



Leptin increases chemosensitivity by inhibiting *CPT1B* in colorectal cancer cells

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Background: Chemoresistance is a major cause of treatment failure in advanced colorectal cancer (CRC), severely impacting patient survival and quality of life. While conventional chemotherapy regimens can somewhat control tumor progression, their effectiveness is frequently compromised by the development of drug resistance in cancer cells. The aim of this study is to verify and elucidate the specific mechanisms by which leptin enhances chemosensitivity in CRC, providing valuable insights for the development of new combination chemotherapy options.

Methods: We examined the link between CRC chemoresistance and fatty-acid metabolism driven by the high expression of carnitine palmitoyltransferase-1b (*CPT1B*) through an integrated approach combining bioinformatics and clinical sample analysis. *In vitro* and *in vivo* experiments were conducted to evaluate the effect of leptin, an adipocyte-derived cytokine, on CRC cells' response to cisplatin.

Results: Leptin significantly enhanced CRC cells' chemosensitivity to cisplatin by downregulating *CPT1B* expression, thereby disrupting the fatty-acid oxidation pathways that support drug resistance. In mouse models, the coadministration of leptin and cisplatin resulted in notable reductions in tumor size and weight compared to cisplatin alone, underscoring leptin's potential to enhance chemotherapy efficacy.

Conclusions: These findings indicate that leptin, through modulation of *CPT1B*, may serve as a promising adjunct to chemotherapy for CRC, addressing the challenge of chemoresistance and improving therapeutic outcomes. The leptin-*CPT1B* axis may be potential therapeutic target, providing new avenues for CRC treatment strategies aimed at overcoming drug resistance.

Keywords: Colorectal cancer (CRC); leptin; chemosensitivity; carnitine palmitoyltransferase-1b (*CPT1B*)

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Introduction

As the third most common cancer globally, colorectal cancer (CRC) poses a significant threat to public health, with over 1.2 million new cases diagnosed annually and approximately 608,700 deaths each year (1). Early detection through cancer screening programs can achieve a survival rate exceeding 90%, but the prognosis for patients with advanced-stage CRC is substantially worse, with survival rates dropping below 10%. Chemotherapy is a primary nonsurgical treatment for advanced CRC; however, it involves adverse effects and the development of resistance, which are major factors contributing to treatment failure (2). Therefore, there is an urgent need to develop strategies that can enhance the efficacy of chemotherapy, thereby improving patient outcomes and survival rates in CRC (3).

Leptin, known to be a hormone associated with obesity, is a cytokine-like hormone predominantly produced by adipose tissue (4). This hormone plays a pivotal role in maintaining energy homeostasis by modulating hunger signals and energy expenditure, in addition to regulating fat

metabolism and storage (5). Beyond its well-documented metabolic functions, leptin has been found to stimulate the proliferation and growth of various cellular and tissue types. It exerts direct effects on a wide array of cancer cells, significantly influencing both the initiation and progression of malignancies. Leptin has been implicated in numerous physiological processes, including energy metabolism, immune system modulation, tissue regeneration, and angiogenesis—the formation of new blood vessels (6).

Moreover, elevated levels of leptin have been observed in several types of cancerous tissues, including those affected by esophageal carcinomas (7), pancreatic neoplasms (8), and colorectal malignancies (9). These findings suggest that leptin may play a critical role not only in cancer development but also in its potential resistance to treatment. Understanding the multifaceted roles of leptin in cancer biology could pave the way for novel therapeutic strategies aimed at targeting leptin signaling pathways, thereby improving patient outcomes across a variety of cancer types.

Leptin has garnered significant attention due to its multifaceted roles in cancer progression, exhibiting a dualistic nature as both a promoter and a suppressor in various malignancies. In the context of gastrointestinal tumors, leptin has demonstrated protective qualities. For instance, in pancreatic cancer, leptin has been shown to curb tumor growth, with the most pronounced inhibitory effect observed at specific dosages (10). Supporting this observation, a case-control study reported a positive correlation between leptin levels and cancer stage, suggesting leptin's potential as a beneficial factor in pancreatic cancer (11). Similarly, in colorectal adenocarcinoma, the presence of leptin has been associated with improved disease-free and overall survival, underscoring its prognostic significance (12). The growth-inhibitory effects of leptin on hepatocellular carcinoma cells have also been documented, with adipokine engaging the *p38-MAPK* signaling pathway to exert its effects *in vitro* (13). Furthermore, innovative drug delivery approaches leveraging leptin's receptor-mediated targeting capabilities in colon cancer have shown promise, with leptin-derived peptides being used to suppress tumor growth (14).

However, conflicting data exist, and some studies suggest that leptin promotes cancer. For example, increased leptin expression has been linked to enhanced adenocarcinoma cell proliferation in lung cancer (15). Additionally, recent findings suggest that leptin may facilitate CRC metastasis through the activation of the *JAK-STAT3* and *PI3K-AKT* pathways (16). This ambiguity in effect has led to the

Highlight box

Key findings

- In this study, leptin enhanced chemosensitivity in colorectal cancer (CRC) cells by inhibiting carnitine palmitoyltransferase-1b (*CPT1B*), a key enzyme in fatty-acid metabolism. Specifically, leptin suppressed *CPT1B* expression, improving CRC cells' sensitivity to cisplatin both *in vitro* and in a xenograft mouse model. These results support leptin as a potential adjunct in CRC chemotherapy to counteract resistance.

What is known and what is new?

- *CPT1B* is often overexpressed in cancer, contributing to chemoresistance through enhanced fatty-acid oxidation. Leptin, predominantly an adipocyte-derived hormone, influences cancer progression through metabolic modulation.
- The study discovered the leptin-*CPT1B* axis that enhances cisplatin efficacy by increasing cancer cell apoptosis in CRC. This work is the first to highlight leptin's role in directly targeting *CPT1B* in mitigating the drug resistance in CRC therapy.

What is the implication, and what should change now?

- The findings imply that targeting the leptin-*CPT1B* pathway could significantly improve cisplatin sensitivity in CRC and address the critical issue of chemoresistance. Clinically, leptin could be explored as an adjunctive therapeutic strategy, and its application could be extended to other cancers with similar metabolic resistance mechanisms.

characterization of a “leptin paradox”, highlighting the need for a more nuanced understanding of leptin’s role in cancer.

Cisplatin, a widely applied chemotherapeutic agent, is known for its DNA-damaging properties, which trigger a cascade of responses leading to cancer cell apoptosis (17). Despite initial therapeutic successes, the emergence of chemoresistance remains a significant clinical challenge. In CRC, understanding and overcoming cisplatin resistance is of paramount importance. This study aimed to characterize the interplay between leptin and cisplatin resistance in CRC and to clarify the underlying mechanisms.

