Am J Cancer Res 2025;15(3):929-945
www.ajcr.us /ISSN:2156-6976/ajcr0162331

Original Article

Salidroside inhibits the invasion and migration of colorectal cancer cells by regulating MMP-12 and WNT signaling pathway

Ye Hong[1], Yanrong Li[1], Xia Liu[2], Jia Deng[3,4], Yanli He[5], Bin Zhao[6,7]

[1]Center of Digestive Endoscopy, Shaanxi Provincial Cancer Hospital, No. 309 Yanta West Road, Yanta District, Xi'an 710061, Shaanxi, China; [2]Department of Gastroenterology, Baoji People's Hospital, No. 24 Xinhua Lane, Jing'er Road, Weibin District, Baoji 721000, Shaanxi, China; [3]Department of Radiation Oncology, Shaanxi Provincial Cancer Hospital, No. 309 Yanta West Road, Yanta District, Xi'an 710061, Shaanxi, China; [4]School of Nuclear Science and Technology, Xi'an Jiaotong University, No. 28 Xianning West Road, Xi'an 710049, Shaanxi, China; [5]Department of Integrated Chinese and Western Medicine, Shaanxi Provincial Cancer Hospital, No. 309 Yanta West Road, Yanta District, Xi'an 710061, Shaanxi, China; [6]Department of Epidemiology, Shaanxi Provincial Cancer Hospital, No. 309 Yanta West Road, Yanta District, Xi'an 710061, Shaanxi, China; [7]The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, No. 28 Xianning West Road, Xi'an 710049, Shaanxi, China

Received November 28, 2024; Accepted February 7, 2025; Epub March 15, 2025; Published March 30, 2025

Abstract: Colorectal cancer (CRC) is a prevalent and highly lethal malignancy, with current therapeutic efficacy limited by the tumor's high invasiveness and metastatic potential. Matrix metalloproteinases (MMPs) and the WNT (Wingless/Integrated) signaling pathway play key roles in the invasion and metastasis of CRC. Salidroside, a natural compound, has demonstrated inhibitory effects in several cancers, but its precise molecular mechanism in CRC cells remains unclear. This study aims to investigate the antitumor effect of salidroside on CRC and its molecular mechanism in influencing epithelial-mesenchymal transition (EMT) by regulating MMP-12 and the WNT signaling pathway. The effects of salidroside on CRC cell proliferation, migration, and invasion were evaluated through in vitro experiments using HCT-116 and SW620 cell lines. The antitumor effects of salidroside were validated using CCK-8, wound healing, and Transwell assays. Expression changes of MMP-12, WNT signaling-related proteins (e.g., β-catenin, GSK-3β), and EMT markers (e.g., E-cadherin, Vimentin) after salidroside treatment were measured by qRT-PCR and Western Blot. Additionally, bioinformatics analysis was performed using TCGA and GEO databases in combination with the BEST online tool to identify differentially expressed genes, followed by GSEA enrichment analysis. Salidroside showed significant antiproliferative and inhibitory effects on the migration and invasion of CRC cells. In vitro experiments demonstrated that salidroside significantly inhibited CRC cell proliferation and reduced their migration and invasion capabilities. qRT-PCR and Western Blot analyses showed that salidroside significantly downregulated MMP-12 expression and led to changes in the expression of WNT signaling and EMT-related proteins, specifically downregulating β-catenin, upregulating E-cadherin, and downregulating Vimentin. Furthermore, bioinformatics analysis indicated that MMP-12 plays a crucial role in salidroside-mediated CRC inhibition, further supporting its potential as a key target. In conclusion, salidroside suppresses CRC invasion and migration by downregulating MMP-12 and modulating the WNT signaling pathway, thereby inhibiting the EMT process. These findings suggest that salidroside holds potential as a therapeutic agent for CRC, offering a novel approach to CRC treatment.

Keywords: Salidroside, colon cancer, MMP-12, WNT signaling pathway, invasion, migration

Introduction

Colorectal cancer (CRC) is a common malignancy worldwide, with its incidence and mortality ranking among the top for all cancer types [1]. In recent years, due to changes in lifestyle and Westernized dietary patterns, especially high-fat, high-calorie diets and lack of exercises, the incidence of CRC has been increasing in many countries [2]. This trend is particularly evident in developed countries, but developing countries are also catching up due to lifestyle chang-

https://doi.org/10.62347/KELA7583

es. According to reports from the World Health Organization (WHO), the global incidence of CRC is approximately 1.9 million cases annually [3]. Despite progress in CRC treatment, surgery remains the primary method for early-stage CRC, while patients with advanced stages often require comprehensive treatments including chemotherapy and radiotherapy [4]. However, tumor recurrence and metastasis are still major challenges in treatment, and the toxic side effects of chemotherapy drugs and drug resistance of cancer cells further limit treatment efficacy [5]. Therefore, finding new anticancer drugs and therapeutic targets is crucial for improving CRC survival rates.

In the development of anticancer drugs, natural products have gained attention due to their low toxicity and multiple target properties. Salidroside, a natural glycoside compound extracted from the traditional medicinal herb Rhodiola rosea, has various biological activities such as antioxidant, anti-inflammatory, and immune regulatory effects, and its application in cancer research has attracted increasing attention in recent years [6]. Salidroside has shown inhibitory effects on various cancer cells, especially in studies on solid tumors such as breast cancer [7], lung cancer [8], and liver cancer [9], achieving significant results. The mechanisms mainly include inhibiting cancer cell proliferation, migration, and invasion, inducing apoptosis, and causing cell cycle arrest [10]. In addition, salidroside exerts antitumor effects by inhibiting inflammatory responses in the tumor microenvironment and reducing oxidative stress [11, 12].

Although salidroside's anticancer effects have been partially validated in multiple types of tumors, there is still a lack of systematic research on its mechanisms in CRC. Preliminary studies have suggested that salidroside can inhibit CRC cell proliferation and migration in vitro, indicating its potential anticancer activity against CRC [13]. However, these studies mostly remain at the level of initial observations, lacking in-depth investigation into the specific molecular mechanisms of salidroside. Epithelial-mesenchymal transition (EMT) is an important cellular biological process that plays a key role in CRC invasion and metastasis [14]. Through EMT, cancer cells change from an epithelial phenotype to a mesenchymal phenotype, losing cell adhesion and gaining stronger migratory and invasive capabilities. Activation of EMT has been found to be closely associated with tumor malignancy, drug resistance, and poor prognosis [15]. Therefore, inhibiting EMT is considered an important strategy for controlling CRC metastasis. The WNT (Wingless/Integrated) signaling pathway plays an important regulatory role in EMT. It is critical in processes like cell differentiation, proliferation, and EMT, and its aberrant activation is considered a major mechanism in CRC progression [16]. Whether salidroside inhibits CRC cell migration and invasion by regulating the WNT signaling pathway and affecting EMT remains to be further investigated.

Matrix metalloproteinases (MMPs) play crucial roles in CRC progression, particularly MMP-12 [17]. MMP-12 is a zinc-dependent protease that degrades the extracellular matrix and promotes cancer cell invasion and metastasis [18]. Studies have shown that MMP-12 is significantly overexpressed in CRC tissues compared to normal tissues, and its high expression is closely related to tumor invasiveness and poor prognosis [19]. Therefore, MMP-12 is considered a potential therapeutic target for CRC. In this study, we combined bioinformatics analysis with in vitro experiments to explore the regulatory effects of salidroside on MMP-12, aiming to further elucidate its anticancer mechanisms against CRC.

The aim of this study is to systematically investigate the antitumor effects of salidroside on CRC cells and its underlying mechanisms, particularly its regulatory effects on MMP-12, the WNT signaling pathway, and EMT. Through these investigations, we hope to provide a scientific basis for salidroside as a potential therapeutic agent for CRC and contribute to the development of new treatment strategies for the disease.

Methods and materials

Cell source and culture

Human CRC cell lines HCT-116 and SW620 were purchased from Cell Bank (Shanghai, China). Cells were cultured in high-glucose and glutamine DMEM (HyClone) supplemented with 10% fetal bovine serum (FBS; VivaCell, Shanghai, China) and 1% penicillin/streptomy-

cin (Gibco, USA). Cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

Differential gene analysis and volcano plot generation

Differential gene expression analysis was performed using The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) microarray data (GSE85871 and GSE110225). The TCGA breast cancer dataset included 1211 samples, with 1098 tumor tissues and 113 adjacent normal tissues; the CRC dataset included 497 samples, with 456 tumor tissues and 41 adjacent normal tissues. In the GSE85871 dataset, GSM2286398-9 served as the control group, while GSM2286318-9 included MCF7 samples treated with salidroside. The GSE110225 dataset contained 60 samples, from which 26 samples (GSM2982906-31) were selected, with odd-numbered samples representing cancer samples and even-numbered samples representing adjacent normal samples. Differential analysis of gene expression between the treatment and control groups was conducted using the DESeq2 package, identifying significantly upregulated and downregulated genes ($P < 0.05$, Fold Change > 2). Volcano plots were generated to visualize differential gene expression. These volcano plots displayed the distribution of differentially expressed genes, providing a visual representation of the regulatory effects of salidroside on cancer cells and further revealing potential molecular mechanisms.

