Acupuncture and Herbal Medicine

Network pharmacology and bioinformatics analysis identify potential therapeutic effects of berberine on colon cancer complicated with radiation enteritis

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Abstract

Objective: Patients with colon adenocarcinoma (COAD) who undergo radiation therapy develop radiation enteritis (RE). The predictive value of RE in COAD is yet to be established. Berberine, an active compound derived from the traditional Chinese medicinal plant, *Coptis chinensis*, has notable anti-inflammatory properties and offers protection to the intestinal mucosa. This study aimed to evaluate the possible therapeutic effect and mechanism of berberine as a treatment for COAD complicated with RE (COAD&RE).

Methods: Relevant genetic features of diverse COAD&RE populations were analyzed using bioinformatics and the Cox proportional hazards regression model. The therapeutic targets of berberine were predicted using network pharmacology and molecular docking. *In vivo* and *in vitro* experiments were conducted to validate the core genes identified using molecular docking.

Results: RE has a certain impact on the prognosis of COAD and berberine may play an important role in the treatment of COAD&RE. In addition, we identified five core therapeutic targets of berberine by network pharmacology and molecular docking: CCND1, MYC, AR, LEP, and CYP19A1. *In vivo* experiments showed that berberine increased short-term survival rate, body weight, and intestinal epithelial cell recovery in mice after radiation. In an *in vitro* study, berberine promoted the proliferation of human intestinal epithelial cells and enhanced the radiosensitivity of HT29 cells after radiation, and the relative mRNA expression levels of *CCND1* and *MYC* closely correlated with these effects.

Conclusions: This study predicted the potential therapeutic effects of berberine on COAD&RE and verified the relevant mechanisms, which may provide insights and suggestions for the clinical treatment of COAD&RE.

Keywords: Berberine, Bioinformatics analysis, Colon adenocarcinoma, Prognosis, Radiation enteritis

Graphical abstract: http://links.lww.com/AHM/A124.

Introduction

According to data published by the American Cancer Society, colon cancer is the second most widespread type of cancer and contributes to being the third primary cause of cancer-related mortality worldwide[1]. There are many different opinions from experts in various countries regarding the treatment guidelines for colon cancer[2]. However, as an important adjuvant treatment, radiotherapy has become an increasingly important part of the colon adenocarcinoma (COAD) treatment landscape before, during, and after colon cancer surgery[3,4].

However, it is widely acknowledged that the administration of radiotherapy dosage substantially influences the occurrence of radiation enteritis (RE). However, strategies implemented to protect other organs from damage are not very effective in protecting the gut, owing to its fixed location. Concurrently, exposure to

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ionizing radiation may induce injury to the intestinal lining, manifesting as clinical symptoms, including nausea, vomiting, abdominal discomfort, and diarrhea, and, in severe cases, can lead to mortality[5,6]. Acute radiation-induced rectal injury occurs in 61% of the patients treated with pelvic radiotherapy[7]. Regrettably, apart from reducing the radiation dosage or discontinuing the treatment, there are limited alternatives to mitigate or prevent these adverse effects. However, these measures can substantially affect the patients' subsequent medical care and overall well-being[8]. Most therapies for RE focus primarily on alleviating symptoms. These include the administration of anti-inflammatory mesalazine, utilization of sucralfate to provide wall protection, application of formalin to prevent further bleeding, and consideration of endoscopic or surgical interventions[9,10]. There is a current lack of universally effective pharmacological treatments for RE is evident. Hence, considering this challenging complexity, there is an urgent need to develop clinical treatment drugs for patients with COAD complicated with RE (COAD&RE) that may be effective and have no negative effects on colon tumors.

Coptidis Rhizoma (Huang Lian) is a common herbal medicine used to treat various gastrointestinal diseases[11–13]. Berberine is the primary bioactive constituent of *Coptidis Rhizoma*. Numerous studies have demonstrated the potential of berberine to treat a wide range of medical conditions, including cancer[14], gastrointestinal disorders[15], and cardiovascular ailments[16,17]. This compound exhibits distinct targeting of intestinal inflammation and possesses antibacterial properties[18]. In gastrointestinal diseases, berberine possesses anti-inflammatory properties through the inhibition of T helper cell 1 (Th1) and T helper cell 17 (Th17) cell proliferation as well as the suppression of nuclear factor kappa-B (NF-κB) activation[19], and reducing the levels of pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α), interferon-γ (INF-γ)[20], and interleukin-10 (IL-10)[21]. It can also regulate opioid receptors to inhibit intestinal peristalsis[22], exert anti-secretory activity in diarrhea[23], avoid intestinal epithelial barrier damage[24], and inhibit bacteria[25]. Berberine is regarded as a candidate drug for the treatment of inflammatory bowel disease[26,27]. This evidence may be partially applicable to the RE, which is also involved in inflammation and barrier damage. However, it can also suppress cancer and improve radiotherapy safety. Berberine prevents tumor spread by inducing p53[28], regulating reactive oxygen species (ROS)[29], inducing mitochondria-mediated apoptosis[30], and inhibiting tumor invasion and metastasis by inhibiting epithelial-mesenchymal transition[31] through the extracellular regulated protein kinases/mitogen activated protein kinase (ERK/MAPK)[32] and Smads[33] pathways. Furthermore, compelling evidence suggests that berberine has an augmented cytotoxic effect against lung cancer when combined with synergistic radiation therapy[34].

Currently, research on the use of berberine for treating COAD&RE is still in its early stages, and the specific targets and mechanisms involved are unknown. In this study, we aimed to comprehensively analyze the mechanism of berberine as an adjuvant therapy for COAD&RE. To accomplish this, we adopted a comprehensive strategy that integrates network pharmacology, bioinformatics, and molecular docking. In addition, we validate the outcomes of the aforementioned predictions through *in vivo* and *in vitro* experiments. The findings of this study offer crucial ramifications for future clinical interventions and investigations.

Materials and methods

Identification of COAD&RE–associated genes

To identify genes linked to COAD and RE, we acquired transcriptomic profiles for patients with colorectal cancer from The Cancer Genome Atlas (TCGA), as recorded on May 5, 2020. The analysis of differential gene expression was performed using the "limma" package in R-Bioconductor, employing a threshold of a false discovery rate <0.05 and |log fold change| >1. To identify the target genes related to rectal endometriosis, we referred to the Genecard database, OMIM database, and NCBI gene function module, because they are not classified as neoplastic disorders. By comparing these genes, we identified the overlapping targets in COAD&RE[35,36].

Clinical analysis of COAD&RE–associated genes

The survival package in R was used to evaluate the correlation between genes associated with COAD&RE and patient survival rates. Prognostic analyses were conducted using univariate Cox proportional hazards regression, whereas a multivariate Cox proportional hazards regression model was used to examine various characteristics of disease-related genes in patients with COAD&RE. Based on their average risk scores, the patients were categorized into low- and high-risk groups[37].

