MMP12*^{-/-} mice compared with *Apc*^{Min/+} mice, which indicating that knocking out MMP12 may suppress the decrease of skeletal muscle.

As a kind of matrix metalloproteinase family, MMP12 is also called macrophage elastase. It was previously reported that MMP12 can decompose various extracellular matrix components and vascular components, and MMP12 is involved in tumor cell invasion and metastasis. In 2014, Lee Jung-Ting had explored the role of MMP12 in white adipose tissue expansion on high-fat feeding(Jung-Ting et al., 2014), while the function of MMP12 under the lack of nutrition yielded has not been studied. We firstly tried to build a model with tumor-bearing environment lacking nutrition in *Apc*^{Min/+} mice hybridized with *MMP12*^{-/-} mice. Unexpectedly, under the condition of tumor-bearing mice, knocking out MMP12 caused a decrease in muscle loss, but not affect white adipose tissue.

A mRNA analysis of the data got from The Cancer Genome Atlas (TCGA) for GTEx, Illumina, BioGPS and SAGE of MMP12 gene in normal human tissues ([Figure 1-figure Supplement 3C](#)). In our

study, we focus on the liver, muscle and fat tissues. We weighed these mice tissues at 24-weeks old, and performed histological evaluation using H&E staining. The liver weight has difference between $Apc^{Min/+}$ mice and $Apc^{Min/+}; MMP12^{-/-}$ mice ([Figure 1-figure Supplement 4 A](#)), however, H&E staining showed that knocking out MMP12 had no more histology effect on the WT mice and $Apc^{Min/+}$ mice ([Figure 1-figure Supplement 4B](#)). Similarly, knocking out MMP12 in $Apc^{Min/+}$ mice did not cause changes in white fat, even though knocking out MMP12 in the wild background can indeed cause white fat increase and expansion, which in agreement with Lee Jung-Ting, who showed in 2014 that knocking out MMP12 can increase fat expansion when performing high-fat feeding in WT mice(Jung-Ting et al., 2014). Notably, the ratio of brown adipose tissue-to-body weight decreased in $Apc^{Min/+}$ mice compared with $Apc^{Min/+}; MMP12^{-/-}$ mice ([Figure 1-figure Supplement 4C](#)), and the area of brown adipose tissue in $MMP12^{-/-}$ mice expansion, and serving the $Apc^{Min/+}$ mice as the control group, the same tendency showed in $Apc^{Min/+}; MMP12^{-/-}$ mice ([Figure 1-figure Supplement 4D](#)), suggesting there may be a tendency to convert to white adipose tissue. However, the reasons have not been extremely explored in the current research. We hypothesized that MMP12 may play an important role in the conversion of brown adipose tissue and white adipose tissue. and it may be associated with a CAC energy consumption and even as useful for researcher focusing on weight loss drug. MMP12 is mainly derived from macrophages. In view of the fact that I have found that knocking out MMP12 can increase muscle weight and cross-sectional area, we conducted related experiments on whether MMP12 is expressed on muscles. Studies have shown that MMP12 is existed and co-labeling with macrophages in our study. Notably, MMP12 levels increase in muscle tissues and peritoneal macrophages not in serum.

IL-6 is mainly secreted by a variety of immune cells and is also highly expressed in a variety of cancer cells(Mauer et al., 2015). Our in vitro studies proved that MC38 cell lines can secrete IL-6. Our animal experiments in vivo confirmed that the serum IL-6 of Apc^{Min/+} mice was also higher than that of WT mice at 15 weeks and 24 weeks, which is consistent with the previous study(Baltgalvis et al., 2008). IL-6 mRNA levels in intestinal tumors are increased compared with normal intestinal epithelial tissue, which echoes our data with the increased serum IL-6 in clinical tumor patients(Nikiteas et al., 2005). In short, maybe, the increased IL-6 in the tumor then circulates into the blood. Of course, the cytokines secreted by MC38 cells also include monocyte chemoattractant protein1(MCP1) and keratinocyte-derived chemokine (KC) which can recruit macrophages(Barcelos et al., 2004; Engin, 2017; Wang et al., 2011). But unfortunately, our experiments have shown that only increased at mRNA levels of intestinal tumors in Apc^{Min/+} mice at CAC stage but serum MCP1 did not change at the CAC stage in Apc^{Min/+} mice (Figure 3-figure Supplement 5A, B). There was no difference in serum KC and KC mRNA levels in late tumors in mice (Figure 3-figure Supplement 5C, D). Similarly, clinically, serum KC did not differ between normal healthy individuals and colorectal cancer patients (Figure 3-figure Supplement 5E). In summary, we suspected that there may be a possibility that MCP1 and KC can also cooperate with IL-6 to recruit macrophages, thereby activating alternative macrophages to polarize M2 macrophages, resulting in MMP12 secretion. The above results suggested that in the period of CAC, the key is that IL-6 secreted by tumor cells plays a major role(Baltgalvis et al., 2008). In Table 2. we summarized the research on IL-6 on body weight and muscle loss, which mostly demonstrated that IL-6 may have indirect effect on body weight and muscle. Especially, Kristen A Baltgalvis pointed out knocking out IL-6 can reduce muscle consumption in Apc^{Min/+} mice(Baltgalvis et al., 2008).

The crosstalk between tumors and inflammatory factors is well known(Talbert et al., 2018b; Zhang et al., 2008). We use co-culture experiments in MC38 cell lines and CT26 with RAW264.7 cell lines to demonstrate that macrophage MMP12 increase treatment with IL-6. We speculated that this is caused by the IL-6 secreted by tumor cells, and we firstly proved that IL-6 can directly upregulate macrophage MMP12. However, we did not determine whether the IL-6 produced by macrophages acts on macrophages themselves. Similarly, we have not explored whether other cytokines secreted by tumors or macrophages themselves play a synergistic or indirect role along with IL-6.

In present study, we used fluorescence intensity measurement and ionization mass spectrometry methods to prove that insulin and IGF-1 can indeed be degraded by MMP12, but the specific sites and amino acids where insulin and IGF-1 were broken have not been further proved. At the same time, we could not prove to completely rule out whether MMP12 itself generates characteristic peaks from fracture ([Figure 5-figure Supplement 2A, B](#)).

MMP12, as a macrophage matrix metalloproteinase, has been repeatedly reported to degrade insulin and affect insulin sensitivity. We verified MMP12 can degrade insulin or IGF-1 in vitro, which is consistent with the previous study(Kettner et al.). As the two key hormones in tumor microenvironment, insulin resistance is correlated with insufficient insulin, lack of insulin receptor, or decreased insulin sensitivity, which will reduce the uptake of glucose in organs, which suggests that MMP12 is closely related to glycolipid metabolism, leading to the loss of skeletal muscle and adipose tissue(Baker Rogers et al., 2020; Dev et al., 2019; Han et al., 2019; Masi and Patel, 2020; Takayama, 2019). The insulin kits and insulin tolerance test and oral glucose tolerance test results showed that the knocking out MMP12 in $Apc^{Min/+}$ mice may reduce insulin levels or increase insulin sensitivity, reversing insulin resistance