As fatty-acid metabolism has a critical role in cancer cell growth and survival, focusing on leptin’s potential to modulate lipid metabolism is well-founded. Fatty acid oxidation (FAO), a key process for nicotinamide adenine dinucleotide phosphate (*NADPH*) generation, has been demonstrated to be increased by leptin via *STAT3* signaling in breast cancer stem cells (18). Inhibiting this pathway, including the downregulation of carnitine palmitoyltransferase-1b (*CPT1B*), a pivotal enzyme in FAO, could disrupt the proliferative and self-renewal capacities of cancer stem cells, thereby degrading tumor cell viability and redox balance. Our findings indicate that leptin enhances the chemosensitivity of CRC cells to cisplatin by suppressing *CPT1B* expression. This novel insight into the molecular interactions between leptin and cisplatin resistance could pave the way for targeted therapeutic strategies that can mitigate drug resistance in CRC chemotherapy. By elucidating these mechanisms, future research can focus on harnessing the beneficial effects of leptin while minimizing its potential adverse impacts, ultimately enhancing the efficacy of cancer treatments. We present this article in accordance with the ARRIVE and MDAR reporting checklists (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2024-950/rc>).

Methods

Patients and tissue specimens

A collection of 20 surgically excised tissue samples, comprising both CRC and adjacent nonneoplastic tissues, was obtained from patients diagnosed with colorectal adenocarcinoma at the First Affiliated Hospital of Soochow University, Suzhou, China, between 2019 and 2020. The selection criteria for the participants included the following: (I) a confirmed histological diagnosis of colorectal

adenocarcinoma with no previous cancer incidence; (II) no prior receipt of chemotherapy, radiotherapy, or other cancer-specific therapies; (III) access to complete medical documentation; and (IV) consent to participate and undergo subsequent follow-up. The tissue samples were preserved by rapid freezing in liquid nitrogen for molecular assessments or preserved in formalin for histological evaluation. Finally, we included 10 tumor samples and 10 adjacent normal tissue samples to provide sufficient statistical power to detect differences between these two groups. This sample size was chosen based on previous studies that used similar sample sizes to achieve meaningful results.

The categorization of CRC stages and the recording of pathological details adhered to the guidelines set forth by the American Joint Committee on Cancer. In accordance with the ethical protocols of Soochow University and the principles outlined in the Declaration of Helsinki (as revised in 2013) by the World Medical Association, the study was conducted with strict compliance to all applicable rules and standards for research involving human participants. The research protocol was approved by the Ethical Review Board of the First Affiliated Hospital of Soochow University (ethics No. 2024737). Informed consent was taken from all the patients.

Cell lines and cell culture

Three human CRC cell lines were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cell lines were RKO, HT29, and SW480. The HT29 and RKO cells were grown in Roswell Park Memorial Institute (RPMI) formulation 1640 media (Gibco, Waltham, MA, USA). Additionally, SW480 cells were routinely cultivated in Dulbecco’s modified Eagle medium (DMEM; HyClone, Logan, UT, USA). Ten percent fetal bovine serum and one percent penicillin/streptomycin were added to this medium to enhance it. The cell lines were maintained in a 5% CO₂ humidified atmosphere at 37 °C with concentration (16). Cisplatin, at a concentration of 30 μM, was prepared by dissolving it in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MI, USA).

Cell apoptosis analysis

In the subsequent phase of the study, the previously mentioned CRC cells were seeded into six-well plates designed for tissue culture. After confluent growth, the cells were detached, rinsed with phosphate-buffered saline (PBS)

that had been precooled, and resuspended in a volume of 500 μ L of binding buffer. The process of apoptosis in the cells was identified using an allophycocyanin (APC)-conjugated Annexin V apoptosis detection kit in conjunction with propidium iodide (PI; Keygenbio, Nanjing, China) according to the guidelines provided by the supplier. The cells were stained using a combination of 5 μ L of Annexin V-APC and 10 μ L of PI, and this mixture was left to stand for a duration of 5 minutes in the absence of light at ambient temperature. Subsequently, a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was used to identify and measure the apoptotic cells. The acquired data were subsequently analyzed using FlowJo version 10.6.2 (BD Biosciences).

Bioinformatic analysis

The Human Genome Microarray datasets of both normal and malignant tissues from a previous study were downloaded from the Gene Expression Omnibus (GEO) database (GSE251845). Differentially expressed genes were defined as those with fold change >2.0 and $P < 0.05$ and were analyzed using the “DEseq2” R package (The R Foundation for Statistical Computing, Vienna, Austria). A volcano plot was used to indicate the distribution of differently expressed genes. Gene Ontology (GO) analysis was applied to investigate the biological functions of the differently expressed genes. Moreover, to predict the possible pathways, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was adopted to map the possible pathways of these differentially expressed genes.

Immunofluorescence assay

The antigens were retrieved using a 6.0-pH sodium citrate buffer after the formalin-fixed paraffin-embedded (FFPE) colorectal tumor sections underwent a series of deparaffinization and hydration treatments with xylene and ethanol. Following a half-hour blocking period with normal goat serum, the sections were stained with *CPT1B* (1:1,000; Abcam, Cambridge, UK) antibodies. Subsequently, they were incubated with secondary antibodies (Alexa Fluor 488 and 555; Invitrogen, Waltham, MA, USA; Thermo Fisher Scientific, Waltham, MA, USA), and the nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; 1:1,000; Sigma-Aldrich). A LSM700 confocal microscope (Zeiss, Jena, Germany) was used to mount and photograph the pictures.

Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. RNA (1 μ g) was reverse transcribed using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). A Power SYBR Green PCR Master Mix and an Applied Biosystems 7500 real-time polymerase chain reaction (PCR) device (Thermo Fisher Scientific) were used to perform real-time qRT-PCR in accordance with the manufacturer's instructions. Fold changes were calculated relative to the internal control, 18-S, via the $2^{-\Delta\Delta CT}$ method.

Protein extraction and Western blot

Proteins were isolated from CRC cell lines, specifically RKO, HT29, and SW480. The extraction process involved lysing the cells in a RIPA lysis buffer (Beyotime Inc., Nantong, China) and was conducted in accordance with the supplier's guidelines. Following centrifugation at 4 °C with a relative centrifugal force of 12,000 rpm for a duration of 10 minutes, the resulting supernatants were carefully collected. The extracted proteins were then resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% gel. Subsequently, the resolved proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (MilliporeSigma, Burlington, MA, USA). The membranes underwent blocking with a 5% skim milk solution for a period of 1 hour at ambient conditions. After blocking, the membranes were exposed to specific primary antibodies at 4 °C overnight. The next step involved washing the membranes with Tris-buffered saline with Tween three times, with each wash lasting 10 minutes. Following the washes, the membranes were further incubated with the appropriate secondary antibodies for 1 hour at room temperature. Finally, the visualization of protein expression was achieved using an enhanced chemiluminescence (ECL) substrate kit (Thermo Fisher Scientific). The captured signals were then quantified using ImageJ software (ImageJ, US National Institutes of Health, Bethesda, MD, USA).