Acquisition of berberine pharmacological targets in COAD&RE

The pharmacological targets of berberine were comprehensively identified and compiled using several on-line resources, including the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), Swiss Target Prediction, TargetNet, Batman-TCM, DrugBank, and HitPick[38]. To ensure data accuracy, we further refined the identified candidate genes using the reviewed (Swiss-Prot) and human settings in the UniProt database[39]. Using these criteria, we identified a set of overlapping candidate genes.

Enrichment analyses and network visualization

Enrichment analysis and the visualization of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for the intersecting gene targets of berberine in COAD&RE were executed using a suite of R-language packages, including "ClusterProfiler," "ReactomePA," "org.Hs.eg.Db," and "GOplot." The "org.Hs.eg.Db" package was specifically employed to retrieve GO data. The analysis was carried out by applying a significance threshold of 0.05 for both *P*- values and *q* values. The results were used to generate bubble charts, bar charts, and Circos plot[40]. To visually represent the network involving drug-target-GO function-pathway-disease associations, Cytoscape

software (version 3.7.1) was employed. This network provided insights into the GO terms and KEGG pathways associated with the intersecting genes in response to the targeting COAD&RE[41].

Identifying core targets of berberine against COAD&RE

The STRING database (version 11.0) was used to identify the common targets of berberine treatment and COAD&RE. We used this database to retrieve an interaction network consisting of functionally associated proteins and a protein–protein interaction (PPI) network map. The Network-Analyzer feature in Cytoscape was used to analyze the topological parameters of the network. The target network exhibits a median degree of freedom of 3.231, with a maximum degree of 7. Based on this information, we set the range for the core target screening condition to 4 to 7 degrees of freedom. Considering the degree value of each target, we applied the MCODE plug-in in Cytoscape for core target identification[42].

Molecular docking

Molecular docking analysis was used to predict and determine the binding conformation and interaction strength between the small molecules and protein receptors. The structural files of protein receptors were obtained from the Protein Data Bank (PDB) database. AutoDock and Vina software were used for the subsequent analyses. The dimensions of each mobile pocket in the target protein were size_x = 40, size_y = 40, and size_z = 40. A genetic algorithm[43] was implemented to perform the docking process. PyMOL was used to visualize the docking results with the lowest binding energies.

Animal groupings and radiation source

Male SPF C57BL/6J mice weighing 20 to 22 g were obtained from Beijing Biotechnology Co., Ltd. (Beijing, China). The mice were randomly divided into three groups: control (CON), model (IR), and berberine (IRB). Thirty mice participated in this experiment, with 10 mice allocated to each group. Following an adaptation period of 3 days, during which they received regular feeding, all mice underwent an 8-hour fasting period prior to irradiation treatment. The study protocol strictly adhered to the ethical guidelines for animal experimentation and was approved by the Ethics Committee of the Academy of Military Medical Science (IACUC-DWZX-2023-P595). From day 3 before radiation to day 5 after irradiation, the IRB group was given 50 mg/kg berberine (S9046; Selleck, TX, USA) suspension with 0.9% (w/v) physiological saline at a dose of 5 mg/mL, and the CON and IR group were given 0.2 mL 0.9% (w/v) physiological saline. All medications were administered once daily. The γ-radiation source was ^{60}Co and procured from the Institute of Academy of Military Medical Science. The mice were securely positioned within an irradiation chamber, and all groups, except for the CON group, received a single whole-body exposure to radiation at a distance of 250 cm from the source to their skin. The radiation dose rate was set at 70 R/min, with a total dose of 8.5 Gy applied to induce RE in the mouse model. Mice exhibiting severe health deterioration and abdominal aortic aneurysms were humanely euthanized using an intraperitoneal overdose of pentobarbital sodium (100 mg/kg, intravenous [IV]).

Hematoxylin-eosin and immunofluorescence (IF) staining

Five patients from each group were randomly selected on day 5 post-irradiation. The small intestine was excised and 3 cm of the upper segment of each jejunum was immersed in 4% (w/v) paraformaldehyde for 72 h. The jejuna were sectioned into 5-μm slices, embedded in paraffin.

The intestinal slides were deparaffinized, rehydrated, and immersed in ethylene diamine tetraacetic acid (EDTA) antigen retrieval buffer at pH 8.0. After blocking, the slides were incubated with anti-Ki67 (GB121141; Guge, Beijing, China), anti-Muc2 (GB11344; Guge), Goat Anti-Rabbit Cy3 (B100802; Baiqiandu, Beijing, China), or Goat Anti-Mouse Cy3 (B100801; Baiqiandu) antibodies. Tunnel staining was performed using the Tunnel Kit (11684817910; Roche, Basel, Switzerland). The images were then analyzed using ImageJ software (Java 1.8.0_172).

Cell lines and cell culture

Berberine with at least 99% purity (S9046, Selleck) was dissolved in dimethyl sulfoxide. Human colon cancer cell lines (HT29 and CBP60011) and human intestinal epithelial cell lines (HIEC and CRL-3266) were purchased from the ATCC Cell Bank. The two cell lines were cultured in high-glucose DMEM (C11965500BT; Gibco, Grand Island, USA) supplemented with 10% fetal bovine serum (C0235; Gibco) and 1% penicillin/streptomycin solution (15140122; Gibco).

CCK8 assay

Cellular viability was quantitatively assessed using the Cell Counting Kit-8 (CA1210; Solarbio, Beijing, China), strictly following the guidelines provided by the manufacturer. Cells were systematically seeded at a calculated density of 4×10^3 cells per well within a 100 μL culture medium in 96-well microplates. This was followed by the administration of berberine at varying concentrations (0, 5, 10, 20, 40, 80, 160, and 320 μM) to the cultured cells for a predetermined duration of 24 h. Post-treatment, an additional incubation phase was conducted with 10 μL of the CCK8 reagent per well for 1 h to facilitate the assessment. The experimental protocol was replicated in triplicates to ensure data reliability. Cell proliferation was quantified from absorbance measurements at a wavelength of 450 nm using a microplate spectrophotometer to determine the cytotoxic and proliferative effects of berberine on the cellular model.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated using TRIzol reagent (15596018; Invitrogen, CA, USA) in strict accordance with the manufacturer's instructions. β-Actin served as the internal reference gene. The reverse transcription process