([Figure 5-figure Supplement 6A-H](#)), but is not related to basic function of islets according to H&E staining and IHC staining ([Figure 5-figure Supplement 4E-H](#)). We tested four blood lipid levels with the kit, and the results showed that when $Apc^{Min/+}$ mice were knocked out MMP12, total triglycerides decreased in the early and middle stages, but high density lipoprotein cholesterol increased in all age groups, total cholesterol and low density lipoprotein cholesterol not changed across the 4 groups ([Figure 5-figure Supplement 6I-L](#)). So, does IL-6 regulating MMP12 help restore muscle loss caused by cachexia? Clinical inhibiting IL-6 may reduce CAC patients with weight loss. However, long-term treatments with high-dose IL-6 may cause additional side effects, such as exacerbating CAC resulting in more muscle loss(Wada et al., 2017). After all, IL-6 acts on muscles indirectly. Surprisingly, MMP12, as the downstream of IL-6, can significantly suppress weight loss when being specifically inhibited in mice, although the effect is not more obvious after combined treatment with the classic colorectal cancer chemotherapy drug 5-FU. Clinically, suppressing MMP12 may reduce the possibility of insulin degradation, suggesting that our findings may as a method to treat directly glucose deficiency, CAC and complications of CAC. It will not only provide a new direction for reducing the blood glucose and blood lipid levels of cancer patients but also bring new research ideas for the clinical treatment of diabetes caused by insulin deficiency.

In summary, our results identified that knocking out MMP12 in $Apc^{Min/+}$ mice significantly reduced muscle loss caused by CAC. We determined a positive correlation with between tumor-derived IL-6 and macrophage MMP12 in colorectal cancer. MMP12 can degrade insulin and IGF-1, reversing the insulin resistance in CAC to regulate tumor glycolipid metabolism. Therefore, MMP12 is a double-edged sword

for tumor microenvironment, but, inhibiting MMP12 may represent a new potential targeting for the treatment of clinical patients with weight loss.

Authors' Contributions

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Ethics Statement for Human Subjects Research or Animal Experimentation

All mouse experimental protocols were approved by the animal experimental ethics committee of Guangdong Pharmaceutical University. The animal ethics approval number was gdpulac2019019. All tests were carried out with the approval of the Guangdong Medical Laboratory Animal Center, Guangzhou, China. All experiments for clinical patients in this study were obtained by the approval of the Guangzhou Human Research Ethics Committee, Provincial First Affiliated Hospital of Guangdong Pharmaceutical University, China. The clinical ethics approval number was EC-AF-019.

References

- Amor, M., and Moreno-Viedma, V.J.M.M. Identification of Matrix Metalloproteinase-12 as a Candidate Molecule for Prevention and Treatment of Cardiometabolic Disease. *22*. PMID: [27385318](#)
- Atlı, Ö. (2017). Matrix metalloproteinases are possible targets in monocrotaline-induced pulmonary hypertension: investigation of anti-remodeling effects of alagebrium and everolimus. *17*, 8-17. PMID: [27182612](#)
- Ayoub, E.A., Dubey, A., Imani, J., Botelho, F., Kolb, M.R.J., Richards, C.D., and Ask, K.J.S.R. Overexpression of OSM and IL-6 impacts the polarization of pro-fibrotic macrophages and the development of bleomycin-induced lung fibrosis. *7*, 13281. PMID: [29038604](#)
- Baker Rogers, J., Syed, K., and Minter, J.F. (2020). Cachexia. In StatPearls (Treasure Island FL: © 2020, StatPearls Publishing LLC.). PMID: [29262118](#)
- Baltgalvis, K.A., Berger, F.G., Peña, M.M.O., Mark Davis, J., White, J.P., and Carson, J.A. (2010). Activity level, apoptosis, and development of cachexia in Apc(Min/+) mice. *Journal of applied physiology* (Bethesda, Md : 1985) *109*, 1155-1161. PMID: [PMC2963323](#)
- Baltgalvis, K.A., Berger, F.G., Pena, M.M., Davis, J.M., Muga, S.J., and Carson, J.A. (2008). Interleukin-6 and cachexia in ApcMin/+ mice. *Am J Physiol Regul Integr Comp Physiol* *294*, R393-401. DOI: [10.1152/ajpregu.00716.2007](#)
- Baracos, V.E., Martin, L., Korc, M., Guttridge, D.C., and Fearon, K.C.H. (2018). Cancer-associated cachexia. *Nature Reviews Disease Primers* *4*, 17105. DOI: [10.1038/nrdp.2017.105](#)
- Barcelos, L.S., Talvani, A., Teixeira, A.S., Cassali, G.D., Andrade, S.P., and Teixeira, M.M. (2004). Production and in vivo effects of chemokines CXCL1-3/KC and CCL2/JE in a model of inflammatory angiogenesis in mice. *Inflammation research : official journal of the European Histamine Research Society* [et al] *53*, 576-584. DOI: [10.1007/s00011-004-1299-4](#)
- Bauters, D., Van Hul, M., and Lijnen, H.R. (2013). Macrophage elastase (MMP-12) in expanding murine adipose tissue. *Biochimica et Biophysica Acta (BBA) - General Subjects* *1830*, 2954-2959. DOI: [10.1016/j.bbagen.2012.12.024](#)

-
- 634 Bing, C. (2011). Lipid mobilization in cachexia: mechanisms and mediators. Current opinion in
635 supportive and palliative care *5*, 356-360. DOI: [10.1097/SPC.0b013e32834bde0e](https://doi.org/10.1097/SPC.0b013e32834bde0e)
- 636 Bonetto, A., Aydogdu, T., Jin, X., Zhang, Z., Zhan, R., Puzis, L., Koniaris, L.G., and Zimmers, T.A.
637 (2012). JAK/STAT3 pathway inhibition blocks skeletal muscle wasting downstream of IL-6 and in
638 experimental cancer cachexia. Am J Physiol Endocrinol Metab *303*, E410-E421.
639 PMCID: [PMC3423125](https://pubmed.ncbi.nlm.nih.gov/23423125/)
- 640 Bonetto, A., Rupert, J.E., Barreto, R., and Zimmers, T.A. (2016). The Colon-26 Carcinoma Tumor-
641 bearing Mouse as a Model for the Study of Cancer Cachexia. Journal of visualized experiments :
642 JoVE, 54893. PMCID: [PMC5226332](https://pubmed.ncbi.nlm.nih.gov/25226332/)
- 643 Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer
644 statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185
645 countries. CA: a cancer journal for clinicians *68*, 394-424. DOI: [10.3322/caac.21492](https://doi.org/10.3322/caac.21492)
- 646 Brody, H. (2015). Colorectal cancer. Nature *521*, S1. DOI: [10.1038/521S1a](https://doi.org/10.1038/521S1a)
- 647 Carson, J.A., and Baltgalvis, K.A. (2010a). Interleukin 6 as a key regulator of muscle mass during
648 cachexia. Exerc Sport Sci Rev *38*, 168-176. DOI: [10.1097/JES.0b013e3181f44f11](https://doi.org/10.1097/JES.0b013e3181f44f11)
- 649 Carson, J.A., and Baltgalvis, K.A. (2010b). Interleukin 6 as a key regulator of muscle mass during
650 cachexia. Exerc Sport Sci Rev *38*, 168-176. PMCID: [PMC3065300](https://pubmed.ncbi.nlm.nih.gov/23065300/)
- 651 Center, M.M., Jemal, A., Smith, R.A., and Ward, E. (2009). Worldwide variations in colorectal
652 cancer. CA: a cancer journal for clinicians *59*, 366-378. DOI: [10.3322/caac.20038](https://doi.org/10.3322/caac.20038)
- 653 Daou, H.N. (2020). Exercise as an anti-inflammatory therapy for cancer cachexia: a focus on
654 interleukin-6 regulation. American journal of physiology Regulatory, integrative and comparative
655 physiology *318*, R296-R310. DOI: [10.1152/ajpregu.00147.2019](https://doi.org/10.1152/ajpregu.00147.2019)
- 656 Deshmukh, Biology, A.S.J.H.M., and Investigation, C. Insulin-stimulated glucose uptake in healthy
657 and insulin-resistant skeletal muscle. DOI: [10.1515/hmbci-2015-0041](https://doi.org/10.1515/hmbci-2015-0041)