Venn diagram and protein-protein interaction (PPI) network analysis

Venn diagrams were used to analyze the intersection of differentially expressed genes in MCF7 cells treated with salidroside and differentially expressed genes in TCGA CRC, breast cancer, and GEO CRC datasets. A PPI network of co-expressed genes was constructed using the STRING database, and hub genes were identified using Cytoscape software. These analyses allowed us to identify key genes that were commonly regulated across different cancer types, potentially playing important roles in the anticancer effects of salidroside. The construction of the PPI network and identification of hub genes laid the foundation for subsequent functional validation and mechanistic studies.

GSEA pathway enrichment analysis

Based on the median expression of MMP-12 in the GSE110225 dataset, 13 CRC samples were divided into high-expression and low-expression groups. Gene set enrichment analysis (GSEA) was performed to identify significantly enriched signaling pathways ($P < 0.01$), with a focus on the regulation of the WNT signaling pathway by salidroside. GSEA analysis helped reveal the potential signaling pathways through which salidroside may exert its effects, particularly those related to cancer cell proliferation and migration, providing important insights into the anticancer mechanisms of salidroside.

BEST online database analysis

The BEST online database (URL: https://rookieutopia.hiplot.com.cn/app_direct/BEST/) was used to analyze the expression data of MMP-12 in CRC, screening potential differential genes as a basis for subsequent functional validation.

Cell proliferation and viability assay

The effects of salidroside on the proliferation and viability of HCT-116 and SW620 cells were assessed using a Cell Counting Kit-8 (CCK-8) assay (Dojindo, Japan). Cells were seeded in 96-well plates at a density of 5000 cells per well and treated for 12 h, 24 h, and 48 h. After each treatment, CCK-8 solution was added to each well, and cells were incubated for 1 hour. Absorbance was measured at 450 nm. The CCK-8 is a simple and efficient method with its kit indirectly reflects cell proliferation by measuring the metabolic activity. The experimental results demonstrated that salidroside significantly inhibited the proliferation of CRC cells, indicating its potent antiproliferative effect.

Cell migration and invasion assay

Wound healing assay: Cells were seeded into 6-well plates and grown to 90% confluence. A sterile pipette tip was used to scratch the cell monolayer, and cells were washed with PBS before adding medium containing different concentrations of salidroside. Wound healing was recorded at 0 h and 24 h using an Olympus microscope (model CKX53) to assess cell migration ability. The wound healing assay

effectively evaluated the impact of salidroside on cell migration by measuring the rate of cell movement into the scratched area.

Transwell invasion assay: Transwell chambers with 8 μm pore membranes coated with Matrigel (BD Biosciences, USA) were used. Serum-free medium containing different concentrations of salidroside was added to the upper chamber, while medium containing 10% FBS was added to the lower chamber as a chemoattractant. After 24 h of incubation, cells were fixed with methanol, stained with crystal violet, and counted. The Transwell invasion assay assessed the ability of cells to invade through the Matrigel-coated membrane, and the results indicated that salidroside treatment significantly inhibited CRC cell invasion.

MMP-12 overexpression and inhibition

Overexpression: MMP-12 overexpression was performed using the pcDNA3.1-MMP-12 plasmid (GeneChem, China) and Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's instructions to transfect HCT-116 and SW620 cells. Forty-eight hours after transfection, MMP-12 mRNA expression levels were determined using quantitative reverse transcription PCR (qRT-PCR) to verify transfection efficiency. MMP-12 overexpression was used to investigate its role in the anticancer effects of salidroside, and the experimental results helped reveal the specific function of MMP-12 in regulating cell proliferation and migration. Inhibition: MMP-12 knockdown was achieved using MMP-12-specific small interfering RNA (siRNA; GenePharma, China) and Lipofectamine 3000 for transfection. Forty-eight hours after transfection, MMP-12 expression levels were assessed by qRT-PCR. Knockdown of MMP-12 helped evaluate its specific role in cellular behavior and further validate its importance in the anticancer effects of salidroside.

qRT-PCR analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, USA), and cDNA synthesis was performed using a reverse transcription kit (Takara, Japan). qRT-PCR was performed using SYBR Green reagent (Takara, Japan) on an ABI 7500 real-time fluorescence quantitative PCR instrument, with GAPDH as the internal reference gene. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. qRT-PCR is a highly sensitive method for quantifying gene expression and can accurately measure MMP-12 and other genes under different treatment conditions, The primer sequences are as described in Table S1.

Western blot analysis

After intervention, cells were lysed on ice with RIPA lysis buffer containing protease and phosphatase inhibitors (Beyotime, China). Protein concentration in the supernatant was measured using a BCA protein assay kit (Beyotime, China). Samples were loaded onto 10% denaturing SDS-PAGE gels for electrophoresis, then transferred onto 0.45 μm polyvinylidene fluoride (PVDF) membranes (Beyotime, China). The membranes were blocked with 5% non-fat milk at room temperature for 2 hours and incubated overnight at 4°C with specific primary antibodies, followed by incubation with HRP-conjugated secondary antibodies (mouse or rabbit). Protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Thermo Scientific, USA). The following primary antibodies were used for Western blotting: MMP-12 (ab52897), E-cadherin (ab-231303), Vimentin (ab137321), β-catenin (ab-32572), c-Myc (ab32072), and Cyclin D1 (ab16663). All antibodies were purchased from Abcam. Western blot analysis provided a direct measurement of protein expression levels, and the results helped verify the regulatory effect of salidroside on the WNT signaling pathway and related proteins.

Statistical analysis

Data analysis was performed using GraphPad Prism 9.0 software. Experimental results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for comparisons among groups, followed by LSD-t post-hoc testing. A P-value < 0.05 was considered statistically significant.

Results

Effect of salidroside on CRC cell proliferation and viability

To evaluate the effect of salidroside on CRC cell proliferation and viability, HCT-116 and SW620 cells were treated with varying concentrations

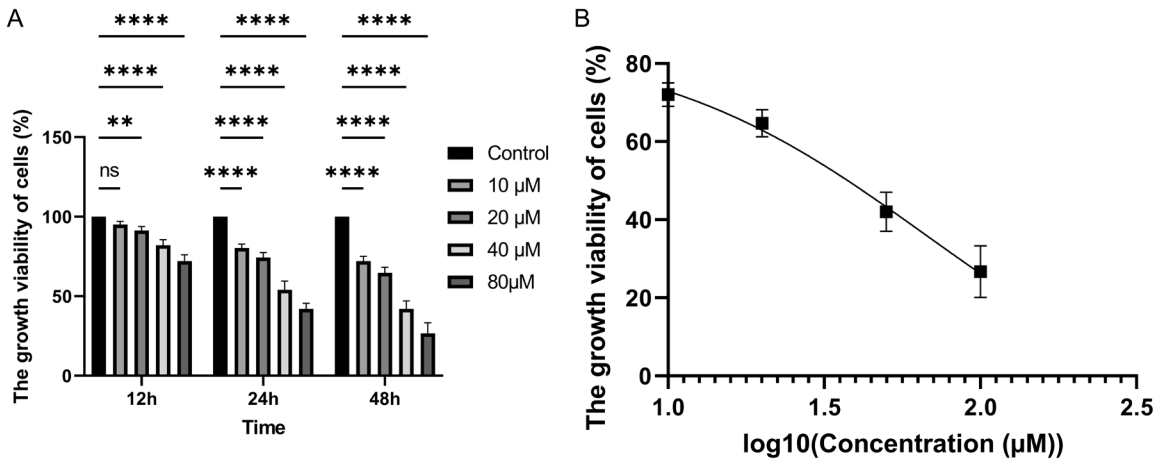

Figure 1. Time-dependent effect of different concentrations of salidroside on CRC cell proliferation and viability. A. CCK-8 analysis of the time-dependent effect of salidroside on cell viability (12 h, 24 h, 48 h). B. Cell proliferation assay showing the effect of different salidroside concentrations on HCT-116 and SW620 cells. Note: CRC, colorectal cancer; nsP > 0.05, **P < 0.01, ****P < 0.0001.

of salidroside (10 μM, 20 μM, 40 μM, 80 μM) and assessed using the CCK-8 assay at 12 h, 24 h, and 48 h (**Figure 1A**). The results showed that salidroside significantly inhibited the viability of CRC cells in a dose- and time-dependent manner (P < 0.05). Furthermore, the cell proliferation assay confirmed the inhibitory effect of salidroside, particularly at high concentrations (40 μM and 80 μM), where cell proliferation was markedly reduced (**Figure 1B**, P < 0.01).

Effect of salidroside on CRC cell migration and invasion

The impact of salidroside at an IC50 concentration of 66.5 μM on the migration and invasion abilities of HCT-116 and SW620 cells was assessed using wound healing and Transwell invasion assays. The results indicated that salidroside treatment significantly decreased cell migration (**Figure 2A**, P < 0.05) and invasion capabilities (**Figure 2B**, P < 0.01), suggesting that salidroside effectively inhibits the migration and invasion of CRC cells.