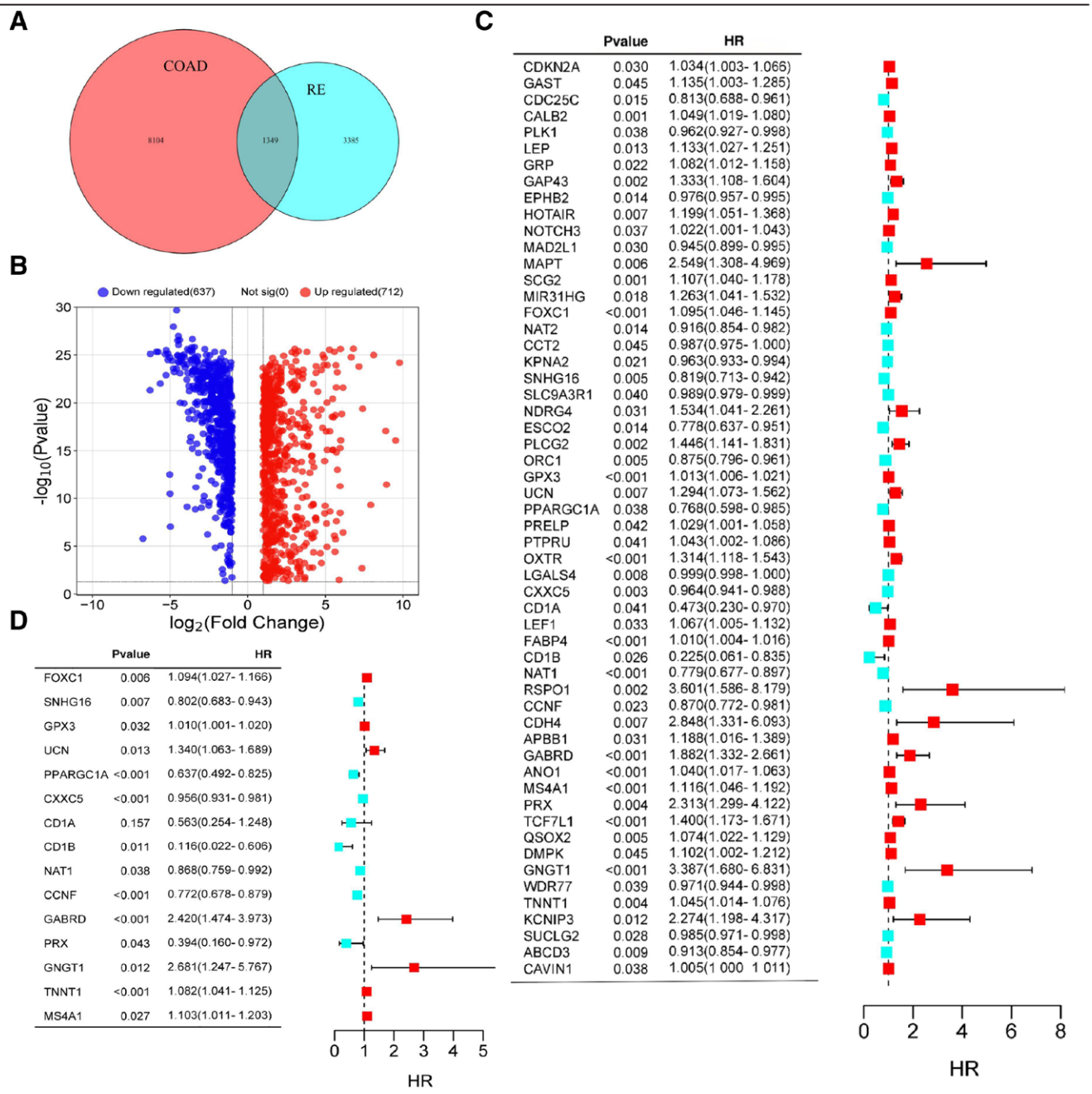

Figure 1. Analysis of intersecting genes in COAD&RE. (A) Venn diagram depicting intersecting genes in COAD&RE. (B) Volcano-plot representation of differential gene expression. (C) Univariate Cox proportional hazards regression analysis of DEGS. (D) Multivariate Cox proportional hazards regression analysis of DEGs. COAD&RE, colon adenocarcinoma complicated with radiation enteritis. COAD&RE: Colon adenocarcinoma complicated with radiation enteritis; DEG: Differentially expressed gene; HR: Hazard ratio; RE: Radiation enteritis.

was facilitated using a cDNA synthesis kit (G3330; Servicebio, Wuhan, China), adhering to the procedural protocols specified by the manufacturer. Supplementary Table S1 (http://links.lww.com/AHM/A123) provides the details of the primers used. mRNA expression levels were standardized using glyceraldehyde-3-phosphate dehydrogenase as a reference.

Results

Identification of targets and clinical analysis of genes for COAD&RE

A total of 4,734 genes associated with RE were retrieved from the Genecard, OMIM, and NCBI databases. We detected 9,453 genes exhibiting differential expression patterns in relation to COAD by leveraging TCGA database. By intersecting these two gene clusters, we identified 1,349 intersection genes in both COAD and RE (Figure 1A). We utilized the R-language to compile the data of the overlapping genes, which indicated an increase in the expression of 712 genes and a decrease in the expression of 637 genes in COAD (Figure 1B). To detect differentially expressed genes (DEGs) in COAD&RE, we performed univariate and multivariate Cox analyses on a set of 1,349 overlapping genes. In the initial step, the univariate Cox analysis identified 56 genes (including *CDKN2A, GAST, CDC25C, CALB2, PLK1,* leptin [*LEP*], and *GRP*) that exhibited significant associations with COAD&RE ($P < 0.05$). A hazard ratio (HR) >1 in red was considered a poor prognostic factor