-
- 658 Dev, R., Del Fabbro, E., and Dalal, S. (2019). Endocrinopathies and cancer cachexia. Current
659 opinion in supportive and palliative care *13*, 286-291. DOI: [10.1097/SPC.0000000000000464](https://doi.org/10.1097/SPC.0000000000000464)
- 660 Engin, A.B. (2017). Adipocyte-Macrophage Cross-Talk in Obesity. Advances in experimental
661 medicine and biology *960*, 327-343. DOI: [10.1007/978-3-319-48382-5_14](https://doi.org/10.1007/978-3-319-48382-5_14)
- 662 Fonseca, G.W.P.D., Farkas, J., Dora, E., Haehling, S.V., and Lainscak, M. (2020). Cancer Cachexia
663 and Related Metabolic Dysfunction. International journal of molecular sciences
664 *21*. PMCID: [PMC7177950](https://pubmed.ncbi.nlm.nih.gov/327177950/)
- 665 Franckhauser, S., Elias, I., Sopasakis, V.R., Ferre, T., Nagaev, I., Andersson, C.X., Agudo, J., Ruberte,
666 J., Bosch, F., and Smith, U. (2008). Overexpression of Il6 leads to hyperinsulinaemia, liver
667 inflammation and reduced body weight in mice. Diabetologia *51*, 1306-1316.
668 DOI: [10.1007/s00125-008-0998-8](https://doi.org/10.1007/s00125-008-0998-8)
- 669 Han, J., Meng, Q., Shen, L., and Wu, G. (2018). Interleukin-6 induces fat loss in cancer cachexia by
670 promoting white adipose tissue lipolysis and browning. Lipids Health Dis *17*, 14-14.
671 PMCID: [PMC5771021](https://pubmed.ncbi.nlm.nih.gov/315771021/)
- 672 Han, X., Møller, L.L.V., De Groote, E., Bojsen-Møller, K.N., Davey, J., Henríquez-Olguin, C., Li, Z.,
673 Knudsen, J.R., Jensen, T.E., Madsbad, S., *et al.* (2019). Mechanisms involved in follistatin-induced
674 hypertrophy and increased insulin action in skeletal muscle. J Cachexia Sarcopenia Muscle *10*,
675 1241-1257. PMCID: [PMC7663972](https://pubmed.ncbi.nlm.nih.gov/327663972/)
- 676 Herremans, K.M., Riner, A.N., Cameron, M.E., and Trevino, J.G. (2019). The Microbiota and Cancer
677 Cachexia. International journal of molecular sciences *20*. PMCID: [PMC6940781](https://pubmed.ncbi.nlm.nih.gov/326940781/)
- 678 Hopkins, B.D., Chantal, P., Du, X., G., W.D., Xiang, L., David, W., C., A.S., D., G.M., Cindy, H., and
679 Nature, L.M.R.J. Suppression of insulin feedback enhances the efficacy of PI3K inhibitors.
680 PMCID: [PMC6197057](https://pubmed.ncbi.nlm.nih.gov/326197057/)
- 681 Hotary, K.B., Allen, E.D., Brooks, P.C., Datta, N.S., Long, M.W., and Cell, S.J.W.J. Membrane Type I
682 Matrix Metalloproteinase Usurps Tumor Growth Control Imposed by the Three-Dimensional
683 Extracellular Matrix. *114*, 0-45. DOI: [10.1016/s0092-8674\(03\)00513-0](https://doi.org/10.1016/s0092-8674(03)00513-0)

-
- 684 Hu, W., Ru, Z., Zhou, Y., Xiao, W., Sun, R., Zhang, S., Gao, Y., Li, X., Zhang, X., and Yang, H. (2019).
685 Lung cancer-derived extracellular vesicles induced myotube atrophy and adipocyte lipolysis via
686 the extracellular IL-6-mediated STAT3 pathway. *Biochim Biophys Acta Mol Cell Biol Lipids* *1864*,
687 1091-1102. DOI: [10.1016/j.bbalip.2019.04.006](https://doi.org/10.1016/j.bbalip.2019.04.006)
- 688 Jung-Ting, Lee, Nathalie, Pamir, Ning-Chun, Liu, Elizabeth, A., Kirk, Michelle, M., and Averill
689 (2014). Macrophage metalloelastase (MMP12) regulates adipose tissue expansion, insulin
690 sensitivity, and expression of inducible nitric oxide synthase. *Endocrinology*.
691 PMID: [PMC4138576](https://pubmed.ncbi.nlm.nih.gov/24138576/)
- 692 Kettner, C., Shaw, E., White, R., and Janoff, A.J.B.J. The specificity of macrophage elastase on the
693 insulin B-chain. *195*, 369-372. PMID: [PMC1162899](https://pubmed.ncbi.nlm.nih.gov/1162899/)
- 694 Kim, T., Choi, S.E., Ha, E.S., Jung, J.G., Han, S.J., Kim, H.J., Kim, D.J., Kang, Y., and Lee, K.W. (2013).
695 IL-6 induction of TLR-4 gene expression via STAT3 has an effect on insulin resistance in human
696 skeletal muscle. *Acta Diabetologica* *50*, 189-200. DOI: [10.1007/s00592-011-0259-z](https://doi.org/10.1007/s00592-011-0259-z)
- 697 Kraen, M., Frantz, S., Nihlén, U., Engström, G., Löfdahl, C.G., Wollmer, P., and Dencker, M. (2019).
698 Matrix Metalloproteinases in COPD and atherosclerosis with emphasis on the effects of smoking.
699 *PloS one* *14*, e0211987-e0211987. PMID: [PMC6383934](https://pubmed.ncbi.nlm.nih.gov/31383934/)
- 700 Langlois, Stephanie, Wild, Benjamin, Cowan, Kyle, N., St-Pierre, and Marie-Eve Elastase and
701 matrix metalloproteinase activities are associated with pulmonary vascular disease in the nitrofen
702 rat model of congenital diaphragmatic hernia. DOI: [10.1016/j.jpedsurg.2017.01.010](https://doi.org/10.1016/j.jpedsurg.2017.01.010)
- 703 Lee, J.-T., Pamir, N., Liu, N.-C., Kirk, E.A., Averill, M.M., Becker, L., Larson, I., Hagman, D.K., Foster-
704 Schubert, K.E., van Yserloo, B., *et al.* (2014a). Macrophage metalloelastase (MMP12) regulates
705 adipose tissue expansion, insulin sensitivity, and expression of inducible nitric oxide synthase.
706 *Endocrinology* *155*, 3409-3420. DOI: [10.1210/en.2014-1037](https://doi.org/10.1210/en.2014-1037)
- 707 Lee, J.T., Pamir, N., Liu, N.C., Kirk, E.A., Averill, M.M., Becker, L., Larson, I., Hagman, D.K.,
708 Fosterschubert, K.E., and Van Yserloo, B. (2014b). Macrophage Metalloelastase (MMP12)
709 Regulates Adipose Tissue Expansion, Insulin Sensitivity, and Expression of Inducible Nitric Oxide
710 Synthase. *Endocrinology* *155*, 3409-3420. PMID: [PMC4138576](https://pubmed.ncbi.nlm.nih.gov/24138576/)