Differential gene analysis under salidroside intervention

Comprehensive differential gene analysis was performed using TCGA and GEO microarray data after salidroside intervention. The differential gene analysis results for TCGA CRC and breast cancer samples are shown in **Figure 3A** and **3B**, respectively. Significant changes in gene expression were observed in both CRC and breast cancer cells after salidroside treatment (P < 0.05). Furthermore, differential gene analysis of MCF7 cells in the GSE85871 dataset showed significant gene expression changes following salidroside intervention (**Figure 3C**, P < 0.01). Differential analysis of CRC samples and controls in the GSE110225 dataset also revealed significant changes (**Figure 3D**, P < 0.05).

Volcano plot analysis of differential gene expression

Volcano plots were used to visualize the upregulation and downregulation of genes after salidroside intervention (**Figure 4**). In both TCGA CRC and breast cancer samples, significantly upregulated and downregulated genes were clearly identified (**Figure 4A**, **4B**, P < 0.05). Volcano plots for GSE85871 and GSE110225 further confirmed the significant changes in gene expression in CRC and breast cancer cells after salidroside treatment (**Figure 4C**, **4D**, P < 0.01).

Intersection analysis of differential genes in MCF7 cells and multiple cancer datasets

To further investigate the target genes of salidroside, Venn diagram analysis was used to examine the intersection between differentially expressed genes in MCF7 cells treated with salidroside and those from TCGA CRC, breast cancer, and GEO CRC datasets (**Figure 5A**, **5B**). The results showed significant intersections

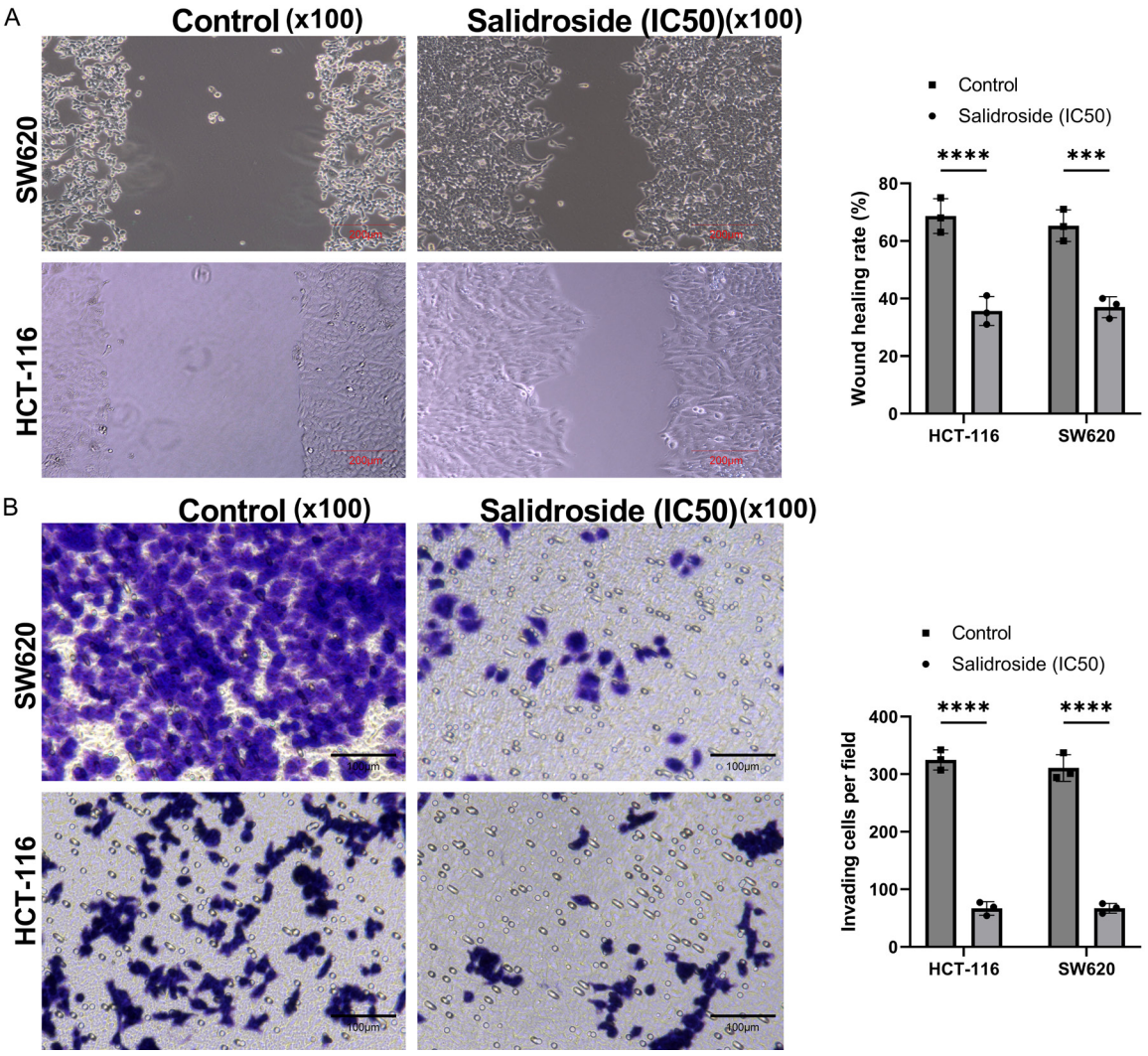

Figure 2. Inhibitory effect of salidroside IC50 on migration and invasion abilities of HCT-116 and SW620 cells. A. Wound healing assay showing the effect of salidroside IC50 treatment on HCT-116 and SW620 cell migration. B. Transwell invasion assay evaluating the effect of salidroside IC50 on cell invasion. Note: CRC, colorectal cancer; ***P < 0.001, ****P < 0.0001.

between highly expressed genes in MCF7 cells and low-expressed genes in TCGA and GEO datasets (P < 0.05). Through PPI network analysis, 17 co-expressed genes were identified. However, further analysis revealed that only BGN, MMP12, AOX1, and ADH1C showed direct connections within the network (**Figure 5C**). The data were subsequently exported and analyzed using the MCC (Maximum Clique Centrality) algorithm in Cytoscape. The results identified MMP-12 as the hub gene within the network, highlighting its critical role in the regulatory processes. These genes may play crucial roles in the anticancer mechanisms of salidroside.

KEGG enrichment analysis after salidroside treatment

KEGG enrichment analysis showed that salidroside treatment significantly affected the WNT signaling pathway and other pathways related to cell proliferation and migration (**Figure 6**, P < 0.01). This suggests that the WNT signaling pathway may play a crucial role in the anticancer effects of salidroside on CRC cells.

Regulation of MMP-12 expression by salidroside and MMP-12 overexpression/inhibition

Analysis of MMP-12 expression levels in nine GEO microarray datasets using the BEST online