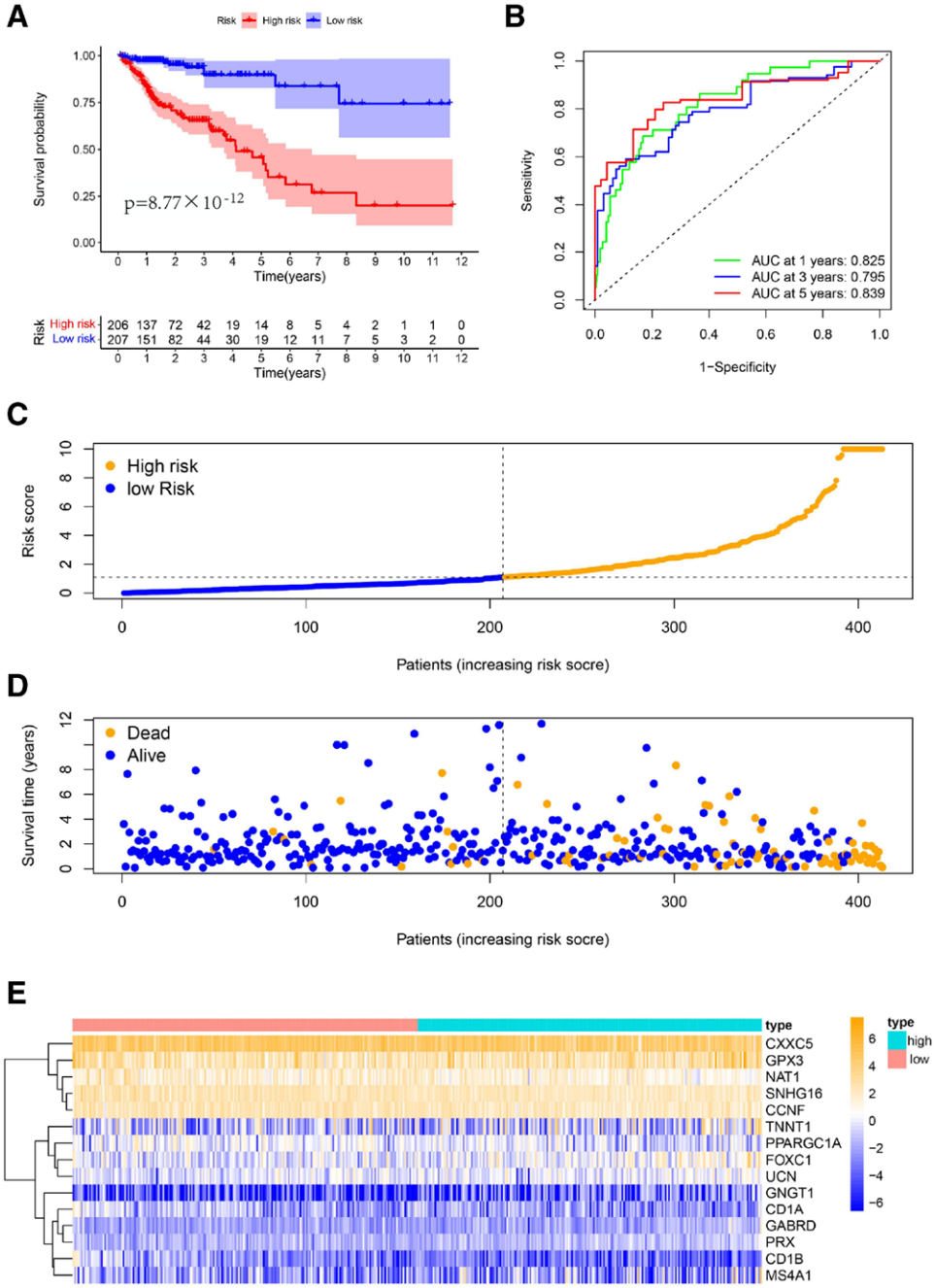

Figure 2. Construction of the clinical prediction model for COAD&RE. (A, B) Survival analysis indicated significant difference in overall survival between high- and low-risk groups. (C, D) Analysis of patients' risk score using Cox proportional hazards regression. (E) Expression levels of genes in the high- and low-risk groups. AUC: Area under the curve; COAD&RE: Colon adenocarcinoma complicated with radiation enteritis.

and indicated a negative correlation with survival time, whereas an HR >1 was colored blue, indicating a good prognostic factor and a positive correlation with survival time (Figure 1C). Multivariate Cox analysis identified 15 target genes among the 56 previously identified genes, namely *FOXC1, SNHG16, GPX3, CN, PPARGC1A, CXXC5, CD1A, CD1B, NAT1, CCNF, GABRD, PRX, GNGT1, TNNT1*, and *MS4A1* (Figure 1D).

Construction of the clinical prediction model for COAD&RE

We also constructed a prognostic model using LASSO Cox regression analysis and found that the model was most effective when the 15 genes in Figure 1D were included. Based on the coefficient values obtained from the LASSO Cox regression analysis, the patients were categorized into high- and low-risk groups. Utilizing the aforementioned set of 15 genes, we developed a COAD&RE model and conducted a survival analysis for both the high- and low-risk groups. The high-risk group exhibited a significantly reduced survival rate compared with the low-risk group at the same time points (Figure 2A). The maximum area under the curve (AUC) values of 1, 3, and 5 years of the risk score reached 0.825, 0.795, and 0.839, respectively, in the training set, which proved that the sensitivity and specificity of the constructed gene signature were qualified (Figure 2B). Furthermore, we analyzed the expression patterns of the aforementioned 15 genes in patients with colon cancer classified into high- and low-risk categories

(Figure 2C–E). Independent univariate and multivariate prognostic evaluations were performed for the 15 genes. The expression levels of *CD1A*, *CD1B*, *CXXC5*, *NAT1*, *SNHG16*, and T*NNT1* significantly differed between patients with varying survival and mortality outcomes (Figure 3A). *GABRD* and *NAT1* expression levels were associated with the primary tumor grade (Figure 3B). The expression of *CXX5*, *GABRD*, *GNGT1*, and *NAT1* correlated with the scope and number of lymph node metastases (Figure 3C). The expression levels of *CD1A*,