-
- 711 Lee, J.T., Pamir, N., Liu, N.C., Kirk, E.A., Averill, M.M., Becker, L., Larson, I., Hagman, D.K.,
712 Fosterschubert, K.E., and Yserloo, B.V. (2016). Macrophage metalloelastase (MMP12) regulates
713 adipose tissue expansion, insulin sensitivity, and expression of inducible nitric oxide synthase.
714 *155*, 3409-3420. PMCID: [PMC4138576](https://pubmed.ncbi.nlm.nih.gov/26811111/)
- 715 Li, J., Xu, J., Yan, X., Jin, K., and Zhang, R. (2018). Targeting Interleukin-6 (IL-6) Sensitizes Anti-
716 PD-L1 Treatment in a Colorectal Cancer Preclinical Model. *Medical ence Monitor International*
717 *Medical Journal of Experimental & Clinical Research* *24*, 5501-5508. PMCID: [PMC6097097](https://pubmed.ncbi.nlm.nih.gov/30011111/)
- 718 Liaqat, A., Rehman, K., Rasul, A., and Akash, M.S.H.J.C.R.E.G.E. (2017). Role of Interleukin-6 in
719 Development of Insulin Resistance and Type 2 Diabetes Mellitus. *27*. DOI: [10.1615/CritRe-](https://doi.org/10.1615/CritRevEukaryotGeneExpr.2017019712)
720 [vEukaryotGeneExpr.2017019712](https://doi.org/10.1615/CritRevEukaryotGeneExpr.2017019712)
- 721 Lin, J.S., Piper, M.A., Perdue, L.A., Rutter, C.M., Webber, E.M., O'Connor, E., Smith, N., and
722 Whitlock, E.P. (2016). Screening for Colorectal Cancer: Updated Evidence Report and Systematic
723 Review for the US Preventive Services Task Force. *Jama* *315*, 2576-2594.
724 DOI: [10.1001/jama.2016.3332](https://doi.org/10.1001/jama.2016.3332)
- 725 Lobato, B., Prado, and Qian (2018). Anti-cytokines in the treatment of cancer cachexia. *Annals of*
726 *palliative medicine*. DOI: [10.21037/apm.2018.07.06](https://doi.org/10.21037/apm.2018.07.06)
- 727 Mahadik, and Sujata, R. (2013). Association between inflammatory cytokines and insulin resistance
728 in Indian hypertensive patients. *Journal of Indian College of Cardiology* *3*, 163-168. DOI:
729 [10.1016/j.jicc.2013.06.003](https://doi.org/10.1016/j.jicc.2013.06.003)
- 730 Masi, T., and Patel, B.M. (2020). Altered glucose metabolism and insulin resistance in cancer -
731 induced cachexia: a sweet poison. *Pharmacological reports : PR*. DOI: [10.1007/s43440-020-](https://doi.org/10.1007/s43440-020-00179-y)
732 [00179-y](https://doi.org/10.1007/s43440-020-00179-y)
- 733 Mauer, J., Chaurasia, B., Goldau, J., Vogt, M.C., Ruud, J., Nguyen, K.D., Theurich, S., Hausen, A.C.,
734 Schmitz, J., Brönneke, H.S., *et al.* (2014). Signaling by IL-6 promotes alternative activation of
735 macrophages to limit endotoxemia and obesity-associated resistance to insulin. *Nat Immunol*
736 *15*, 423-430. PMCID: [PMC4161471](https://pubmed.ncbi.nlm.nih.gov/25111111/)

-
- 737 Mauer, J., Denson, J.L., and Brüning, J.C. (2015). Versatile functions for IL-6 in metabolism and
738 cancer. *Trends in Immunology* *36*, 92-101. DOI: [10.1016/j.it.2014.12.008](https://doi.org/10.1016/j.it.2014.12.008)
- 739 Narsale, A.A., Carson, J.A.J.C.O.i.S., and Care, P. (2014). Role of interleukin-6 in cachexia:
740 therapeutic implications. *8*, 321-327. PMCID: [PMC4323347](https://pubmed.ncbi.nlm.nih.gov/PMC4323347/)
- 741 Nicholson, T., Church, C., Tsintzas, K., Jones, R., Breen, L., Davis, E.T., Baker, D.J., and Jones, S.W.
742 (2019). Vaspin promotes insulin sensitivity of elderly muscle and is upregulated in obesity. *J*
743 *Endocrinol.* DOI: [10.1530/JOE-18-0528](https://doi.org/10.1530/JOE-18-0528)
- 744 Nikiteas, N.I., Tzanakis, N., Gazouli, M., Rallis, G., Daniilidis, K., Theodoropoulos, G., Kostakis, A.,
745 and Peros, G. (2005). Serum IL-6, TNF α and CRP levels in Greek colorectal cancer patients:
746 Prognostic implications. *World Journal of Gastroenterology* *11*, 1639-1643.
747 PMCID: [PMC4305945](https://pubmed.ncbi.nlm.nih.gov/PMC4305945/)
- 748 Patel, H.J., and Patel, B.M. (2017). TNF- α and cancer cachexia: Molecular insights and clinical
749 implications. *Life Sciences* *170*, 56-63. DOI: [10.1016/j.lfs.2016.11.033](https://doi.org/10.1016/j.lfs.2016.11.033)
- 750 Peixoto da Silva, S., Santos, J.M.O., Costa E Silva, M.P., Gil da Costa, R.M., and Medeiros, R.
751 (2020a). Cancer cachexia and its pathophysiology: links with sarcopenia, anorexia and asthenia.
752 *Journal of cachexia, sarcopenia and muscle* *11*, 619-635. DOI: [10.1002/jcsm.12528](https://doi.org/10.1002/jcsm.12528)
- 753 Peixoto da Silva, S., Santos, J.M.O., Costa, E.S.M.P., Gil da Costa, R.M., and Medeiros, R. (2020b).
754 Cancer cachexia and its pathophysiology: links with sarcopenia, anorexia and asthenia. *J*
755 *Cachexia Sarcopenia Muscle*. PMCID: [PMC7296264](https://pubmed.ncbi.nlm.nih.gov/PMC7296264/)
- 756 Pettersen, K., Andersen, S., Degen, S., Tadini, V., Grosjean, J., Hatakeyama, S., Tesfahun, A.N.,
757 Moestue, S., Kim, J., Nonstad, U., *et al.* (2017). Cancer cachexia associates with a systemic
758 autophagy-inducing activity mimicked by cancer cell-derived IL-6 trans-signaling. *Sci Rep* *7*,
759 2046-2046. PMCID: [PMC5435723](https://pubmed.ncbi.nlm.nih.gov/PMC5435723/)