Transfection of short hairpin RNA (shRNA)

The negative control shRNA (shNC; 5'-TTCTCCGAACGTGTCACGT-3') and antihuman *CPT1B* shRNA (sh*CPT1B*) were designed, manufactured, and cloned into the pFU-GW-016 vector by Ganache Co.,

(Shanghai, China). Using lentiviral constructs that express sh*CPT1B*, we created stable *CPT1B*-silenced RKO, HT29, and SW480 cells, and the shNC was added to parallel cultures. The media that included 2 µg/mL puromycin (Sigma-Aldrich) was used to maintain the cells. The sequence of antihuman sh*CPT1B* was as follows: forward oligo, 5'-CCGGAACAGGTGGTTTGACAAATCCCTC GAGGGATTTGTCAAACACCTGTTTTTTTG-3'; and reverse oligo, 5'-AATTCAAAAAACAGGTGGTTT GACAAATCCCTCGAGGGATTTGTCAAACACCTG TT-3'.

Mouse subcutaneous xenograft model

To assess the effect of leptin on drug sensitivity in CRC, we used a mouse subcutaneous tumor cell transplantation model. Male BALB/c nude mice (specific-pathogen free grade; 16–18 g) that were 3–5 weeks old, were purchased from Jicuiyaokang Experimental Animal Co., (Shanghai, China). All mice were kept in a specific pathogen-free environment with free sterile food and water under the standard 12-hour light-dark cycle at 22±1 °C. Mice that exhibited signs of illness, stress, or abnormalities were excluded from the study. Additionally, any animals that did not tolerate the treatment well or experienced adverse effects unrelated to the experimental interventions were excluded. A random number table was used to assign mouse to different groups. Notably, team members who conducted and analyzed the experiment were blinded to the allocation. SW480 human intestinal cancers in a logarithmic growth phase were selected, the concentration was adjusted to 5×10⁷/mL, and 0.2 mL PBS containing 1×10⁷ SW480 cells was injected subcutaneously into the right armpit of each nude mouse. One week after tumor formation, the mice were divided into a cisplatin group (CIS group) and a cisplatin-plus-leptin group (CIS + leptin group) with 5 mice in each group. The mice were injected with cisplatin (30 µM) once a week; meanwhile, the CIS + leptin group was treated with a supplement of 2 mg/kg of leptin every 3 days. Mice were killed via cervical dislocation on day 20, and the volume and the weight of the whole tumor were measured. This sample size was determined through a priori power analysis to ensure adequate power to detect significant differences between the treatment groups, while also considering ethical guidelines to minimize the number of animals used.

Strategies were implemented to minimize potential confounders. The order of treatments and measurements

was randomized using a stochastic-generated sequence to avoid sequence effects. Additionally, animal cages were randomly allocated and rotated every 2 weeks to control for location-specific environmental factors. Standardized anesthetic agents were used, and effective analgesia was administered throughout the experiment to minimize animal suffering. All animal experimental procedures were approved by the Ethical Review Board of the First Affiliated Hospital of Soochow University (ethics No. 2024737) and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition. A protocol was prepared before the study without registration.

Statistical analyses

Each experiment was done three or more times, and the results are shown as the mean ± standard error of the mean (SEM). SPSS 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 7.0 (GraphPad Software, Boston, MA, USA) were used to assess significant differences in the one-way analysis of variance (ANOVA) or the Mann-Whitney test. For the survival analysis, the log-rank test was employed. The Spearman rank correlation test was used to evaluate the correlations between two variables. P<0.05 was considered to be a significant.

Results

Leptin enhanced the chemosensitivity of CRC cells to cisplatin

A previous study found that individuals with breast cancer, particularly those with the human epidermal growth factor receptor 2 (HER2)⁺ subtype, benefited therapeutically from neoadjuvant chemotherapy when leptin and leptin receptor (*LEPR*) were overexpressed (19). However, the connection between leptin and the chemosensitivity of gastroenteric tumor remains unclear. To investigate the role of leptin in the treatment of CRC, we first detected the apoptosis of two CRC cell lines (HT29 and SW480) via flow cytometry after the addition of the optimal concentration of leptin. The number of apoptotic cells did not significantly change between the 200 ng/mL leptin group and the blank control group, indicating that leptin itself did not promote apoptosis (Figure 1A).

In a follow-up experiment, the same CRC cell lines (HT29 and SW480) were treated with cisplatin or cisplatin combined with leptin for 48 hours. It was found that the