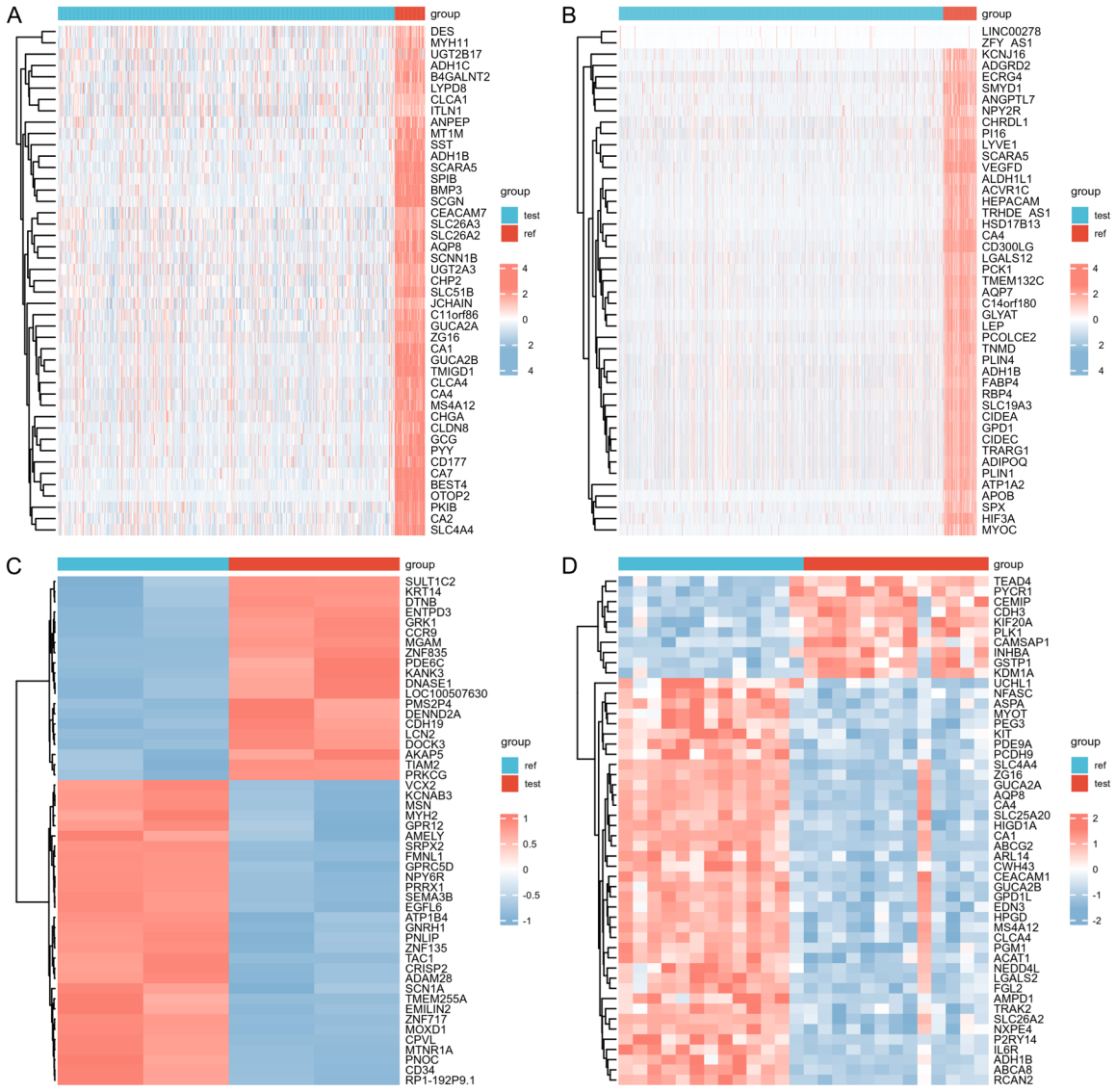

Figure 3. Differential gene analysis under salidroside intervention. A. TCGA CRC samples. B. TCGA breast cancer samples. C. MCF7 cells from the GSE85871 dataset following salidroside intervention. D. GSE110225 CRC samples compared to controls. Note: CRC, colorectal cancer.

database showed that MMP-12 expression was significantly higher in CRC tissues compared to normal tissues, except in the GES77953 dataset where no difference was found (**Figure 7A**, $P < 0.01$). qRT-PCR and Western blot analysis further demonstrated that salidroside treatment significantly inhibited MMP-12 expression, whereas MMP-12 overexpression significantly increased its mRNA levels in cells (**Figure 7B-D**, $P < 0.05$).

Regulation of cyclin D1, c-Myc, and β-catenin expression by salidroside and MMP-12

The effects of salidroside (66.5 μM) and MMP-12 on WNT signaling-related proteins were also analyzed. Salidroside treatment significantly downregulated the expression of Cyclin D1 and c-Myc, while MMP-12 overexpression reversed this inhibition (**Figure 8A**, $P < 0.05$). Additionally, β-catenin expression showed significant changes across salidroside treatment and MMP-12 overexpression/inhibition groups (**Figure 8B**, $P < 0.01$).

Effect of salidroside on EMT marker expression via MMP-12 regulation

To evaluate the effect of salidroside (66.5 μM) on EMT markers, the expression levels of E-cadherin and Vimentin were examined (**Figure**

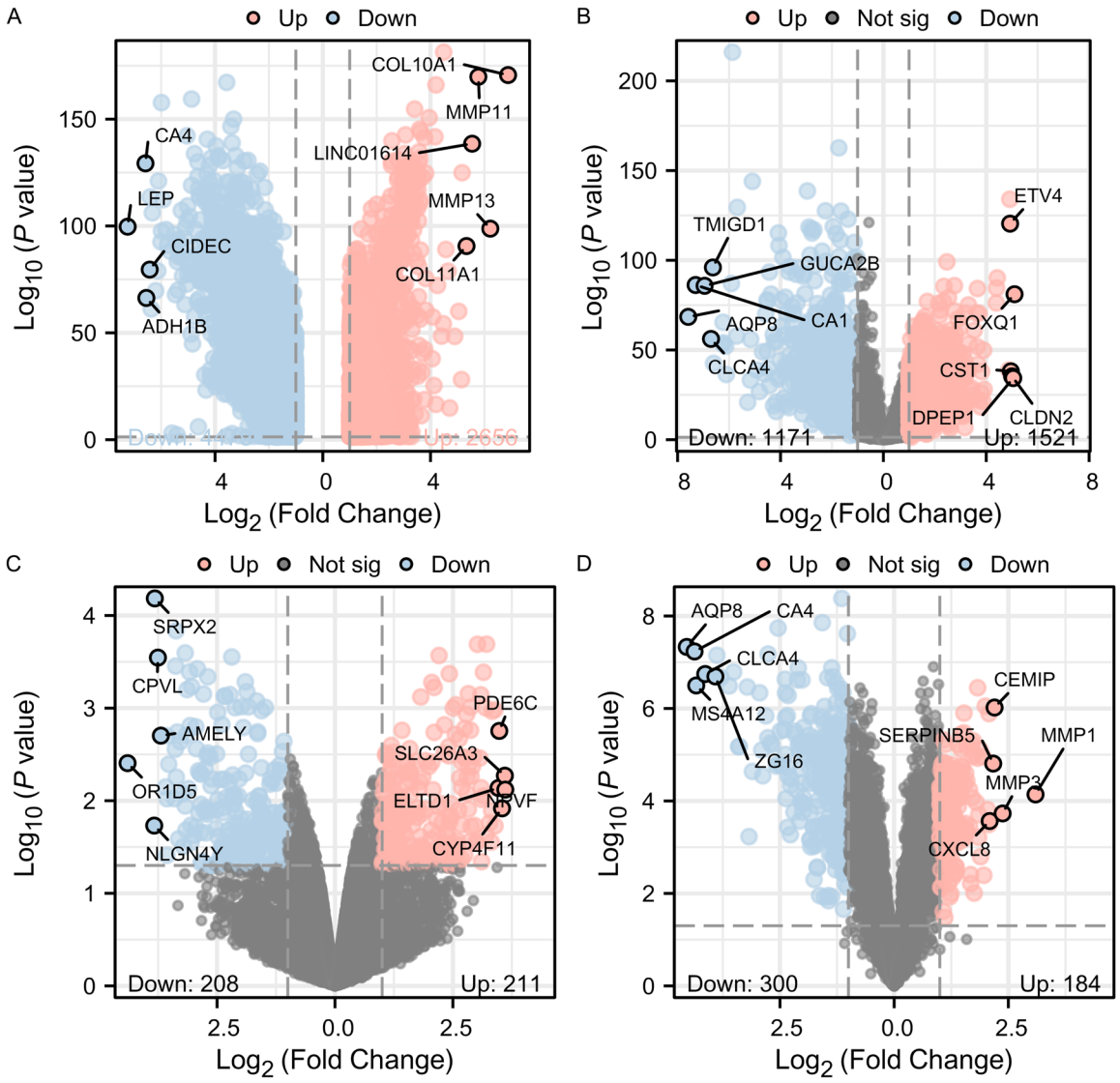

Figure 4. Volcano plots of differential gene expression. A. TCGA CRC samples showing upregulated and downregulated genes. B. TCGA breast cancer samples showing differential gene expression. C. Differential gene expression in MCF7 cells from the GSE85871 dataset following salidroside intervention. D. GSE110225 CRC samples. Note: CRC, colorectal cancer.

9). Salidroside treatment significantly upregulated E-cadherin expression and downregulated Vimentin expression (**Figure 9A**, **9B**, P < 0.01), while MMP-12 overexpression partially reversed these changes, further demonstrating the role of MMP-12 in salidroside-mediated EMT regulation.

Synergistic effect of MMP-12 regulation and salidroside on cell migration and invasion

Finally, wound healing and Transwell invasion assays were performed to assess the effect of MMP-12 regulation combined with salidroside (66.5 μM) on the migration and invasion abilities of HCT-116 and SW620 cells (**Figure 10**). The results indicated that MMP-12 overexpression significantly enhanced cell migration and invasion, while salidroside treatment effectively inhibited these effects (**Figure 10A**, **10B**, P < 0.05). This supports the hypothesis that salidroside inhibits CRC cell migration and invasion through the regulation of MMP-12.