-
- 760 Puppa, M.J., White, J.P., Velázquez, K.T., Baltgalvis, K.A., Sato, S., Baynes, J.W., Carson,
 761 J.A.J.J.o.C.S., and Muscle (2012). The effect of exercise on IL-6-induced cachexia in the ApcMin/+
 762 mouse. *3*, 117-137. PMCID: [PMC3431229](#)
- 763 Qu, P., Yan, C., and Du, H. (2011). Matrix metalloproteinase 12 overexpression in myeloid lineage
 764 cells plays a key role in modulating myelopoiesis, immune suppression, and lung tumorigenesis.
 765 *Blood* *117*, 4476-4489. PMCID: [PMC3099569](#)
- 766 Siegel, R., Desantis, C., and Jemal, A. (2014). Colorectal cancer statistics, 2014. *CA: a cancer*
 767 *journal for clinicians* *64*, 104-117. DOI: [10.3322/caac.21220](#)
- 768 Song, W., Kir, S., Hong, S., Hu, Y., Wang, X., Binari, R., Tang, H.-W., Chung, V., Banks, A.S.,
 769 Spiegelman, B., *et al.* (2019). Tumor-Derived Ligands Trigger Tumor Growth and Host Wasting
 770 via Differential MEK Activation. *Developmental Cell* *48*, 277-286.e276. PMCID: [PMC6368352](#)
- 771 Suzuki, K., Meguro, K., Nakagomi, D., and Nakajima, H. (2017). Roles of alternatively activated M2
 772 macrophages in allergic contact dermatitis. *Allergology international : official journal of the*
 773 *Japanese Society of Allergology* *66*, 392-397. DOI: [10.1016/j.alit.2017.02.015](#)
- 774 Takayama, K. (2019). [Overview of Cancer-Associated Cachexia]. *Gan to kagaku ryoho Cancer &*
 775 *chemotherapy* *46*, 1814-1817. PMID: [31879394](#)
- 776 Talbert, E.E., Lewis, H.L., Farren, M.R., Ramsey, M.L., Chakedis, J.M., Rajasekera, P., Haverick, E.,
 777 Sarna, A., Bloomston, M., Pawlik, T.M., *et al.* (2018a). Circulating monocyte chemoattractant
 778 protein-1 (MCP-1) is associated with cachexia in treatment-naïve pancreatic cancer patients.
 779 *Journal of cachexia, sarcopenia and muscle* *9*, 358-368. DOI: [10.1002/jcsm.12251](#)
- 780 Talbert, E.E., Lewis, H.L., Farren, M.R., Ramsey, M.L., Chakedis, J.M., Rajasekera, P., Haverick, E.,
 781 Sarna, A., Bloomston, M., Pawlik, T.M., *et al.* (2018b). Circulating monocyte chemoattractant
 782 protein-1 (MCP-1) is associated with cachexia in treatment-naïve pancreatic cancer patients. *J*
 783 *Cachexia Sarcopenia Muscle* *9*, 358-368. DOI: [10.1002/jcsm.12251](#)
- 784 Tisdale, and Michael, J. (2002). Cachexia in cancer patients. *Nature Reviews Cancer* *2*, 862-
 785 871. [1441 Accesses](#)

-
- 786 Utsumi, K., Takai, Y., Tada, T., Ohzeki, S., and Hamaoka, T.J.J.o.I. (1990). Enhanced production of
787 IL-6 in tumor-bearing mice and determination of cells responsible for its augmented production.
788 *145*, 397-403. PMID: [1972720](#):
- 789 van der Werf, A., van Bokhorst, Q.N.E., de van der Schueren, M.A.E., Verheul, H.M.W., and
790 Langius, J.A.E. (2018). Cancer Cachexia: Identification by Clinical Assessment versus International
791 Consensus Criteria in Patients with Metastatic Colorectal Cancer. *Nutrition and cancer* *70*, 1322-
792 1329. DOI: [10.1080/01635581.2018.1504092](#)
- 793 Wada, E., Tanihata, J., Iwamura, A., Takeda, S., Hayashi, Y.K., and Matsuda, R. (2017). Treatment
794 with the anti-IL-6 receptor antibody attenuates muscular dystrophy via promoting skeletal
795 muscle regeneration in dystrophin-/utrophin-deficient mice. *Skelet Muscle* *7*, 23.
796 PMCID: [PMC5660454](#)
- 797 Wagner, C.J., Schultz, C., and Mall, M.A. (2016). Neutrophil elastase and matrix metalloproteinase
798 12 in cystic fibrosis lung disease. *Molecular and cellular pediatrics* *3*, 25-25.
799 PMCID: [PMC4960106](#)
- 800 Wallenius, V., Wallenius, K., Ahrén, B., Rudling, M., Carlsten, H., Dickson, S.L., Ohlsson, C., and
801 Jansson, J.-O. (2002). Interleukin-6-deficient mice develop mature-onset obesity. *Nature*
802 *Medicine* *8*, 75-79. DOI: [10.1038/nm0102-75](#)
- 803 Wang, J., An, F., Cao, Y., Gao, H., Sun, M., Ma, C., Wu, H., Zhang, B., Liu, W., and Wang, J. (2019).
804 Association of TIMP4 gene variants with steroid-induced osteonecrosis of the femoral head in
805 the population of northern China. *PeerJ* *7*, e6270-e6270. PMCID: [PMC6348097](#)
- 806 Wang, M., Spinetti, G., Monticone, R.E., Zhang, J., Wu, J., Jiang, L., Khazan, B., Telljohann, R., and
807 Lakatta, E.G. (2011). A local proinflammatory signalling loop facilitates adverse age-associated
808 arterial remodeling. *PLoS One* *6*, e16653. PMCID: [PMC3035650](#)
- 809 Wang, Q., He, Z., Huang, M., Liu, T., Wang, Y., Xu, H., Duan, H., Ma, P., Zhang, L., and Zamvil, S.S.
810 (2018). Vascular niche IL-6 induces alternative macrophage activation in glioblastoma through
811 HIF-2 α . *Nature Communications* *9*, 559. PMCID: [PMC5805734](#)

812 Wetzl, V., Tiede, S.L., Faerber, L., Weissmann, N., Schermuly, R.T., Ghofrani, H.A., and Gall, H.J.L.
813 Plasma MMP2/TIMP4 Ratio at Follow-up Assessment Predicts Disease Progression of Idiopathic
814 Pulmonary Arterial Hypertension. DOI: [10.1007/s00408-017-0014-5](https://doi.org/10.1007/s00408-017-0014-5)

815 Yang, J., Cao, R.Y., Li, Q., and Zhu, F. (2018). Muscle Atrophy in Cancer. *Advances in experimental*
816 *medicine and biology* *1088*, 329-346. DOI: [10.1007/978-981-13-1435-3_15](https://doi.org/10.1007/978-981-13-1435-3_15)

817 Yang, Q., Yan, C., Wang, X., and Gong, Z. (2019). Leptin induces muscle wasting in a zebrafish
818 kras-driven hepatocellular carcinoma (HCC) model. *Disease models & mechanisms* *12*,
819 dmm038240. PMCID: [PMC6398506](https://pubmed.ncbi.nlm.nih.gov/3138506/)