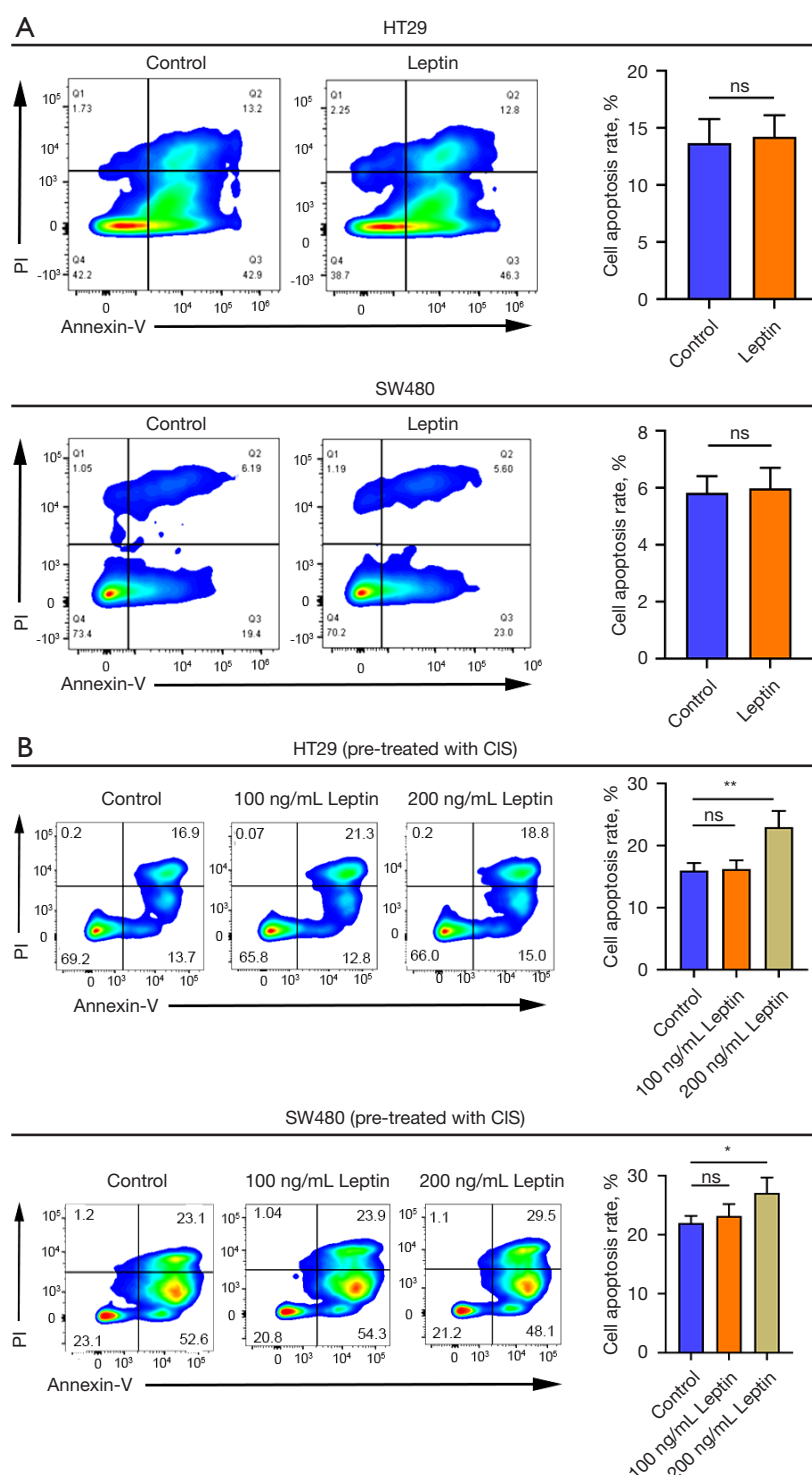


Figure 1 Leptin promoted the sensitivity of CRC to cisplatin in chemotherapy. (A) The percentages of apoptotic cells in two CRC cell lines (HT29 and SW480) treated with 200 ng/mL leptin for 48 hours were compared with those in the blank control. (B) Comparison of apoptotic cell numbers in two CRC cell lines (HT29 and SW480) treated with cisplatin alone or combined with 200 ng/mL leptin for 48 hours. $n=4$ for each group. The differences were analyzed with two-tailed Student t -tests. The data are presented as the mean $\pm$ SEM. *, $P<0.05$; **, $P<0.005$; ns, not significant. PI, propidium iodide; CIS, cisplatin; CRC, colorectal cancer; SEM, standard error of the mean.

number of apoptotic cells in the experimental group was significantly greater than that in the control group, and the number of apoptotic cells increased with increasing leptin concentration (Figure 1B). These results indicated that leptin combined with chemotherapy accelerated the progression of CRC cell apoptosis.

To further verify the effect of leptin on drug sensitivity, we employed a xenograft mouse model and administered cisplatin chemotherapy with or without leptin intervention. We observed that compared to cisplatin chemotherapy alone, the addition of leptin resulted in a significant reduction in both the weight and volume of subcutaneous tumors in mice (Figure S1A-S1C). These findings indicate that leptin can enhance the chemosensitivity of CRC to cisplatin, potentially offering a new avenue for improving treatment efficacy in patients with CRC.

CPT1B was upregulated in patients with clinical CRC and may serve as the target gene of leptin

Given the evidence that leptin can heighten the chemosensitivity of CRC to the drug cisplatin, our study aimed to further investigate the potential target and underlying mechanism of leptin in modulating CRC cells' sensitivity to cisplatin. To clarify leptin-associated oncogenes, we first screened differentially expressed genes between CRC tissue and normal tissue through bioinformatics analysis of the GSE251845 database, which revealed 1,586 upregulated genes in CRC tissues (Figure 2A). It has been reported that leptin enhances the resistance of breast cancer stem cells to chemotherapy by modulating the FAO pathway (18). As a result, the lipid metabolism-associated genes and pathways were also specially focused in this study. According to the GO analysis, fatty-acid metabolic pathways are particularly activated in CRC, including lipid transport, lipid localization, and organic acid transmembrane transport (Figure 2B). The KEGG pathways analysis indicated similar results, indicating fatty-acid metabolism is crucial in the tumorigenesis of CRC (Figure 2C). In particular, the dysregulated KEGG pathways fatty-acid degradation and adipocytokine signaling pathway were of particular note, which may serve as important regulator of tumor lipid metabolism. Notably, *CPT1B* was identified as a differentially expressed gene in all three pathways and upregulated in CRC compared with normal tissue (Figure 2D). Analysis of hub genes reveals that *CPT1B* interacts with multiple lipid metabolism-related genes, suggesting that *CPT1B* is extensively involved in the regulation of lipid metabolism in tumor cell (Figure 2E).

Consequently, we hypothesized that leptin can regulate the tumor lipid metabolism by inhibiting *CPT1B* thereby increasing tumor sensitivity to chemotherapeutics.

To further test this, we conducted immunofluorescence detection analysis on clinical samples from CRC and normal intestinal tissues. This analysis confirmed the enhanced expression of *CPT1B* in malignant tissue (Figure 2F). To validate these observations, we analyzed 10 clinical samples of CRC tissue using Western blot techniques. In seven out of the 10 pairs of samples, the cancerous tissue exhibited a higher *CPT1B* expression compared to adjacent noncancerous tissue (Figure 2G).

These results suggest a potential mechanism by which leptin modulates CRC chemosensitivity to cisplatin through the *CPT1B* pathway. This study underscores the importance of further exploring the leptin-*CPT1B* axis as a therapeutic target, which could lead to improved treatment strategies for patients with CRC resistant to conventional chemotherapy.

Leptin reduced the expression of CPT1B in CRC cells

We then explored the impact of leptin on *CPT1B* expression in distinct CRC cell lines using Western blot and qRT-PCR. The data indicated a significant reduction in *CPT1B* messenger RNA (mRNA) levels in CRC cells following leptin exposure, with a dose-dependent decrease in *CPT1B* expression as leptin concentration increased (Figure 3A, 3B).

To further elucidate leptin's role in regulating *CPT1B* expression, we pretreated CRC cells with antileptin antibodies, which was followed by either leptin treatment or no leptin treatment. Western blot and qRT-PCR analyses demonstrated that compared to cells treated exclusively with leptin, the application of leptin antibodies notably reversed leptin's inhibitory impact on *CPT1B* expression (Figure 3C, 3D). These experimental findings collectively confirmed that leptin can suppress the expression of *CPT1B* in CRC cells. Consequently, we proceeded with additional experiments to ascertain whether leptin enhances the chemosensitivity of CRC cells to cisplatin through the regulation of *CPT1B*.

Leptin promoted the chemosensitivity of CRC to cisplatin by reducing CPT1B expression

In the initial phase of our studies, we transfected pLKO vectors into HT29 cells to establish a baseline for comparison. We observed that the induction of apoptosis