Discussion

This study explores the anticancer potential of salidroside, a natural compound known for its

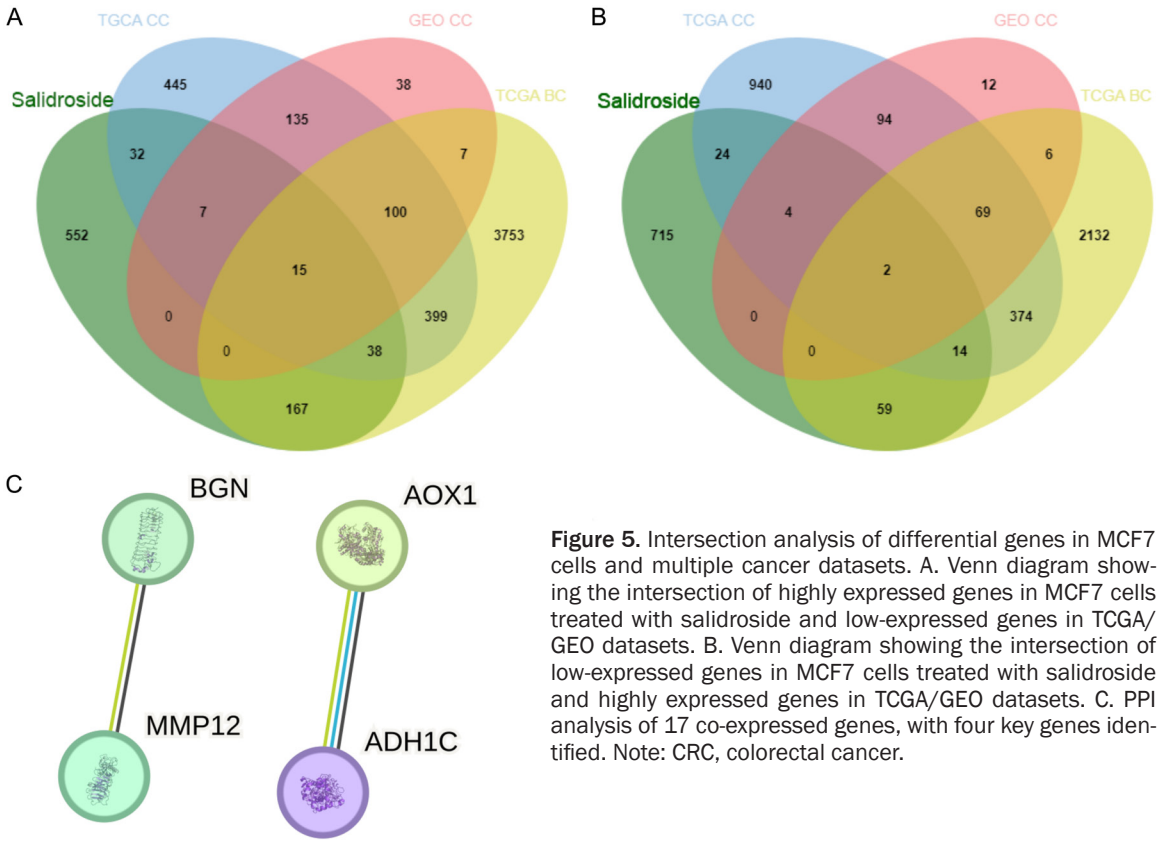

Figure 5. Intersection analysis of differential genes in MCF7 cells and multiple cancer datasets. A. Venn diagram showing the intersection of highly expressed genes in MCF7 cells treated with salidroside and low-expressed genes in TCGA/GEO datasets. B. Venn diagram showing the intersection of low-expressed genes in MCF7 cells treated with salidroside and highly expressed genes in TCGA/GEO datasets. C. PPI analysis of 17 co-expressed genes, with four key genes identified. Note: CRC, colorectal cancer.

antiproliferative, anti-invasive, and apoptosis-inducing effects across various pathologies, including cardiovascular diseases, bone metabolism disorders, and cancer. Korbozova et al. [20] demonstrated that salidroside effectively regulated thyroid hormone levels in a hypothyroid model, highlighting its significant biological activity. Similarly, Wojdasiewicz et al. [21] reported that salidroside could promote osteoblast proliferation and differentiation, reduce oxidative stress, and modulate bone metabolism-related pathways, thereby playing a critical role in bone tissue repair and metabolism. Fei et al. [22] further established that salidroside exerted protective effects in atherosclerosis by inhibiting vascular smooth muscle cell proliferation, improving endothelial function, and regulating lipid metabolism. In the context of cancer, Liu et al. [23] found that salidroside significantly inhibited proliferation, migration, and clonogenic ability while enhancing apoptosis in prostate cancer cells by suppressing the PI3K/AKT signaling pathway. Building upon these findings, our research confirms the anticancer potential of salidroside in CRC cells, demonstrating its ability to inhibit proliferation, migration, and invasion of HCT-116 and SW620 cells in a dose- and time-dependent manner. These results underscore the potential application of salidroside in CRC therapy and provide a foundation for further exploration of its molecular mechanisms.

In this study, differential gene analysis was performed on CRC samples by integrating data from the TCGA and GEO databases, alongside breast cancer samples. This cross-cancer comparative strategy was employed for two primary reasons: first, despite being distinct cancer types, breast cancer and CRC may share key molecules and pathways involved in tumor invasion and migration. Michelli et al. [24] identified significant changes in the WNT signaling pathway gene expression in both breast cancer and CRC, closely relating to tumor invasion and migration. Zougros et al. [25] further demonstrated the co-expression of WNT-related genes such as β-catenin, Wnt2, and FZD4 in both cancers, suggesting similarities in their tumorigenesis mechanisms. Second, numerous studies have elucidated the molecular targets and pathways of salidroside in breast cancer, pro-

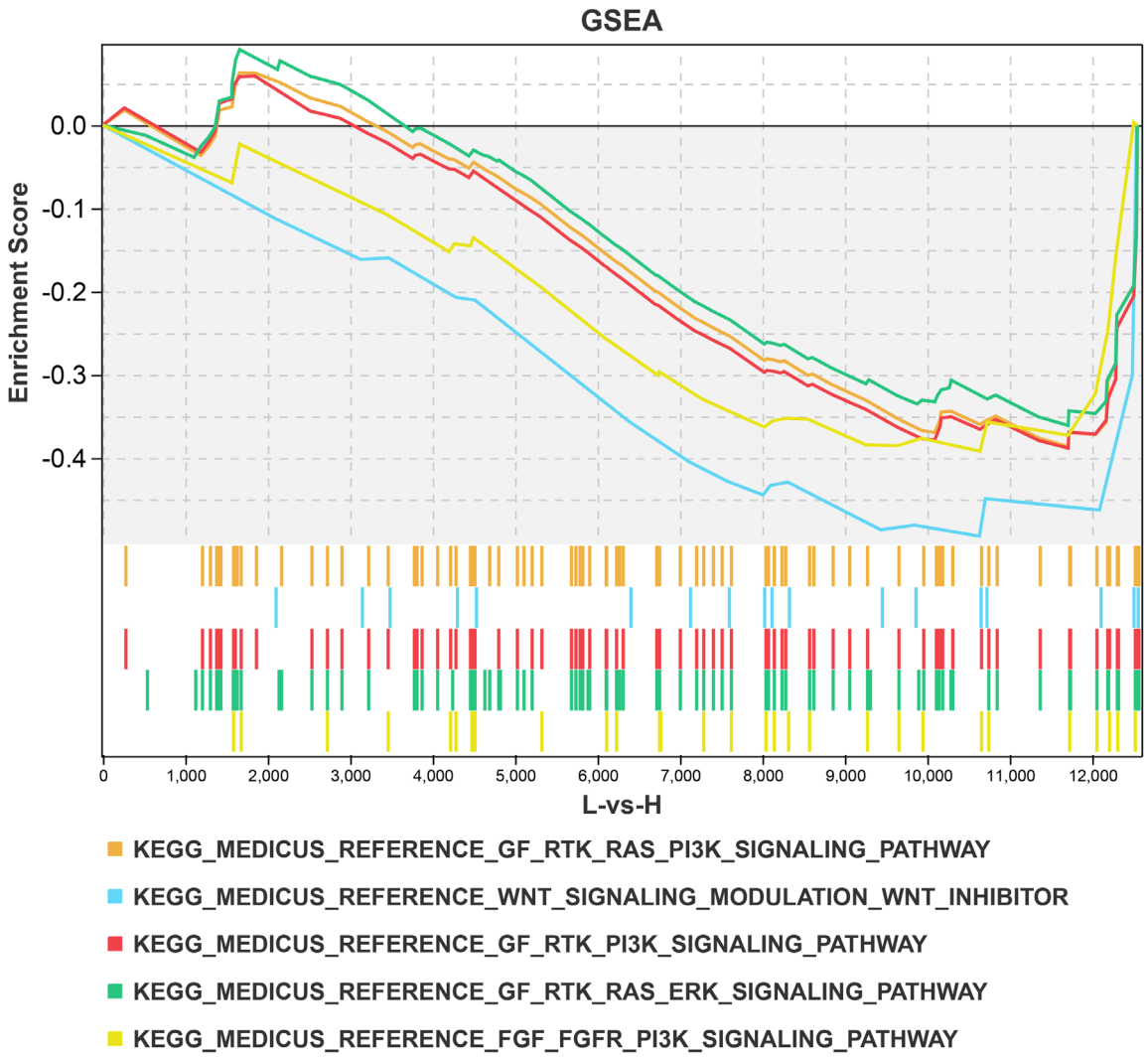

Figure 6. KEGG enrichment analysis revealing the regulatory effect of salidroside on the WNT signaling pathway.

viding essential references for target analysis in CRC. Telang et al. [26] showed that using breast cancer and CRC stem cell models could uncover new molecular mechanisms and support drug development. Through this cross-cancer analysis, several differentially expressed genes commonly regulated by salidroside were identified, particularly MMP-12. This finding suggests that salidroside may exert anticancer effects by modulating common molecular mechanisms.