820 Yarla, N.S., Polito, A., and Peluso, I. (2018). Effects of Olive Oil on TNF- α and IL-6 in Humans:
821 Implication in Obesity and Frailty. *Endocrine, metabolic & immune disorders drug targets* *18*, 63-
822 74. DOI: [10.2174/1871530317666171120150329](https://doi.org/10.2174/1871530317666171120150329)

823 Yuan, L., Han, J., Meng, Q., Xi, Q., Zhuang, Q., Jiang, Y., Han, Y., Zhang, B., Fang, J., and Wu, G.
824 (2015). Muscle-specific E3 ubiquitin ligases are involved in muscle atrophy of cancer cachexia: an
825 in vitro and in vivo study. *Oncology reports* *33*, 2261-2268. DOI: [10.3892/or.2015.3845](https://doi.org/10.3892/or.2015.3845)

826 Zhang, Y., Wang, S., Li, Y., Xiao, Z., Hu, Z., and Zhang, J. (2008). Sophocarpine and matrine inhibit
827 the production of TNF- α and IL-6 in murine macrophages and prevent cachexia-related
828 symptoms induced by colon26 adenocarcinoma in mice. *International immunopharmacology* *8*,
829 1767-1772. DOI: [10.1016/j.intimp.2008.08.008](https://doi.org/10.1016/j.intimp.2008.08.008)

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Figure legends

Figure 1

Figure 1. Knockout of MMP12 in $Apc^{Min/+}$ mice prevents weight and muscle loss

(A-C) Plots of the body weight of wild-type (WT), $Apc^{Min/+}$, $Apc^{Min/+}; MMP12^{-/-}$ and $MMP12^{-/-}$ mice from 5 to 24 weeks ($***P < 0.001$; $**P < 0.01$; $*P < 0.05$; data are shown as the means $\pm$ SD; n = 6 per group). (D) The ratio of white adipose tissue to body weight ($***P < 0.001$; $**P < 0.01$, n=5). (E) The ratio of skeletal muscle to body weight ($*P < 0.05$, n=5). (F) Hematoxylin and eosin staining of white adipose tissue in WT, $Apc^{Min/+}$, $Apc^{Min/+}; MMP12^{-/-}$ and $MMP12^{-/-}$ mice at 24 weeks of age. Scale bars, 5 μ m. (G) Evaluation of the white adipose tissue across 4 groups by ImageJ software(40X) ($***P < 0.001$; $**P < 0.01$, data are shown as the means $\pm$ SD; n = 5 mice each group). (H) Hematoxylin and eosin staining of muscle in $Apc^{Min/+}$, $Apc^{Min/+}; MMP12^{-/-}$ at 24 weeks of age. Scale bars, 5 μ m. (I) Evaluation of the cross-sectional area of the gastrocnemius from $Apc^{Min/+}$ mice and $Apc^{Min/+}; MMP12^{-/-}$ mice by ImageJ software (40X) ($*P < 0.05$; data are shown as the means $\pm$ SD; n = 3 mice each group).

Figure 2

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Figure 2. MMP12 was upregulated in muscle tissues and macrophages of $Apc^{Min/+}$ mice

(A) Immunostaining of MMP12-positive in muscle of clinical individuals and WT mice. Scale bar, 2 μ m. (B) Immunostaining of MMP12-positive in muscle tissue of WT mice. Scale bar, 2 μ m (C) Immunostaining of MMP12-positive in muscle (gastrocnemius) from WT mice and $Apc^{Min/+}$ mice at 24 weeks of age. Scale bar, 5 μ m. (D) Quantification of MMP12-positive in gastrocnemius tissues was performed by ImageJ software (40X) (* $P < 0.05$, data are shown as the means $\pm$ SD; $n = 4$ per group). (E) Representative images of double immunofluorescent staining of macrophages (F4/80 in green) and MMP12 (in red) in WT mice are shown. The yellow areas in the merged images indicate overlapping localization of the red and green signals, indicated by the white arrows. Scale bars, 2 μ m. (F) Quantification of MMP12 mRNA expression level in peritoneal macrophages isolated from WT mice and $Apc^{Min/+}$ mice by qPCR (* $P < 0.05$; data are shown as the means $\pm$ SD; $n = 3$ per group). (G) The serum MMP12 levels detected in WT and $Apc^{Min/+}$ mice at 9-, 15-, and 24 weeks by enzyme-linked immunosorbent assay ($P > 0.05$; data are shown as the means $\pm$ SD; $n = 6$ per group).

Figure 3

Figure 3. Tumor cells can secrete interleukin 6

(A) Serum interleukin 6 (IL-6) levels in normal individuals and patients with colorectal cancer aged 30-50 years detected by enzyme-linked immunosorbent assay (** $P < 0.001$; data are shown as the means $\pm$ SD; $n = 26$ per group). (B) The IL-6 levels in serum in WT mice and Apc^{Min/+} mice were detected at 9-, 15-, and 24 weeks old by enzyme-linked immunosorbent assay (* $P < 0.05$; data are shown as the means $\pm$ SD; $n = 5$ per group). (C) IL-6 mRNA expression was validated in normal intestinal epithelium isolated from Apc^{Min/+} mice versus that in intestinal tumors isolated from WT mice by qPCR (* $P < 0.05$; data are shown as the means $\pm$ SD; $n = 4$ per group). (D) Cytokine array detects inflammatory cytokines in fresh untreated medium (-MC38 cells) and cultured MC-38 cells (+MC38 cells); arrows indicate the significantly increased cytokines. (E) The relative quantification of the significantly upregulated cytokine to positive quality control density ratio by ImageJ software. The positive quality control density was determined for normalization purposes (** $P < 0.01$, * $P < 0.05$; data are shown as the means $\pm$ SD).

Figure 4

Figure 4. Tumor-derived IL-6 can upregulate MMP12 in macrophages.

(A) Schematic diagram of tumor cells (MC38/CT26 cell lines) coculture with macrophage cells (RAW264.7 cell lines). All quantifications use image J software grayscale statistics. (B, C) Representative western blots showing the secreted MMP12 protein levels from RAW264.7 cell lines ($1-2 \times 10^5$) cultured alone or cocultured with MC38 cell lines (control, 1×10^4 , 3×10^4 , 5×10^4). β -Actin was used as the internal control for normalization purposes. (D, E) Representative western blots showing the secreted MMP12 protein levels from RAW264.7 cell lines ($1-2 \times 10^5$) cultured alone or cocultured with CT26 cell lines (control, 1×10^4 , 3×10^4 , 5×10^4). GAPDH was used as the internal control for normalization purposes. (F) Schematic diagram of IL-6 treated macrophages. RAW264.7 cells incubated with fresh media were served as untreated negative controls. Using western blotting to detect MMP12 in RAW 264.7 cells and GAPDH was used as the internal control. (G, H) RAW264.7 cells ($1-2 \times 10^5$) were seeded into 6-well plates and treated with increasing doses of IL-6 (0, 2, 5, 10, 30 ng/mL) for 72 hours. (I, J) RAW264.7 cells ($1-2 \times 10^5$) were treated continuously with IL-6 (30 ng/ml) for 0, 3, 6, and 9 hours. (K) Immune gene data proved that IL-6 receptor is expressed on myeloid cells and the red box represents F480⁺ macrophages. The colored bars refer to the expression level of IL-6 receptors on macrophages. Red represents high expression of IL-6 receptors, and green represents low expression of IL-6 receptors.