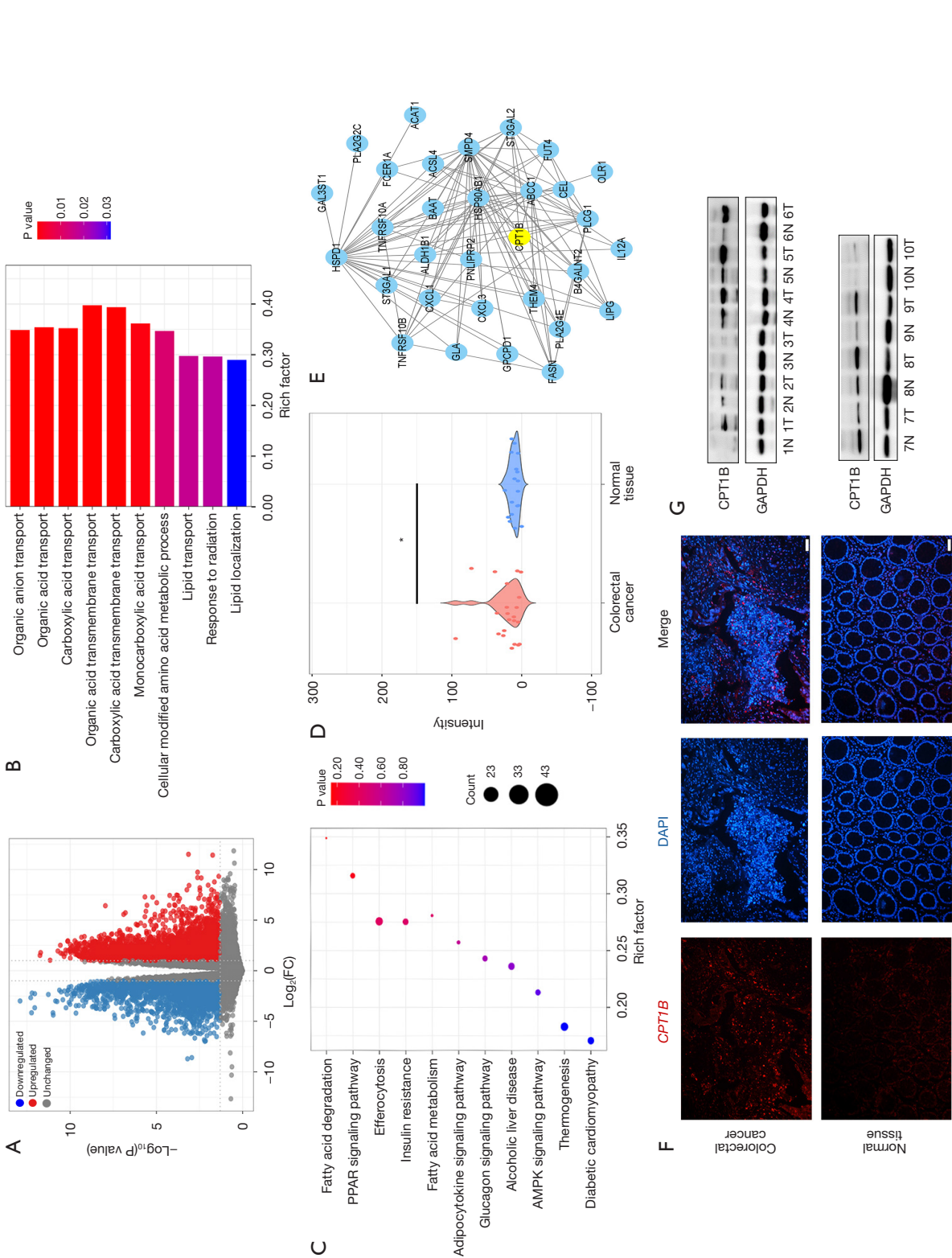


Figure 2 *CPT1B* was highly expressed in CRC. (A-E) Bioinformatics techniques were used to analyze the expression of *CPT1B* and related pathways in colon cancer. (F) Immunofluorescence was used to show the expression of *CPT1B* in colon cancer tissue samples. The scale bar is 10 μm . (G) Analysis of *CPT1B* expression in clinically collected colon cancer samples using Western blot. The data are presented as the mean $\pm$ SEM. Asterisks indicate statistically significant differences compared to the control group. *, $P < 0.05$. FC, fold change; *CPT1B*, carnitine palmitoyltransferase-1b; DAPI, 4',6-diamidino-2-phenylindole; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; N, normal colorectal tissues; T, CRC tissues; CRC, colorectal cancer; SEM, standard error of the mean.