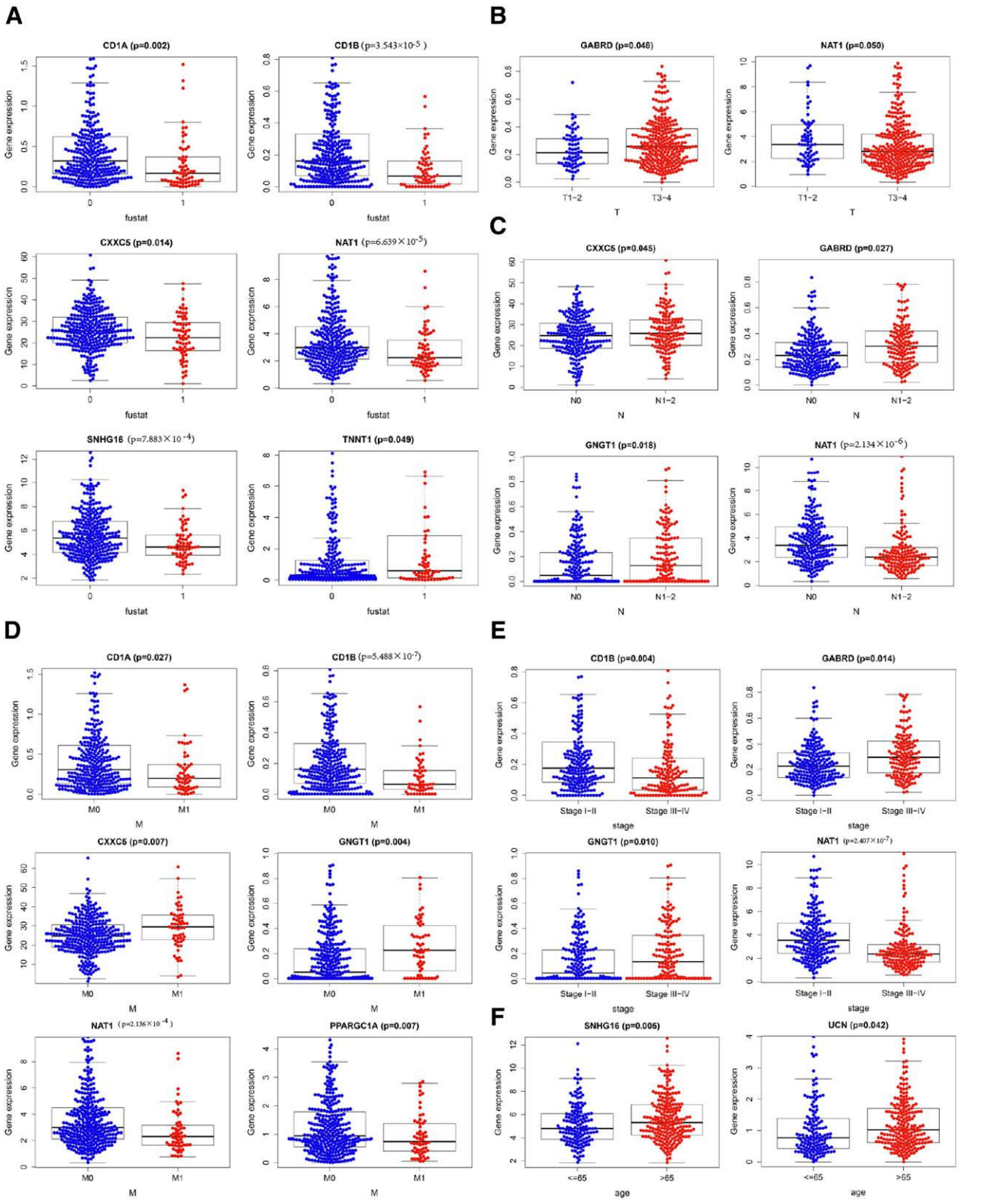

Figure 3. Clinical prognostic analysis of differential genes. (A) The expression of *CD1A, CD1B, CXXC5, NAT1, SNHG16,* and *TNNT1* is significantly different between the survival and death of patients. (B) *GABRD* and *NAT1* are correlated with the grade of primary tumor. (C) The expression of *CXX5, GABRD, GNGT1,* and *NAT1* is related to the number and extent of lymph node metastasis. (D) The expression of *CD1A, CD1B, CXXC5, GNGT1, NAT1,* and *PPARGC1A* is correlated with the degree of tumor metastasis. (E) The expression of *CD1B, GABRD, GNGT1,* and *NAT1* is related to cancer stage. (F) *SNHG16* and *UCN* expression are higher in older patients than in younger patients.

CD1B, CXXC5, GNGT1, NAT1, and *PPARGC1A* were linked to the extent of tumor metastasis (Figure 3D). *CD1B, GABRD, GNGT1,* and *NAT1* expression levels were related to the cancer stage (Figure 3E). In addition, the expression levels of *SNHG16* and *UCN* were higher in older patients than in their younger counterparts (Figure 3F).

Identification of core targets of berberine against COAD&RE and the interaction between the targets

A total of 1,349 drug targets for berberine hydrochloride were discovered using the TCMSP and SWISS target databases, while a set of 425 target genes was obtained through normalization using the UniProt database. A total of 103 differential expressed genes between berberine, COAD, and RE were identified by taking the intersection (Figure 4A). In the KEGG pathway analysis, 48 signaling pathways were identified as significantly relevant, including neuroactive ligand–receptor interactions, lipid metabolism and atherosclerosis, phosphatidylinositol-3-kinase-protein kinase B (PI3K-Akt) signaling, and the p53 pathway (Figure 4B). Furthermore, enrichment analysis of 103 DEGs revealed that berberine affected various biological processes, such as regulating responses to xenobiotic stimuli, promoting kinase activity, and influencing gland development (Figure 4C). Berberine also affects cellular components, such as the cyclin-dependent protein kinase holoenzyme complex, cell projection membranes, and protein kinase complexes. Additionally, it influences molecular functions, such as histone kinase activity, neurotransmitter receptor activity, and catecholamine binding (Figure 4C). The PPI network mediated by 103 intersection genes of berberine in the treatment of COAD&RE was identified using STRING analysis. All intersection genes were input into Cytoscape, the PPI network topology parameters related to berberine in the treatment of COAD&RE were calculated, and the interaction network of therapeutic targets was generated (Figure 4D, E). According to the degree value and correlation between the target genes, seven core gene targets were determined: cyclin D1 (*CCND1*), *MYC, LEP,* androgen receptor (*AR*), cytochrome p450 enzyme (*CYP19A1*), bone gamma-carboxyglutamate protein (*BGLAP*), and ATP binding cassette subfamily G member 2 (*ABCG2*) (Figure 4F).

Molecular docking between berberine and therapeutic targets for COAD&RE

To investigate the potential binding between berberine and the core therapeutic targets in COAD&RE, molecular docking analysis was performed. The crystal structures of the core therapeutic targets for COAD&RE were collected from the PDB database for docking analysis. Using computer-simulated molecular docking techniques, we determined that berberine could bind to protein crystals associated with five core targets: *MYC, CCND1, LEP, AR,* and *CYP19A1*. In detail, the binding energy between berberine and ARG-181 residue on the protein crystal of CCND1 (PDBid: 2W96) was −7.45 (Figure 5A). The binding energy between berberine and residue LYS-30 on the protein crystal of MYC (PDBid: 1A93) was −6.14 (Figure 5B). The binding energy between berberine and residue GLY-44 on the protein crystal of LEP (PDBid: 1AX8) was −6.83 (Figure 5C). The binding energy between berberine and residues LYS-808/TYR-763 on the protein crystal of AR (PDBid: 1E3G) was −8.39 (Figure 5D). The binding energy between berberine and residues LYS-440/VAL-422 on the protein crystal of CYP19A1 (PDBid: 5JKW) was −7.85 (Figure 5E). These results indicate a high affinity between berberine and the protein crystals, corresponding with the core therapeutic targets of COAD&RE.

Validation of the action and mechanism of berberine in vivo and in vitro experiments

In the *in vivo* experiment, berberine reduced the mortality of the irradiated animals and improved their survival status (Figure 6A). Food and water intake decreased after radiation, and the animals showed pus and blood in their stools and decreased physical strength on the third day after radiation. The overall condition of the IRB group, especially body weight, was better than that of the control group (Figure 6B). Simultaneously, there was a significant reduction in colon length following irradiation ($P < 0.01$). However, the IRB group exhibited a significant increase in colon length compared with that in the IR group ($P < 0.01$; Figure 6C, D). The intestinal pathological changes observed after radiation exposure included decreased villi length ($P < 0.01$), significantly reduced numbers of Ki67-and MUC-positive cells ($P < 0.01$), and an increased number of apoptotic cells as indicated by tunnel staining ($P < 0.01$). Berberine demonstrated a certain degree of alleviation of the aforementioned pathological damage with statistically significant differences ($P < 0.01$; Figure 6E–H).

The effects of berberine on HIEC and human colon cancer cells (HT29) were examined *in vitro* using the CCK8 assay. Berberine promoted the proliferation HIEC in the concentration range of 5 to 80 μM, and inhibit the proliferation of HT29 in the concentration range of 40 to 320 μM (Figure 6I). Then we treated the two cell lines with 80 μM berberine after radiation, and the five predicted core genes were detected using quantitative real-time polymerase chain reaction (qPCR). In HIEC, radiation led to a significant reduction in the relative mRNA expression of *MYC* and *CCND1* compared with that in the CON group ($P < 0.01$). Conversely, the IRB group exhibited significantly higher levels of *MYC* and *CCND1* mRNA than the IR group ($P < 0.01$). The relative mRNA expression levels of MYC and CCND1 significantly decreased in HT29 cells after irradiation ($P < 0.01$), and the expression levels of these genes further decreased after berberine treatment in HT29 cells ($P < 0.01$; Figure 6J).