MMP-12, a zinc-dependent protease, is integral to extracellular matrix degradation and remodeling, playing a crucial role in tumor invasion and metastasis [27]. Previous studies have shown that MMP-12 is significantly upregulated in various malignancies, with high expression often associated with increased tumor invasiveness, poor prognosis, and metastasis [28]. He et al. [19] reported that MMP-12 influenced the tumor microenvironment by modulating inflammation and immune cell activity in the development of inflammatory bowel disease-related CRC. In our study, qRT-PCR and Western blot analyses revealed that MMP-12 expression was significantly higher in CRC cell lines HCT-116 and SW620 compared to normal controls. Salidroside treatment resulted in a significant downregulation of both mRNA and protein levels of MMP-12. Furthermore, functional experiments involving MMP-12 overexpression and inhibition demonstrated that MMP-12 overexpression significantly enhanced the proliferation, migration, and invasion of CRC cells, whereas MMP-12 inhibition significantly re-

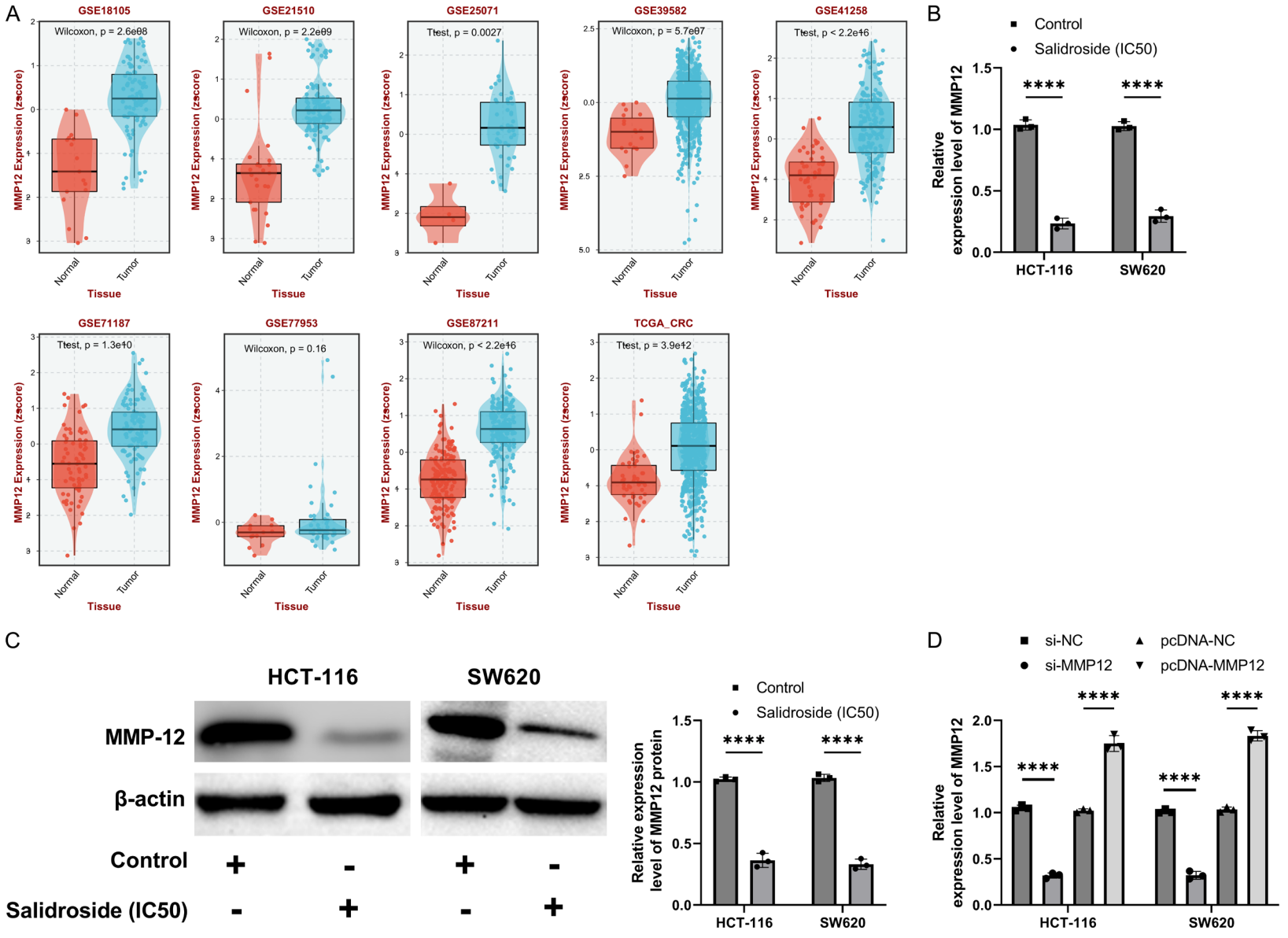

Figure 7. Effect of salidroside and MMP-12 overexpression/inhibition on CRC. A. Screening of MMP-12 expression in CRC tissues using the BEST online database. B. qRT-PCR analysis of MMP-12 expression in different treatment groups. C. Western blot analysis of MMP-12 protein levels in different treatment groups. D. Relative expression of MMP-12 mRNA in transfected cells. Note: IC50 = 66.5 μM, CRC, colorectal cancer; MMP-12, matrix metalloproteinase-12; ****P < 0.0001.

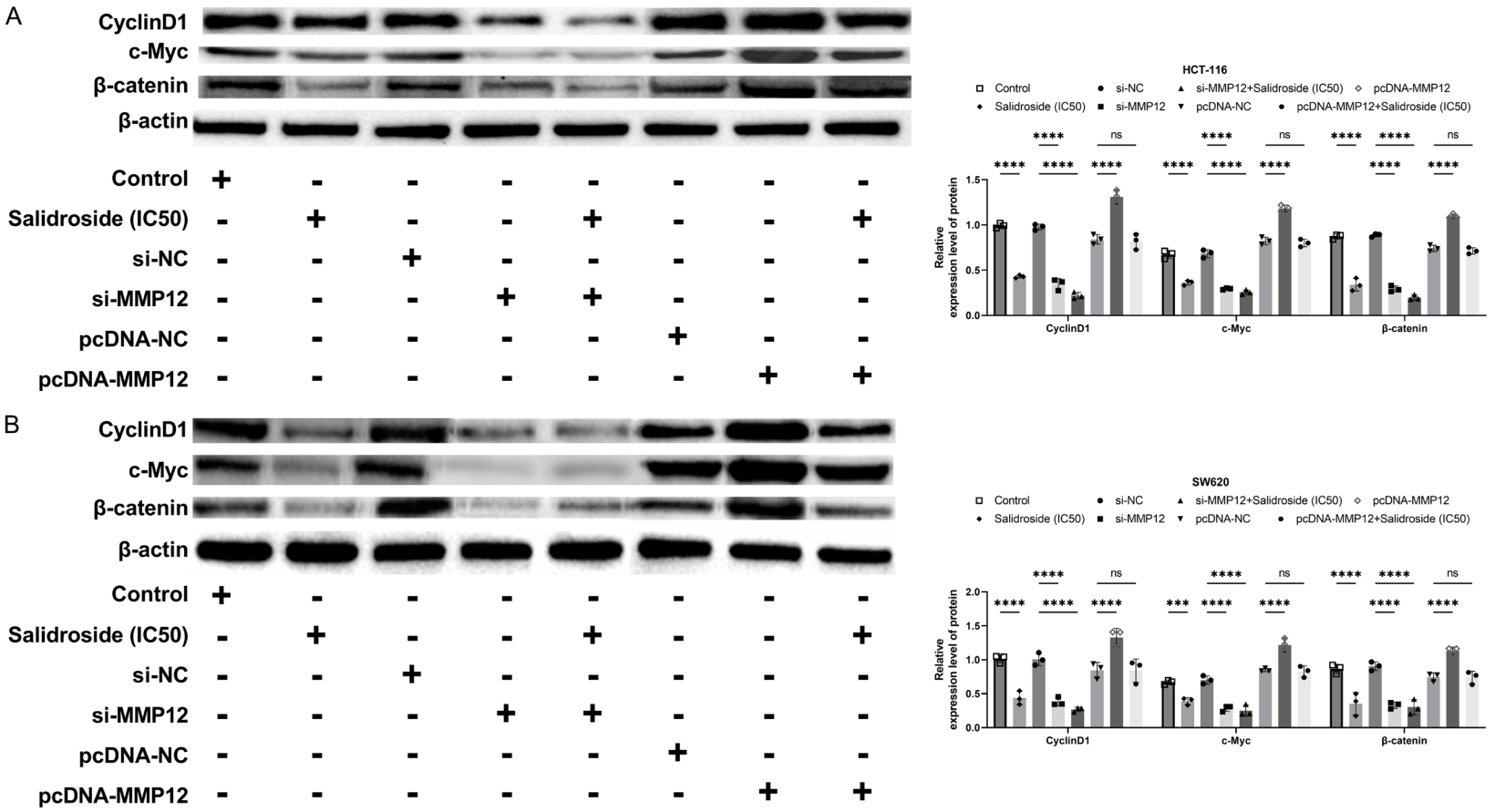

Figure 8. Regulation of Cyclin D1, c-Myc, and β-catenin protein expression by salidroside and MMP-12. A. Western Blot analysis of Cyclin D1, c-Myc, and β-catenin protein levels in HCT-116 cells under different treatment conditions: Control, Salidroside (IC50), si-NC, si-MMP-12, pcDNA-NC, and pcDNA-MMP-12. B. Western Blot analysis of Cyclin D1, c-Myc, and β-catenin protein levels in SW620 cells under the same treatment conditions as in 8A. Note: IC50 = 66.5 μM, CRC, colorectal cancer; MMP-12, matrix metalloproteinase-12; nsP > 0.05, ****P < 0.0001.