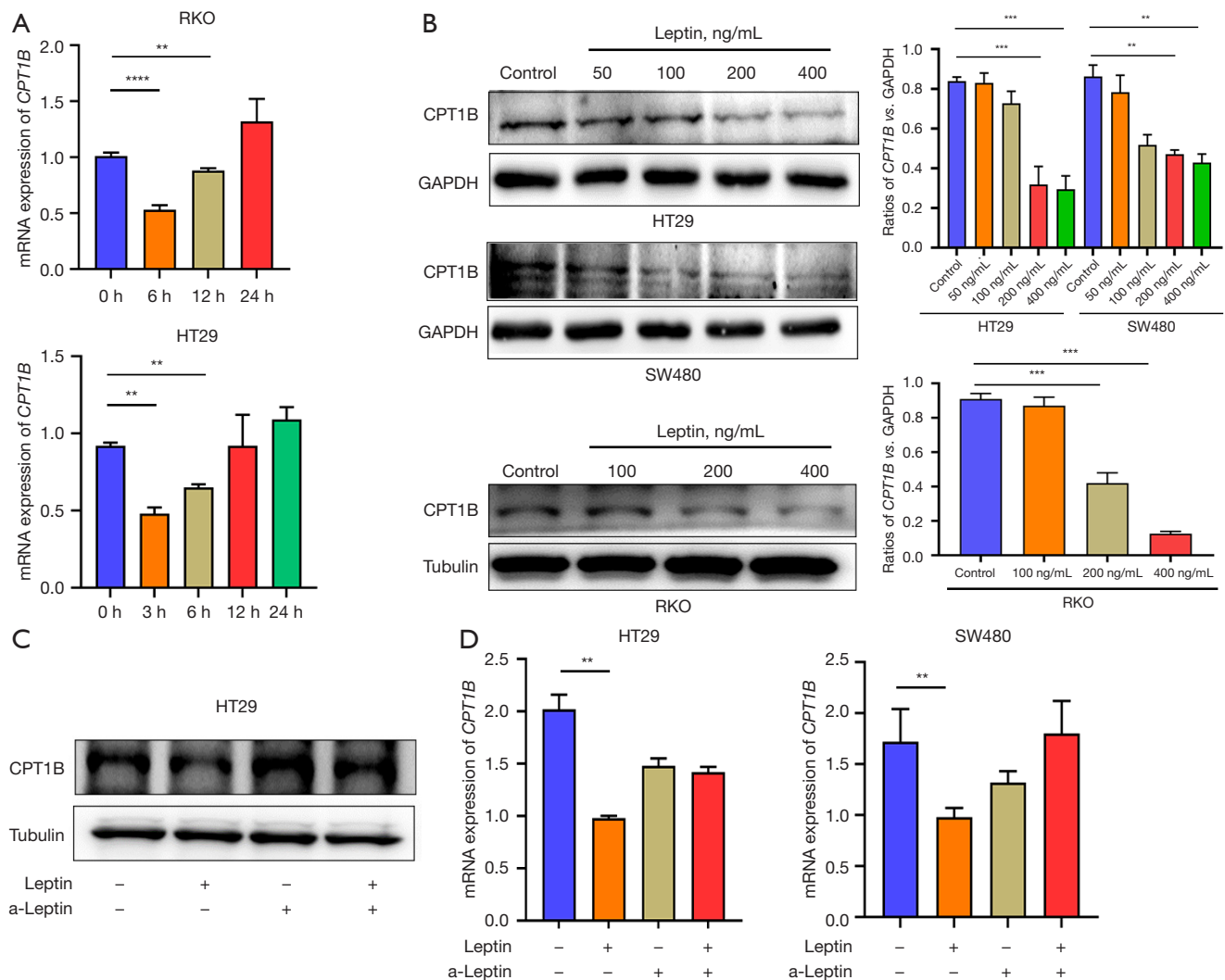


Figure 3 Leptin reduced *CPT1B* expression in CRC. (A) mRNA expression of *CPT1B* in two CRC cell lines (RKO and HT29) at different time points after leptin treatment. (B) *CPT1B* expression in three CRC cell lines (HT29, SW480, and RKO) after treatment with leptin at different concentrations. (C) Changes in *CPT1B* expression after HT29 cells were treated with or without leptin or leptin combined with a leptin-neutralizing antibody. (D) Changes in *CPT1B* mRNA expression in two CRC cell lines (HT29 and SW480) treated with or without leptin or leptin combined with a leptin-neutralizing antibody. $n=4$ for each group. The differences were analyzed using two-tailed Student t -tests. The data are presented as the mean $\pm$ SEM. **, $P<0.005$; ***, $P<0.001$; ****, $P<0.0001$. mRNA, messenger RNA; *CPT1B*, carnitine palmitoyltransferase-1b; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; a-Leptin, leptin-neutralizing antibody; CRC, colorectal cancer; SEM, standard error of the mean.

in HT29 cells was enhanced when they were subjected to a combination treatment of cisplatin and leptin, as opposed to CIS alone. Following this, we used shRNA techniques to knockdown *CPT1B* in both HT29 and SW480 CRC cells. As depicted in Figure 4A, the knockdown of *CPT1B* was successful, leading to a significantly diminished expression level of *CPT1B* in these cells.

After the knockdown, the two CRC cell lines were exposed to cisplatin and leptin. Apoptosis assays indicated that the number of apoptotic cells in the experimental group (sh*CPT1B* #1/2) significantly decreased compared to the negative control (NC) group, suggesting that after the reversal of the mediated effect of *CPT1B*, leptin was unable to enhance chemotherapy sensitivity (Figure 4B).

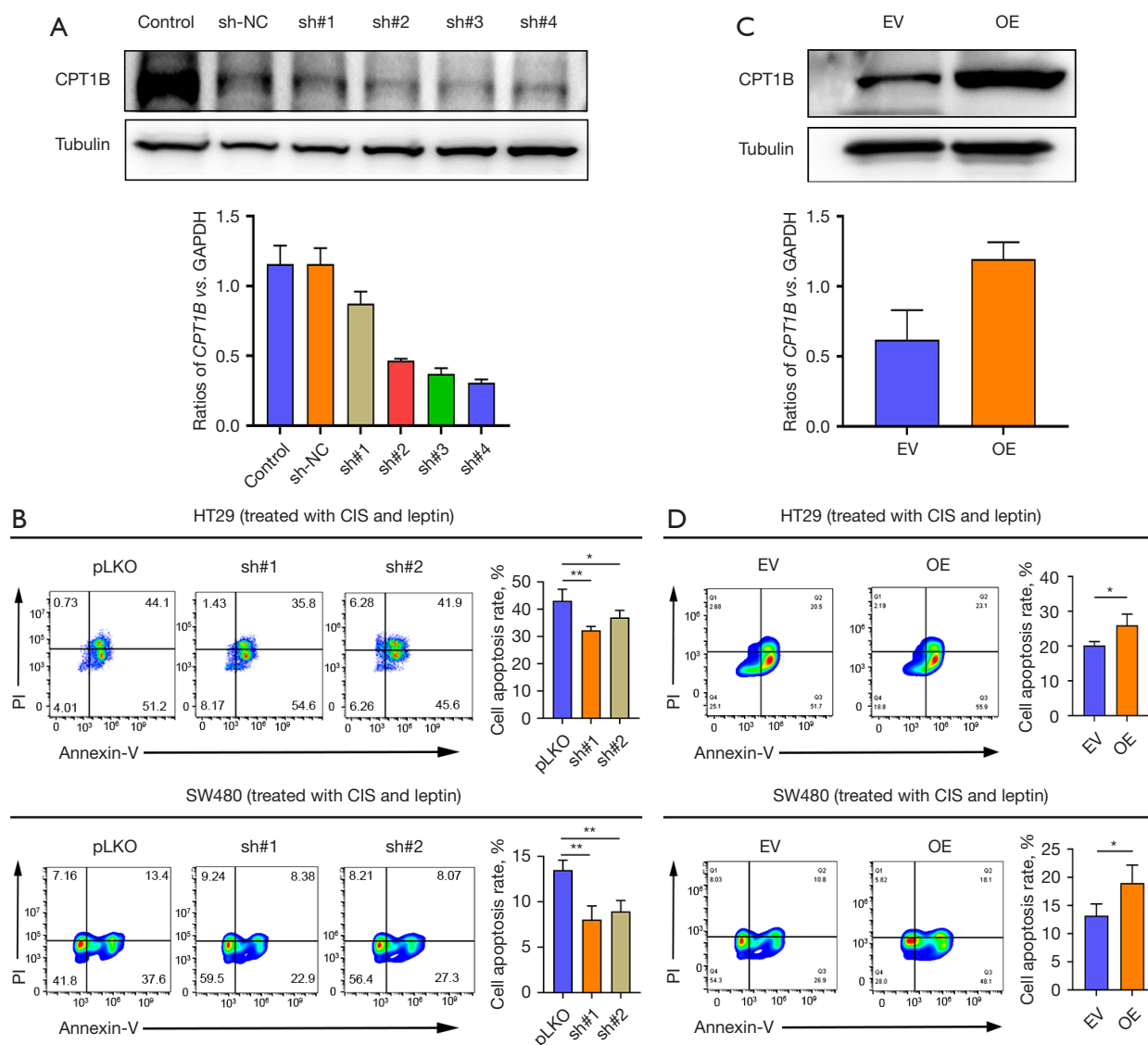


Figure 4 Leptin promoted the chemotherapeutic sensitivity of CRC to cisplatin through the reduction of *CPT1B*. (A) The expression of *CPT1B* and tubulin was evaluated after HT29 cells were transfected with *CPT1B*-shRNA-NC (#1/#2/#3/#4). (B) Apoptosis of HT29/SW480 cells after transfection with pLKO/*CPT1B*-shRNA-#1/#2 after CIS and leptin treatment. (C) The expression of *CPT1B* and tubulin in HT29 cells was evaluated after the transfection of EV/*CPT1B*-OE. (D) Apoptosis of HT29/SW480 cells after transfection with EV/*CPT1B*-OE after CIS and leptin treatment. The differences were analyzed using two-tailed Student *t*-tests. The data are presented as the mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.005$. *CPT1B*, carnitine palmitoyltransferase-1b; shNC, negative control shRNA; shRNA, short hairpin RNA; sh, shRNA *CPT1B*; EV, empty vector; OE, overexpression; CIS, cisplatin; PI, propidium iodide; CRC, colorectal cancer; NC, negative control; SEM, standard error of the mean.