Discussion

COAD represents a substantial global health challenge and is characterized by high morbidity and mortality rates. In 2020, the incidence of colorectal cancer reached

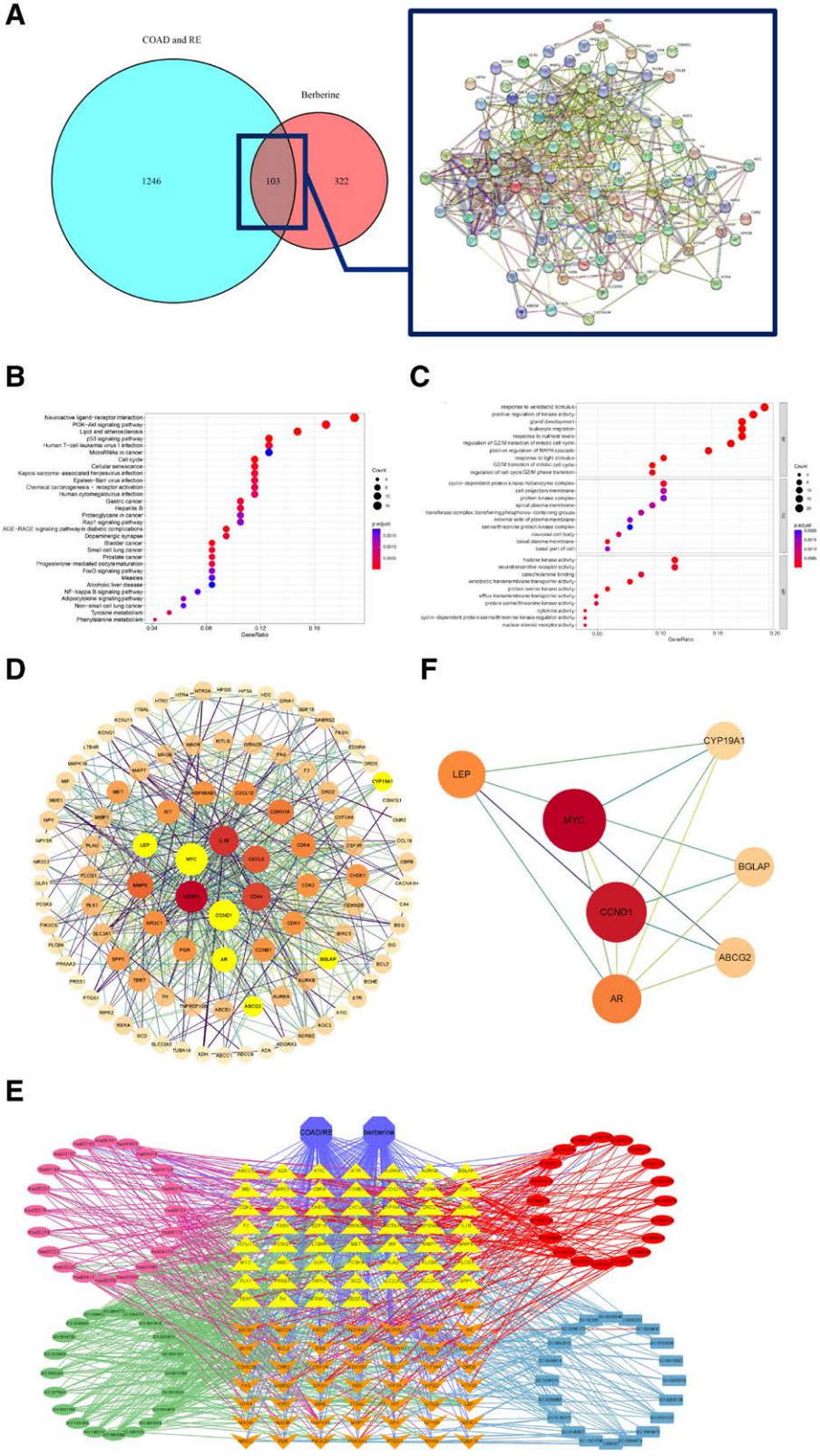

Figure 4. Identification of core targets of berberine against COAD&RE and the interaction between the targets. (A) Venn diagram depicting intersecting genes of berberine and COAD&RE, STRING analysis indicating protein–protein interaction networking mediated by 103 intersecting targets of berberine against COAD&RE. (B) KEGG pathway of intersecting genes of berberine and COAD&RE. (C) Gene Ontology analysis of intersecting genes of berberine and COAD&RE. (D) Cytoscape analysis representing the protein interaction network related to the action of berberine against COAD&RE. (E) Interaction network showing core biotargets, pharmacological functions, and signaling pathways of berberine against COAD&RE. (F) Seven core targets—MYC, CCND1, AR, LEP, CYP19A1, BGLAP, ABCG2 are highlighted. AGE-RAGE: Advanced glycation end products-receptor for advanced glycation end products; COAD&RE: Colon adenocarcinoma complicated with radiation enteritis; KEGG: Kyoto Encyclopedia of Genes and Genomes; MAPK: Mitogen activated protein kinase; NF-κB: Nuclear factor kappa-B; PI3K-Akt: Phosphatidylinositol-3-kinase-protein kinase B.