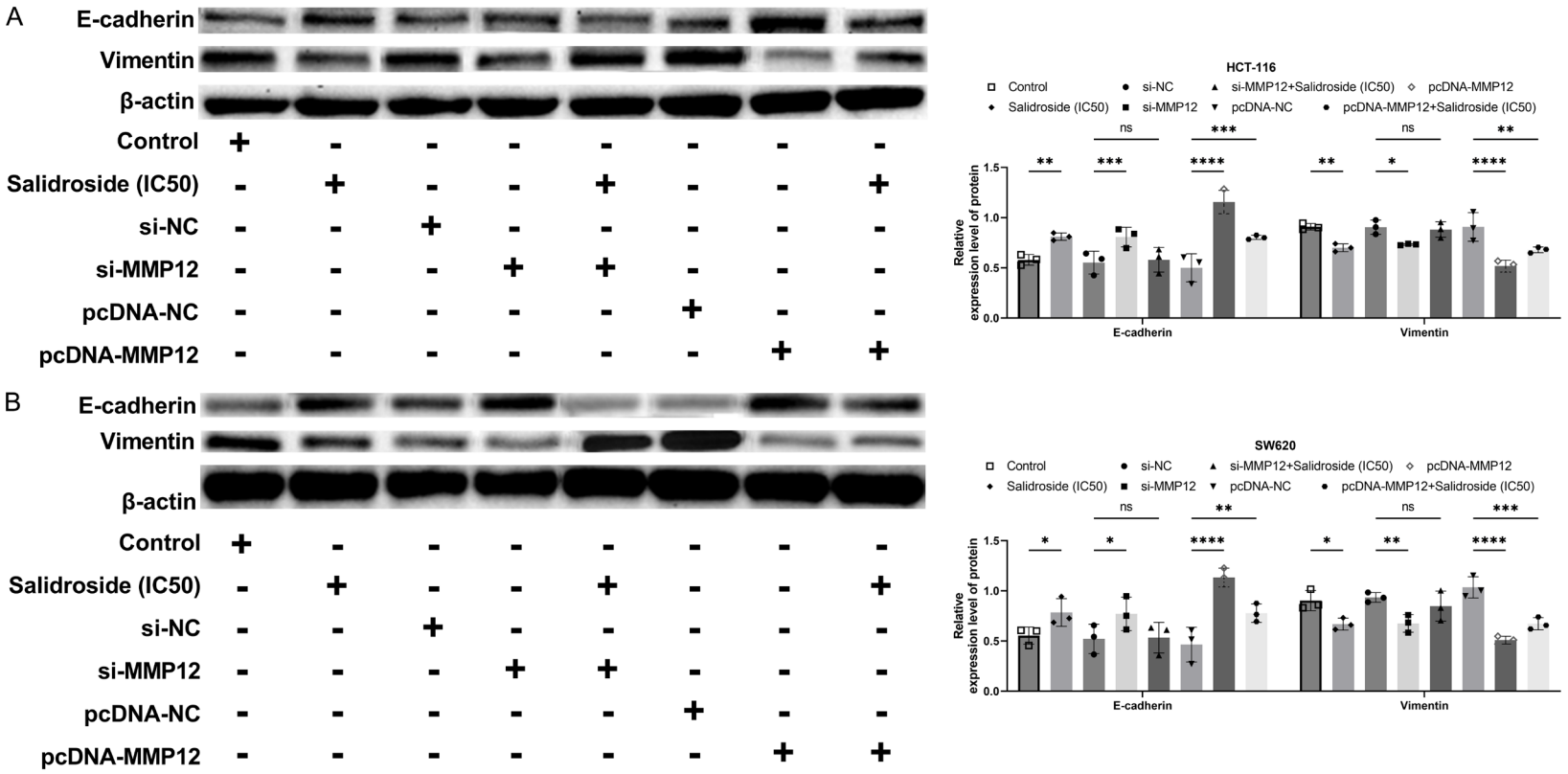

Figure 9. Effect of salidroside on EMT marker E-cadherin and Vimentin expression through MMP-12 regulation. A. Western Blot analysis of E-cadherin and Vimentin protein levels in HCT-116 cells under different treatment conditions: Control, Salidroside (IC50), si-NC, si-MMP-12, pcDNA-NC, and pcDNA-MMP-12. B. Western Blot analysis of E-cadherin and Vimentin protein levels in SW620 cells under the same treatment conditions as in 9A. Note: IC50 = 66.5 μM, CRC, colorectal cancer; MMP-12, matrix metalloproteinase-12; nsP > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

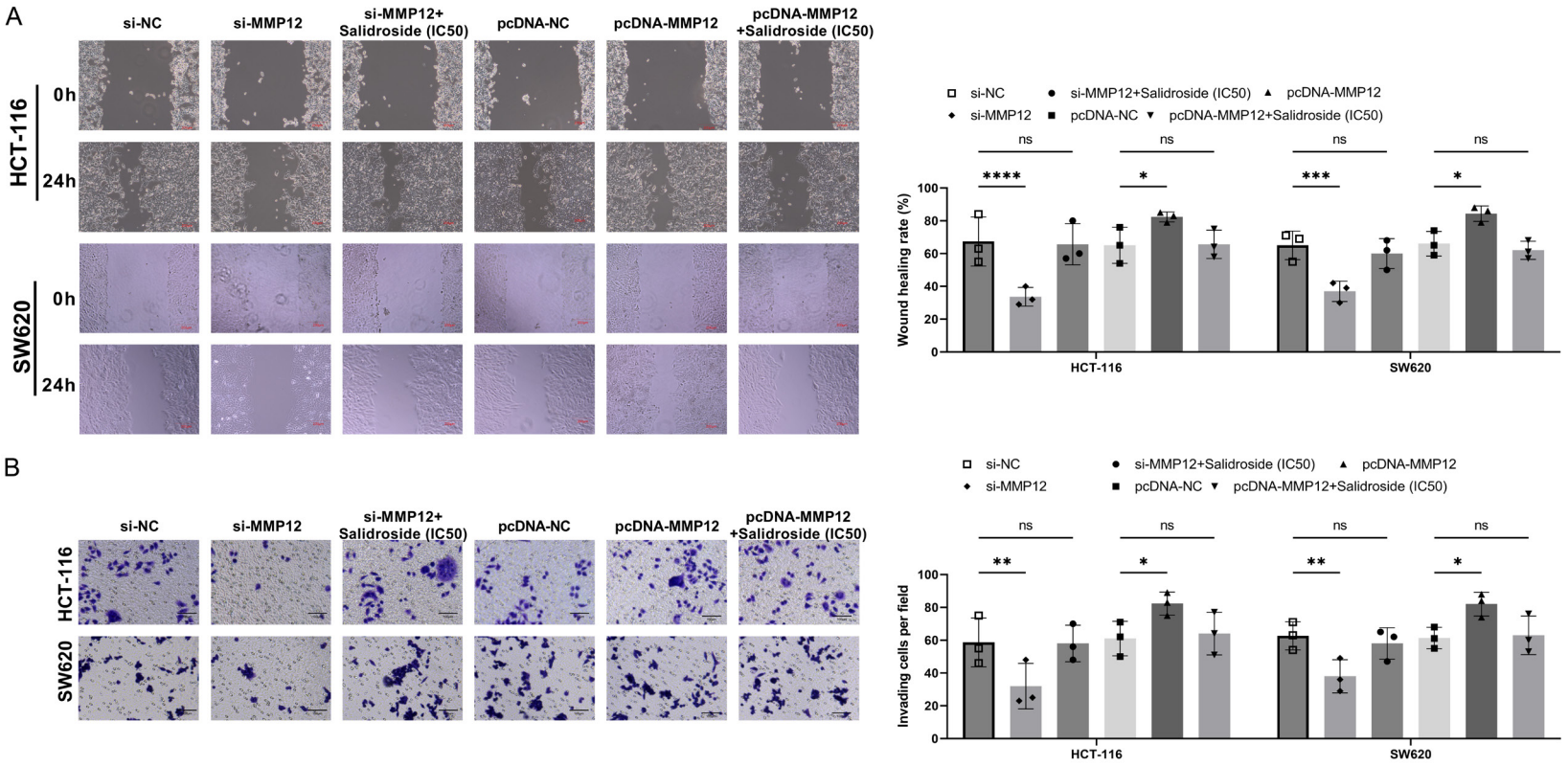

Figure 10. Synergistic effect of MMP-12 regulation and salidroside on the migration and invasion abilities of HCT-116 and SW620 cells. A. Wound healing assay showing the effect of MMP-12 overexpression or knockdown combined with salidroside treatment on cell migration. B. Transwell invasion assay showing the effect of different treatments on cell invasion abilities. Note: IC50 = 66.5 μM, CRC, colorectal cancer; MMP-12, matrix metalloproteinase-12; nsP > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