To further elucidate the role of *CPT1B*, we created overexpression (OE) and control empty vector (EV) cell lines for *CPT1B*. After transfection and Western blot validation, we achieved OE of *CPT1B* (Figure 4C). Upon the addition of cisplatin and leptin to these cell lines, the

OE-*CPT1B* group exhibited a significantly higher number of apoptotic cells compared to the EV group, indicating that high expression of *CPT1B* could significantly inhibit chemotherapy sensitivity (Figure 4D).

These findings collectively support the role of leptin in

augmenting the chemosensitivity of CRC to cisplatin, which appears to be mediated through the downregulation of *CPT1B*. Our results also suggest the potential for targeting of the leptin-*CPT1B* axis as a novel therapeutic strategy to enhance the efficacy of chemotherapy in CRC.

Discussion

Leptin, a cytokine primarily secreted by adipocytes, has garnered significant attention in cancer research in recent years (20). Although leptin is sometimes viewed as a procancer factor, an increasingly growing body of research suggests it also holds substantial potential in inhibiting cancer progression and enhancing the efficacy of immunotherapy. First, in CRC, studies have shown that decreased leptin levels are associated with better prognoses following surgical treatment (12). This finding implies that leptin may play an inhibitory role in the tumor microenvironment. Furthermore, research has indicated that leptin can suppress hepatocellular carcinoma development by blocking the p38-MAPK-dependent signaling pathway. *In vitro* experiments have demonstrated that leptin effectively reduces the proliferation of liver cancer cells through this mechanism, highlighting its potential as an anticancer agent (13).

In clinical studies on breast cancer, leptin expression levels have been shown to be significantly higher in patients responding to chemotherapy compared to those with disease progression (19). This suggests that leptin may enhance chemotherapy sensitivity. Additionally, the statistically significant difference in *LEPR* expression within the chemotherapy response group further indicates a positive relationship between leptin and chemotherapy (19). These studies indicate that leptin not only helps inhibit tumor growth but also improves cancer cells' responsiveness to chemotherapy, thereby enhancing treatment outcomes. What's more, the positive relationship between leptin and cisplatin treatment sensitivity reveals similar results. Patients with lower serum leptin at diagnosis and an increase during cisplatin chemotherapy predict a better clinical prognosis of advanced lung adenocarcinoma (21), which shows that leptin may be a useful marker for the prognosis of cancer patients undergoing cisplatin treatment. Meanwhile, recent studies have reported that a high-fructose diet boosts leptin production from adipocytes, which in turn prevents CD8 T cells from becoming terminally exhausted, thereby enhancing antitumor CD8 T cell responses and controlling lung cancer progression (22).

Leptin also shows potential as an anticancer agent in other cancer types. For instance, in esophageal cancer, leptin effectively inhibits cancer cell growth and spread by regulating signaling pathways related to cell proliferation and apoptosis. Although these studies are still in their early stages, initial results indicate that leptin holds promise for diverse cancer treatments.

CPT1B, a key enzyme in fatty-acid metabolism, plays a crucial role in the β -oxidation process of fatty acids, which is significant for tumor cell energy metabolism. The NADH and FADH₂ produced by fatty-acid β -oxidation are used in oxidative phosphorylation to generate ATP, providing the energy necessary for rapid tumor cell proliferation. Numerous studies have shown that high *CPT1B* expression is associated with various cancer types, including breast cancer (19), liver cancer (23), and CRC (24). In these cancers, elevated *CPT1B* expression not only promotes tumor cell energy metabolism but also enhances their antioxidant capacity, aiding tumor cells in surviving and proliferating in harsh microenvironments. Furthermore, high *CPT1B* expression is linked to resistance to several chemotherapy drugs. By regulating antioxidant pathways, *CPT1B* enhances tumor cell survival and reduces chemotherapy-induced apoptosis. *CPT1B* can also affect intracellular drug efflux mechanisms, further increasing chemotherapy resistance (25,26).

Our study, combining bioinformatics analysis and clinical sample detection, found that CRC is closely related to highly active fatty-acid metabolism and is dependent on high *CPT1B* expression. Flow cytometry results similarly showed significant responsiveness to cisplatin in CRC cells treated with leptin. Thus, we hypothesized that leptin might enhance CRC's sensitivity to cisplatin by regulating *CPT1B* expression. We found that leptin can inhibit *CPT1B* expression in CRC cells. Furthermore, OE of *CPT1B* in CRC cells via plasmids resulted in significantly increased apoptosis following cisplatin treatment. Conversely, a reduction in *CPT1B* expression suppressed leptin's ability to enhance cisplatin's cytotoxic effect on tumor cells. These results indicate that leptin CRC cells' chemosensitivity to cisplatin by regulating *CPT1B*.

To further validate leptin's positive effect on enhancing cisplatin treatment in CRC cells, we hypothesized that leptin supplementation might also induce chemosensitivity *in vivo*. We evaluated the combined effect of cisplatin and leptin in a mouse subcutaneous tumor experiment. The representative graphs in [Figures S1,S2](#) clearly show that leptin treatment, in combination with cisplatin, significantly

reduced tumor size compared to cisplatin treatment alone. Consistent with the reduced tumor size, quantitative analysis showed decreased tumor volume and weight in the CIS + leptin group. These findings further confirmed that leptin can induce the chemosensitivity of CRC cells to cisplatin.

Although these findings suggest the value of adjunctive treatments for CRC, our study has several limitations. For instance, leptin's effect on CRC drug sensitivity was only studied with cisplatin, leaving it unclear whether other drugs might be similarly affected. Additionally, the specific mechanisms by which leptin inhibits *CPT1B* and the downstream molecular changes were not examined. Moreover, *in vivo* knockout experiments in mice were not conducted, highlighting the need for further research to address these limitations and better understand leptin's role in CRC chemotherapy response, as well as to comprehensively explore its therapeutic potential.

In summary, our study provides preliminary insights into how leptin might enhance CRC chemosensitivity by suppressing the *CPT1B* pathway. These findings offer important evidence for leptin as a potential target for cancer prevention and treatment. Further research will help to fully elucidate leptin's role in cancer therapy and provide stronger support for its clinical application, potentially offering valuable strategies for increasing chemotherapy sensitivity in patients with CRC.