Figure 5

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Figure 5. MMP12 can degrade insulin and insulin-like growth factor-1

(A) Representative picture of the peak shift after the insulin polypeptide interacts with serum. (A) The synthetic insulin (or insulin-like growth factor-1) peptide was labeled with FAM and DABCLY as shown; if the insulin (or insulin-like growth factor-1) peptide was degraded, the FAM signal was detected. This is based on fluorescence resonance energy transfer (FRET); Detection of characteristic peaks of insulin alone and the mixture of insulin (or insulin-like growth factor-1) and MMP12 by ionization mass spectrometry. (C)The coexistence of MMP12 and insulin (or insulin-like growth factor-1) peptide led to a fluorescence signal and appeared dose-dependent ($***P < 0.001$, $**P < 0.01$; data are shown as the means $\pm$ SD).

Figure 6

Figure 6. Inhibiting MMP12 in Apc^{Min/+} mice reduces weight loss

(A)Schematic diagram of the administration process of 17-week Apc^{Min/+} mice. The drug was given every two days (MMP408-5mg/kg, 5-FU-30mg/kg). The saline group was used as a control. (B) Percentage of weight gain compared to the basal weight after administration of drugs in Apc^{Min/+} mice ($**P < 0.01$, data are shown as the means $\pm$ SD; n = 5 per group).

Figure 7. The role of MMP12 in the crosstalk between tumor and muscular macrophage in cancer cachexia.

Supplementary data

Figure S1

Figure S1 Mouse crossbreeding and genotype identification.

A. Schematic of the crossbreeding of $Apc^{Min/+}$ mice with $MMP12^{-/-}$ mice to obtain $Apc^{Min/+}; MMP12^{-/-}$ mice. B. The APC gene mutant PCR product size was 340 bp, and the PCR product size of wild-type (WT) mice was 600 bp. The MMP12 knockout (mutation) PCR product size was 460 bp, and the WT (WT) PCR product size was 350 bp. In detail, $Apc^{Min/+}$: 1#, 4#, 5#, 9#; $MMP12^{-/-}$: 1#, 2#, 3#, 4#, 5#, 6#, 10#, 11#; $Apc^{Min/+}; MMP12^{-/-}$: 1#, 4#, 5#.

Figure S2

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Figure S2 Ionization mass spectrometry analysis report: the characteristic peak of insulin (insulin-like growth factor 1) disappears after incubating with MMP12.

(A) The characteristic peak of insulin ($m/z = 436.99$) and disappeared after incubation with MMP12.

(B) The characteristic peak of insulin-like growth factor 1 ($m/z = 436.98$) disappeared after incubation with MMP12.

Figure S3

Figure S3 Knockout of MMP12 does not affect the food intake of $Apc^{Min/+}$ mice and MMP12 is expressed in bone marrow, muscle, liver and adipose tissue.

(A) There was no difference in food intake between $Apc^{Min/+}$ mice and $Apc^{Min/+}; MMP12^{-/-}$ mice. The weight of food consumed after fasting for 8 h was measured every day starting on 17-week. Each group of mice had three cages, and each cage had 5 mice. (B) Immunostaining of MMP12-positive in muscle (gastrocnemius) from the clinical individual. Scale bar, 50 μ m. (C) A mRNA analysis of the data got from The Cancer Genome Atlas (TCGA) for GTEx, Illumina, BioGPS and SAGE of MMP12 gene in normal human tissues.

Figure S4

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Figure S4 The effect of knocking out MMP12 in $Apc^{Min/+}$ mice on liver, brown adipose tissue and pancreatic islets.

(A) The liver-to-body weight ratio. (B) Hematoxylin and eosin staining (H&E) of the liver at 24 weeks. Scale bar, 20 μ m (* $P < 0.05$ data are shown as means $\pm$ SD; $n = 6$ per group). (C) Brown adipose tissue-to-body weight ratio was higher in $Apc^{Min/+}$; $MMP12^{-/-}$ mice than in $Apc^{Min/+}$ mice (* $P < 0.05$ data are shown as means $\pm$ SD; $n = 6$ per group). (D) The results of H&E indicate that white fat increased in brown fat in MMP12 knockout mice. Scale bar, 10 μ m. (E) The pancreas-to-body weight ratio (* $P < 0.05$ data are shown as means $\pm$ SD; $n = 6$ per group). (F) The number of islets between WT and $MMP12^{-/-}$ mice ($P > 0.05$; data are shown as the means $\pm$ SD; $n = 6$ per group). (G) The staining results of H&E for pancreas WT and $MMP12^{-/-}$ mice at 24 weeks. Scale bar, 20 μ m. (H) Immunostaining of

insulin-positive in WT mice and in MMP12^{-/-} pancreases at 24 weeks of age. Scale bar, 20μm.

Figure S5

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976 **Figure S5** Serum monocyte chemoattractant protein 1 (MCP1) and keratinocyte-de-
977 rived chemokine(KC) did not change of Apc^{Min/+} mice in cancer cachexia at 24 weeks of
978 age.

979 (A, C) The mRNA expression of MCP1 and KC was validated in normal intestinal epithelium isolated
980 from WT mice versus that in intestinal tumors isolated from Apc^{Min/+} mice by quantitative PCR
981 (***) $P < 0.001$ data are shown as means $\pm$ SD; $n = 4$ per group). (B, D) Serum MCP1 and KC in
982 Apc^{Min/+} mice versus WT mice at 9, 15 and 24 weeks (** $P < 0.01$, * $P < 0.05$ data are shown as means $\pm$
983 SD; $n = 6$ per group). (E) Serum KC in normal healthy individuals and colorectal cancer patients by
984 enzyme-linked immunosorbent assay ($P > 0.05$; data are shown as the means $\pm$ SD; $n = 6$ per group).

Figure S6

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Figure S6 Knockout MMP12 affects glycolipid metabolism in $Apc^{Min/+}$ mice.

(A) Fasting plasma glucose levels at 9, 15 and 24 weeks of age in WT mice, $MMP12^{-/-}$ mice, $Apc^{Min/+}$ mice and $Apc^{Min/+}; MMP12^{-/-}$ mice ($***P < 0.001$, $*P < 0.05$; data are shown as the means $\pm$ SD; $n = 4$ per group). (B) Enzyme-linked immunosorbent assay was used to detect fasting serum insulin levels in $Apc^{Min/+}$ and $Apc^{Min/+}; MMP12^{-/-}$ mice at approximately 9, 15 and 24 weeks of age ($***P < 0.001$; $*P < 0.05$; data are shown as the means $\pm$ SD; $n = 4$ per group). (C) Oral Glucose Tolerance Test (OGTT): WT, $MMP12^{-/-}$ mice were fasted for 4 h and then administered glucose (75 IU/kg), ($*P < 0.05$; data are shown as the means $\pm$ SD; $n = 6$ per group). (D) Area under the curves for the OGTT-AUG, which was increased in $MMP12^{-/-}$ mice compared with that in WT mice ($*P < 0.05$ data are shown as means $\pm$ SD; $n = 6$ per group). (E) OGTT: $Apc^{Min/+}$ and $Apc^{Min/+}; MMP12^{-/-}$ mice were fasted for 4 h and then administered glucose (75 IU/kg), ($**P < 0.01$, $*P < 0.05$ data are shown as means $\pm$ SD; $n = 6$ per group). (F) Areas under the curves for the OGTT-AUG, which was significantly increased in $Apc^{Min/+}; MMP12^{-/-}$ mice compared with that in $Apc^{Min/+}$ mice ($**P < 0.01$, data are shown as means $\pm$ SD; $n = 6$ per group). (G) Insulin tolerance test (ITT): mice were fasted for 4 hours; $Apc^{Min/+}$ and $Apc^{Min/+}; MMP12^{-/-}$ mice then received an ip injection of insulin ($**P < 0.01$, data are shown as means $\pm$ SD; $n = 6$ per group). (H) Areas under the curves for the ITT-AUG, which was significantly decreased in $Apc^{Min/+}; MMP12^{-/-}$ mice compared with that in $Apc^{Min/+}$ mice ($*P < 0.05$ data are shown as means $\pm$ SD; $n = 6$ per group). (I-L) Quantitative determination of serum total cholesterol (TC), total triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) in WT mice, $MMP12^{-/-}$ mice, $Apc^{Min/+}$ mice and $Apc^{Min/+}; MMP12^{-/-}$ mice by kits at 9, 15 and 24 weeks of age ($***P < 0.001$,