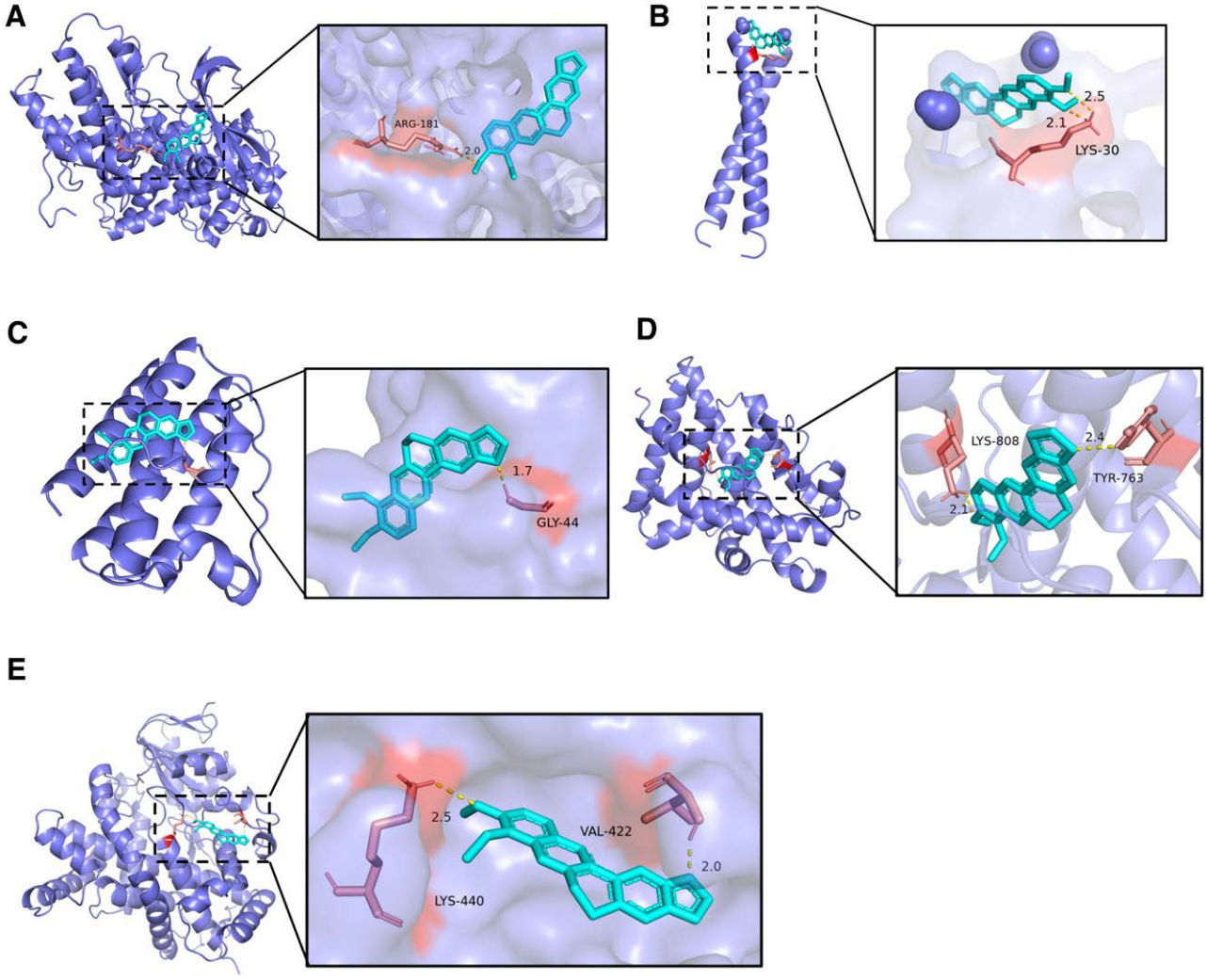

Figure 5. Molecular docking between berberine and therapeutic targets for COAD&RE. (A) Binding between berberine and ARG-181 residue on the protein crystal of CCND1 (PDBid: 2W96). (B) Binding between berberine and residue LYS-30 on the protein crystal of MYC (PDBid: 1A93). (C) Binding between berberine and residue GLY-44 on the protein crystal of LEP (PDBid: 1AX8). (D) Binding between berberine and residues LYS-808/TYR-763 on the protein crystal of AR (PDBid: 1E3G). (E) Binding between berberine and residues LYS-440/VAL-422 on the protein crystal of CYP19A1 (PDBid: 5JKW). COAD&RE: Colon adenocarcinoma complicated with radiation enteritis.

approximately 1.9 million new cases, with approximately 930,000 fatalities globally. The gastrointestinal tract is particularly vulnerable to the detrimental effects of ionizing radiation, which leads to a spectrum of symptoms of intestinal radiation toxicity. These include vomiting, diarrhea, abdominal pain, bleeding, obstruction, perforation, malabsorption disorders, and other complications. These adverse effects can considerably diminish quality of life and can result in mortality. Approximately 90% of patients with cancer who receive radiotherapy to the abdomen and pelvis experience permanent changes in their habits, and 50% experience a reduction in their quality of life[44]. Many different treatment strategies have been developed to relieve symptoms and manage complications, such as radiation colitis. However, patient prognosis remains poor[45]. Given the previous evidence of the inhibitory effects of berberine on COAD cells[46] and its therapeutic potential for intestinal inflammation[47,48], we aimed to investigate the potential efficacy of berberine in treating COAD&RE.

In this study, we performed an extensive analysis to detect genes that are differentially expressed in COAD&RE. Our findings indicate that within the group of patients with COAD&RE, there was an increase in the expression of 712 genes and a decrease in the expression of 637 genes. We then employed clinical modeling and bioinformatics methods to predict 15 genes that may play crucial roles in the development of RE following radiotherapy for COAD. Univariate and multivariate analyses were performed to evaluate the prognostic significance of these genes, considering the various clinical characteristics of patients diagnosed with COAD&RE. Alterations in the expression of genes, such as *CD1A, CD1B, CXXC5, NAT1, SNHG16*, and *TNNT1* demonstrate the potential to distinguish patients with favorable or unfavorable prognoses. Additionally, we investigated the potential therapeutic effects of berberine and identified seven potential core targets for the treatment of COAD&RE. These targets included *CCND1, MYC, LEP, AR, CYP19A1, BGLAP,* and *ABCG2*. To better understand the therapeutic effects of berberine on COAD&RE, GO and KEGG enrichment analyses were performed. Furthermore, molecular docking analysis revealed that berberine exhibited a strong binding