Conclusions

This study examined the role of leptin in enhancing the chemosensitivity of CRC to cisplatin, with a particular focus on the underlying molecular mechanisms. We observed that when leptin was combined with cisplatin, it not only significantly increased the apoptosis rate of CRC cells *in vitro* but also led to a marked reduction in tumor volume and weight in a xenograft mouse model. Further bioinformatics analysis, along with experimental validation, revealed that leptin enhances CRC chemosensitivity to cisplatin by downregulating *CPT1B*, which is associated with fatty-acid metabolism. Therefore, targeting the leptin-*CPT1B* axis may represent a promising therapeutic strategy for improving the chemotherapy outcomes in patients with CRC who are resistant to conventional treatments.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE and MDAR reporting checklists. Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2024-950/rc>

Data Sharing Statement: Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2024-950/dss>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2024-950/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. In accordance with the ethical protocols of Soochow University and the principles outlined in the Declaration of Helsinki (as revised in 2013) by the World Medical Association, the study was conducted with strict compliance to all applicable rules and standards for research involving human participants. The research protocol was approved by the Ethical Review Board of the First Affiliated Hospital of Soochow University (ethics No. 2024737). Informed consent was taken from all the patients. All animal experimental procedures were approved by the Ethical Review Board of the First Affiliated Hospital of Soochow University (ethics No. 2024737) and carried out in compliance with the Guide for the Care and Use of Laboratory Animals, 8th edition.

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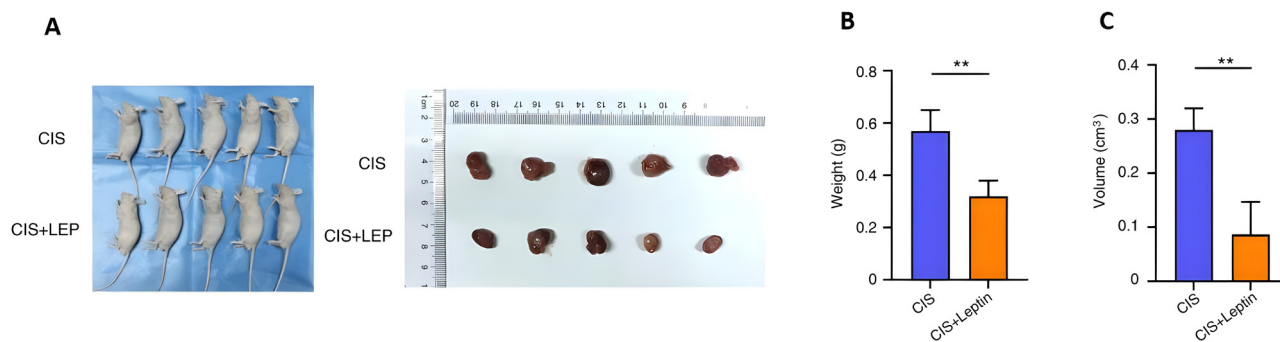


Figure S1 A mouse subcutaneous xenotransplantation model was established to verify the ability of leptin to enhance the chemotherapeutic sensitivity of CRC to cisplatin *in vivo*. (A) Average tumor size in the two groups (scale =1 cm). (B) Average tumor weight in both groups. (C) Average tumor volume in both groups. n=5 mice for each group. The differences were analyzed using two-tailed Student *t*-tests. The data are presented as the mean $\pm$ SEM. **, P<0.005. CIS, cisplatin; LEP, leptin; CRC, colorectal cancer; SEM, standard error of the mean.

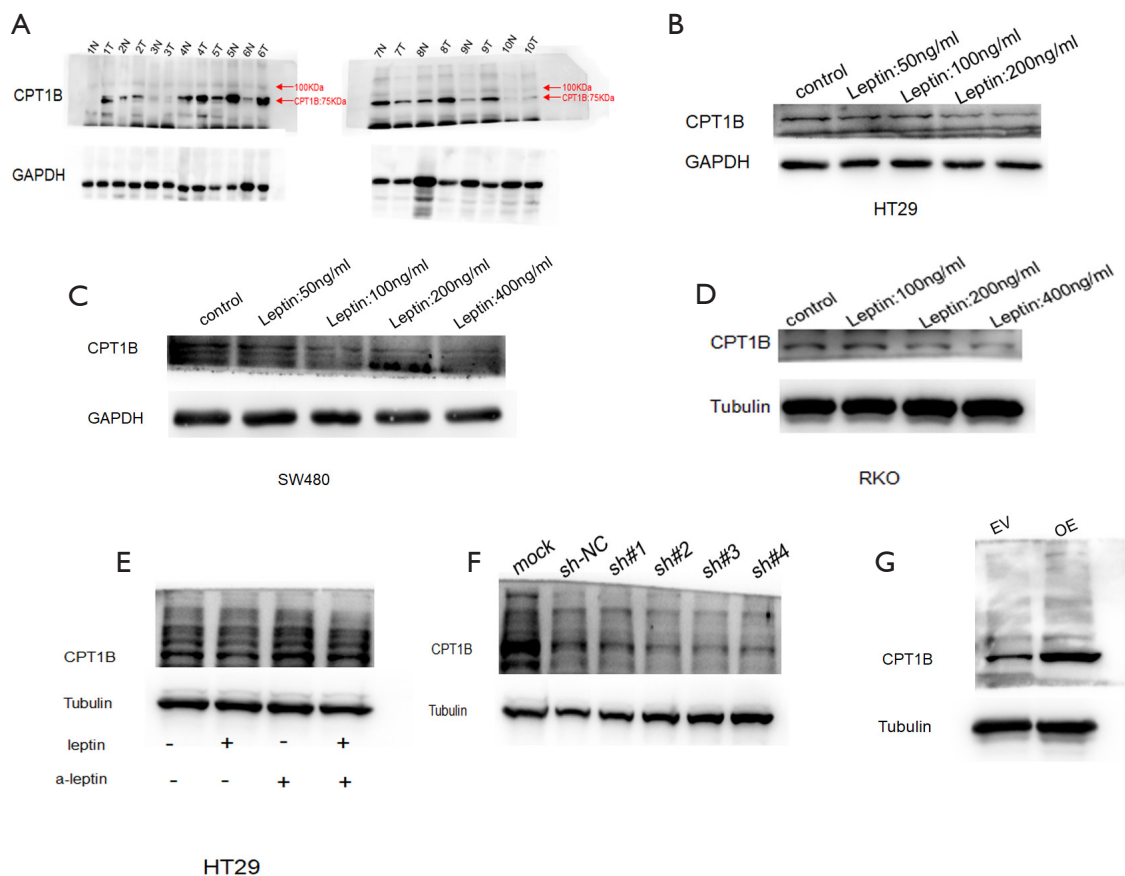


Figure S2 Full uncropped and unedited Western blot pictures. (A) Correspond to the blot pictures in Figure 2G. (B-D) Correspond to the blot pictures in Figure 3B. (E) Correspond to the blot pictures in Figure 3C. (F) Correspond to the blot pictures in Figure 4A. (G) Correspond to the blot pictures in Figure 4B. The molecular mass of *CPT1B* used for Western blot is 75 kDa. *CPT1B*, carnitine palmitoyltransferase-1b; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; N, normal colorectal tissues; T, CRC tissues; CRC, colorectal cancer; a-Leptin, leptin-neutralizing antibody; shNC, negative control shRNA; shRNA, short hairpin RNA; sh, shRNA *CPT1B*; EV, empty vector; OE, overexpression.