** $P < 0.01$, * $P < 0.05$ data are shown as means $\pm$ SD; n = 7 per group).

Figure S7

Cytokine Assay

BLC (CXCL13)	CD30 Ligand (TNFSF8)	Eotaxin-1 (CCL11)	Eotaxin-2 (MPIF-2/CCL24)	Fas Ligand (TNFSF6)
Fractalkine (CX3CL1)	GCSF	GM-CSF	IFN-gamma	IL-1 alpha (IL-1 F1)
IL-1 beta (IL-1 F2)	IL-2	IL-3	IL-4	IL-6
IL-9	IL-10	IL-12 p40/p70	IL-12 p70	IL-13
IL-17A	I-TAC (CXCL11)	KC (CXCL1)	Leptin	LIX
Lymphotoctin (XCL1)	MCP-1 (CCL2)	M-CSF	MIG (CXCL9)	MIP-1 alpha (CCL3)
MIP-1 gamma	RANTES (CCL5)	SDF-1 alpha (CXCL12 alpha)	I-309 (TCA-3/CCL1)	TECK (CCL25)
TIMP-1	TIMP-2	TNF alpha	TNF RI (TNFRSF1A)	TNF RII (TNFRSF1B)

Figure S7 Specific detection factors in the protein chip. Protein chip contains 40 kinds of cytokine detection indicators.

1019 **Table1: Quantitative PCR primers.**

S Table1 List of quantitative PCR primers			
Gene Name	Accession Number	Forward (5'-3')	Reverse (5'-3')
m-MMP12	nm_001320076.1	GAGTCCAGCCACCAACATTAC	GCGAAGTGGGTCAAAGAC
m-GAPDH	nm.001289726.1	CGTCCCGTAGACAAAATGGT	TCAATGAAGGGGTCGTTGAT
m-IL6	nm.31168	TAGTCCTTCCTACCCCAATTTC	TTGGTCCTTAGCCACTCCTTC
m-MCP1(CCL2)	nm.11333	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
m-KC(CXCL1/IL8)	nm.008176	CTGGGATTACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
Note: m, mouse.			

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1028 **Table 2 The effect of IL-6 on body weight**

S Table 2		
Various studies on IL-6	Highlights of Impact on Body Weight (fat or muscle)	PMID
Overexpression IL-6	Circulating IL-6 levels were increased and the animals rapidly lost both weight and body fat (often when mice bear tumors); Chronically elevated IL-6 levels lead to hyperinsulinemia, reduced body weight, impaired insulin-stimulated glucose uptake by the skeletal muscles. Systemic IL-6 overexpression in tumor-bearing Apc ^{Min/+} mice accelerated cachexia development, which coincided with suppressed basal and eccentric contraction-induced muscle protein synthesis.	18437347 ; 29641213
IL-6 Inhibited (Inhibit the STAT3/JAK/IL-6R)	Mice body weight increase to resistant cancer cachexia. Therapeutic effects of IL-6R blockade on promoting muscle regeneration. IL-6R blockade has therapeutic effects on the dystrophic skeletal muscle.	31002945 ; 11786910
Cytokine IL-6 Treatment	Centrally acting IL-6 exerts anti-obesity effects in rodents.	11786910 ;
IL-6 Neutralizing antibody Treatment	IL-6 may indirectly cause muscle wasting.	20871233
IL-6 KO under tumor-bearing	Centrally acting IL-6 exerts anti-obesity effects in rodents; Apc ^(Min/+) /IL-6 ^(-/-) mice did not lose gastrocnemius muscle mass or epididymal fat pad mass while overall polyp number decreased compared with Apc ^(Min/+) mice.	11786910 18056981

IL-6 overexpression under tumor-bearing	Administration of an IL-6 receptor antibody to cachectic male Apc ^{Min/+} mice can attenuate further cachexia progression to increase body weight. IL-6 over-expression in pre-cachectic mice accelerated body weight loss and muscle wasting. IL-6 overexpression did not induce cachexia in non-tumor-bearing mice.	25555992 ; 22769563 ; 18056981
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1042 **Table 3: Abbreviations**

Abbreviations	Full name
MMP12	Matrix Metalloproteinases 12
CRC	Colorectal Cancer
CAC	Cancer Cachexia
Apc ^{Min/+} ; MMP12 ^{-/-}	Apc ^{Min/+} ; MMP12 ^{-/-} mice
WAT	White Adipose Tissue
BAT	Brown Adipose Tissue
HRP	Horseradish Peroxides
H&E	Hematoxylin & Eosin
qPCR	Quantitative Polymerase Chain Reaction
IHC	Immunohistochemistry
IF	Immunofluorescence
PBS	Phosphate Buffered Saline
MCP1(CCL2)	Monocyte Chemoattractant Protein 1
KC(CXCL1)	Keratinocyte-derived Chemokine
RANTES	Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted
IL-6	Interleukin 6
IGF-1	Insulin-like Growth Factor 1

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Figure 1

Figure 2

A

B

C

D

E

F

G

Figure 3

Figure 4

Figure 5

A

B

C

Figure 6

Figure 7

Tumor-derived IL-6 to MMP12 in Muscle macrophage Metastasis

Figure S1

A

B

Figure S2

A

Insulin

Insulin+MMP12

B

IGF-1

IGF-1+MMP12

Figure S3

Figure S4

Figure S5

Figure S6

Figure S7

Cytokine Assay

BLC (CXCL13)	CD30 Ligand (TNFSF8)	Eotaxin-1 (CCL11)	Eotaxin-2 (MPIF-2/CCL24)	Fas Ligand (TNFSF6)
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Lymphotactin (XCL1)	MCP-1 (CCL2)	M-CSF	MIG (CXCL9)	MIP-1 alpha (CCL3)
MIP-1 gamma	RANTES (CCL5)	SDF-1 alpha (CXCL12 alpha)	I-309 (TCA-3/CCL1)	TECK (CCL25)
TIMP-1	TIMP-2	TNF alpha	TNF RI (TNFRSF1A)	TNF RII (TNFRSF1B)