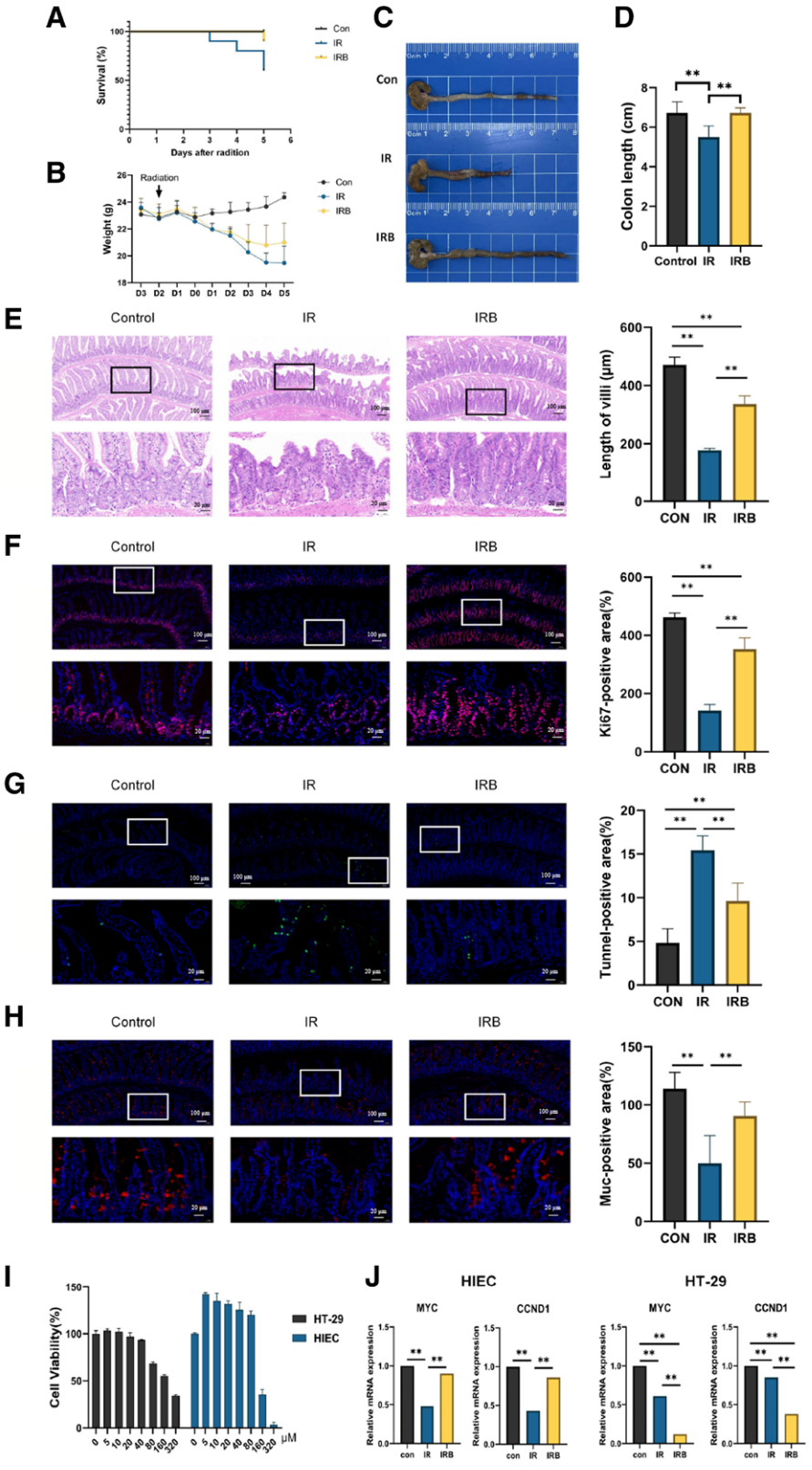

Figure 6. Validation of the action and mechanism of berberine *in vivo* and *in vitro* experiments. (A) Survival curves of mice in the different treatment groups after irradiation. (B) Weight changes in mice in different treatment groups after radiation. (C, D) Colon length was significantly shortened after irradiation ($P < 0.01$), whereas the colon length in the IRB group was significantly increased compared with that in the IR group ($P < 0.01$). (E) HE staining revealed villus length in the different treatment groups. (F) Tunnel staining of the intestines in the different treatment groups. (G) Ki67 immunofluorescence staining of the intestines in different treatment groups. (H) Muc-2 immunofluorescence staining of the intestines in the different treatment groups. Bars represent 100 μm (10×) and 20 μm (40×), respectively; $^{**}P < 0.01$. (I) Using the CCK8 assay, berberine was found to promote the proliferation HIEC in the concentration range of 5–80 μM, and inhibit the proliferation of HT29 in the concentration range of 40–320 μM. (J) Relative mRNA expression of *MYC* and *CCND1* in HIEC and HT29 cells after treatment of berberine. HE: Hematoxylin-eosin; HIEC: Human intestinal epithelial cells; IR: Model group; IRB: Berberine group.

affinity to the protein crystals associated with the five key targets of COAD&RE: MYC, CCND1, LEP, AR, and CYP19A1. Additionally, the crystal structure of BGLAP remains elusive, hindering the performance of computer simulations for molecular docking techniques. ABCG2 and berberine lack tightly bound conformations or hydrogen bonds.

Among these core targets and pathways, CCND1, a key cell cycle regulator responsible for the G1-to-S transition of the cell cycle, is frequently expressed and localized in human tumor cells. Polymorphisms in this gene are associated with an increased risk of colorectal cancer[49,50]. Aberrant upregulation of MYC family members (MYC, MYCN, or MYCL) promotes metabolic reprogramming, which leads to the sustained proliferation of cancer cells[51,52]. MYC promotes tryptophan uptake and metabolism in colon cancer cells *via* the kynurenine pathway[53]. The cytokine LEP, which is produced by adipose tissue, plays a role in promoting tumorigenesis[54], and its polymorphisms lead to an increased risk of colorectal cancer and are associated with poor prognosis[55,56]. The AR and variants in the aromatase *CYP19A1* gene, which converts estrone to estradiol, are associated with the risk of colon cancer[57–59]. Estrogens and androgens may operate through an inflammation-related pathway[60].This inflammatory process also plays a role in RE. Berberine inhibits the activation of NF-κB, a common pro-inflammatory mediator, and exerts anti-colon cancer-RE effects. The activation of BGLAP upon treatment with berberine has also been observed in other studies. Liu and Xu[61] found that berberine exerted a stimulatory effect on the proliferation and osteogenic differentiation of human alveolar osteoblasts by down-regulation miRNA214 expression and upregulating BGLAP expression. The close association between ABCG2 and intestinal diseases is exemplified by its identification as a diagnostic gene for ulcerative colitis through single-cell sequencing, which indicates a strong correlation with immune infiltration[62]. Berberine potentially targets ABCG2, as evidenced by the observed elevation of ABCG2 in the colon tissues of rats treated with berberine[63].

We then validated the therapeutic effects and underlying mechanisms of berberine in *in vivo* and *in vitro* experiments and validated the prediction by quantifying the above-mentioned core genes. Berberine administration significantly enhanced the post-radiation survival rate and average body weight of mice, while effectively ameliorating pathological damage to the small intestine. Additionally, we discovered that 5 to 320 μM berberine exhibits inhibitory effects on HT29 proliferation and synergistically enhances radiotherapy efficacy. Conversely, 5 to 80 μM berberine promotes HIEC cell proliferation and efficiently restores radiation-induced epithelial cell damage. qPCR validation revealed that MYC and CCND1 were potential key targets for the proliferative effect of berberine on HIEC cells and its antiproliferative effect on HT29 cells. These findings provide additional evidence supporting the potential of berberine as a targeted therapy for COAD&RE. The limitation of this study lies in its exclusive reliance on network pharmacology and bioinformatics for predictions. In future studies, we aim to enhance the clinical relevance by selecting colon cancer patients with RE as research subjects. Additionally, we plan to conduct double-blind clinical controlled experiments to investigate the therapeutic efficacy of berberine while incorporating evidence-based medical data to substantiate the conclusions drawn from this study.

In this study, we predicted the core targets of COAD&RE and preliminarily validated the potential therapeutic effects of berberine on COAD&RE. These findings offer valuable insights and recommendations for the clinical diagnosis and treatment of COAD&RE and RE. Therefore, berberine holds promise as a potential therapeutic agent for combined treatment of COAD&RE.

Conflict of interest statement

Yue Gao is the editorial board member of this journal. None of the other authors declare any conflicts of interest.

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Author contributions

Yue Gao: Conception and design, financial support, administrative support, final approval of manuscript. Yongqi Dou: Conception and design, financial support, administrative support, provision of study material or patients. Zhexin Ni: Final approval of manuscript, conception and design, provision of study material or patients. Ziqiao Yan: Provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing. Hongyang Yu: Provision of study material or patients, collection and/or assembly of data, data analysis and interpretation. Liangliang Zhang: Collection and/or assembly of data, data analysis and interpretation. Zebin Liao: Provision of study material or patients, data analysis and interpretation. Xiangwei Ge: Articles editing, data analysis and interpretation. Yuguo Wang: Collection and/or assembly of data, data analysis and interpretation. Peiyu Tian: Collection and/or assembly of data, data analysis and interpretation.

Ethical approval of studies and informed consent

All experiments complied with the relevant experimental animal welfare principles, approved by the Ethics Committee of the Academy of Military Medical Science (IACUC-DWZX-2020-783).

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None.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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