duced these capabilities. Study of Fan et al. [29] suggests that MMP-12$^+$ macrophages play a key role in colorectal cancer liver metastasis by regulating the TGF-β signaling pathway and promoting angiogenesis, providing further evidence for the role of MMP-12 in tumor metastasis. Additional experiments showed that salidroside could reverse the pro-invasive effects induced by MMP-12 overexpression, highlighting the crucial role of salidroside in inhibiting tumor invasion by regulating MMP-12.

The WNT signaling pathway is a highly conserved network essential for embryonic development, tissue regeneration, and tumorigenesis [30]. It regulates cell proliferation, differentiation, and migration, playing a pivotal role in tumor invasion and metastasis. Activation of the canonical WNT signaling pathway is typically associated with the accumulation of β-catenin, leading to enhanced transcriptional activity and upregulation of downstream target genes such as Cyclin D1 and c-Myc. This promotes cell cycle progression and maintains the invasive phenotype of tumors [31]. Pan et al. [32] demonstrated that aberrant activation of the WNT/β-catenin pathway is a major driver of tumorigenesis in multiple cancers, with its downstream targets serving as potential intervention points for cancer treatment. Furthermore, Zhou et al. [33] noted that the WNT signaling pathway played a crucial role in immune evasion by tumors, with its aberrant activation hindering T cell-mediated antitumor immune responses and thereby enhancing the immunosuppressive tumor environment. In our GSEA analysis, we found that high MMP-12 expression was significantly enriched in the WNT signaling pathway gene set, indicating that MMP-12 may contribute to CRC cell invasion and migration by promoting WNT pathway activity. Additionally, Tejeda-Muñoz et al. [34] emphasized that WNT pathway activation in colorectal cancer could further promote tumor invasion and metastasis by regulating cell adhesion and extracellular matrix remodeling. Experimental results demonstrated that high MMP-12 expression was accompanied by significant upregulation of key WNT signaling molecules, including β-catenin, Cyclin D1, and c-Myc, potentially creating a favorable microenvironment for WNT signaling by promoting extracellular matrix degradation and ultimately enhancing tumor invasiveness. Salidroside treatment significantly suppressed MMP-12 expression and indirectly blocked abnormal WNT signaling activation by downregulating WNT pathway-related molecules such as β-catenin, Cyclin D1, and c-Myc. This inhibition may be achieved by directly reducing MMP-12 expression, thereby weakening its activation effect on the WNT signaling pathway and leading to a significant reduction in tumor invasion and migration abilities. This mechanism was further validated by MMP-12 overexpression and inhibition experiments: MMP-12 overexpression reversed the inhibitory effect of salidroside on WNT signaling molecules, whereas MMP-12 inhibition enhanced the effect of salidroside.

This study is the first to systematically investigate the anticancer effects and molecular mechanisms of salidroside in CRC cells, demonstrating that salidroside inhibits EMT, invasion, and migration by downregulating MMP-12 expression and modulating the WNT signaling pathway. By combining bioinformatics analysis and experimental validation, we found that high MMP-12 expression significantly promoted WNT signaling activity, while salidroside effectively inhibited this process, thereby suppressing the malignant behavior of CRC cells. These findings not only expand the potential application of salidroside in cancer therapy but also highlight the importance of MMP-12 as a target for CRC treatment. However, there are limitations in this study. First, in vivo experiments are lacking to verify the anticancer effects of salidroside and its impact on the tumor microenvironment. Second, the study focused solely on MMP-12 and the WNT signaling pathway, without exploring other potentially relevant pathways, such as PI3K/AKT and TGF-β. Future research should incorporate animal models and multi-omics technologies to explore the multitarget mechanisms of salidroside and investigate its synergy with existing therapeutic approaches, providing a solid foundation for its clinical application.

Conclusion

Salidroside inhibits the invasion and migration of CRC cells by downregulating MMP-12 and regulating the WNT signaling pathway, effectively suppressing EMT. This study provides a scientific basis for the potential use of salidroside as a therapeutic agent for CRC and offers

new insights for optimizing CRC treatment strategies.

Acknowledgements

This work was supported by Shaanxi Provincial Key R&D Program (No. 2024SF-YBXM-133) and Shaanxi Provincial Health Commission, No 2021B007.

Disclosure of conflict of interest

None.

Address correspondence to: Bin Zhao, Department of Epidemiology, Shaanxi Provincial Cancer Hospital, No. 309 Yanta West Road, Yanta District, Xi'an 710061, Shaanxi, China. E-mail: billness@stu.xjtu.edu.cn

References

[1] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022; 72: 7-33.

[2] Kratzer TB, Jemal A, Miller KD, Nash S, Wiggins C, Redwood D, Smith R and Siegel RL. Cancer statistics for American Indian and Alaska Native individuals, 2022: including increasing disparities in early onset colorectal cancer. CA Cancer J Clin 2023; 73: 120-146.

[3] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74: 229-263.

[4] Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, Laurent-Puig P, Quirke P, Yoshino T, Taieb J, Martinelli E and Arnold D; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2020; 31: 1291-1305.

[5] Wei M, Huang X, Liao L, Tian Y and Zheng X. SENP1 decreases RNF168 phase separation to promote DNA damage repair and drug resistance in colon cancer. Cancer Res 2023; 83: 2908-2923.

[6] Yang S, Wang L, Zeng Y, Wang Y, Pei T, Xie Z, Xiong Q, Wei H, Li W, Li J, Su Q, Wei D and Cheng W. Salidroside alleviates cognitive impairment by inhibiting ferroptosis via activation of the Nrf2/GPX4 axis in SAMP8 mice. Phytomedicine 2023; 114: 154762.

[7] Huang G, Cai Y, Ren M, Zhang X, Fu Y, Cheng R, Wang Y, Miao M, Zhu L and Yan T. Salidroside sensitizes Triple-negative breast cancer to ferroptosis by SCD1-mediated lipogenesis and NCOA4-mediated ferritinophagy. J Adv Res 2024; [Epub ahead of print].

[8] Zhu X, Liu D, Wang Y and Dong M. Salidroside suppresses nonsmall cell lung cancer cells proliferation and migration via microRNA-103-3p/Mzb1. Anticancer Drugs 2020; 31: 663-671.

[9] Jiang B, Cui Y, Ma X, Zhang Y, Feng X, Yang T, Feng L, Guo W, Li Y, Wang T, Guo H, Li H, Duan Y and Su H. Crosstalk between autophagy inhibitor and salidroside-induced apoptosis: a novel strategy for autophagy-based treatment of hepatocellular cancer. Int Immunopharmacol 2023; 124: 111040.

[10] Yuetong L, Shangzhu L, Qinglin H and Pingping H. Salidroside inhibits proliferation, migration and invasion of human pancreatic cancer PANC1 and SW1990 cells through the AKT and ERK signaling pathway. Pharmazie 2020; 75: 385-388.

[11] He S, Xie F, Su W, Luo H, Chen D, Cai J and Hong X. Anti-inflammatory salidroside delivery from chitin hydrogels for NIR-II image-guided therapy of atopic dermatitis. J Funct Biomater 2023; 14: 150.

[12] Zhang X, Xie L, Long J, Xie Q, Zheng Y, Liu K and Li X. Salidroside: a review of its recent advances in synthetic pathways and pharmacological properties. Chem Biol Interact 2021; 339: 109268.

[13] Sun KX, Xia HW and Xia RL. Anticancer effect of salidroside on colon cancer through inhibiting JAK2/STAT3 signaling pathway. Int J Clin Exp Pathol 2015; 8: 615-621.

[14] Mao L, Yang J, Yue J, Chen Y, Zhou H, Fan D, Zhang Q, Buraschi S, Iozzo RV and Bi X. Decorin deficiency promotes epithelial-mesenchymal transition and colon cancer metastasis. Matrix Biol 2021; 95: 1-14.

[15] Taniguchi N, Ohkawa Y, Kuribara T, Abe J, Harada Y and Takahashi M. Roles of glyco-redox in epithelial mesenchymal transition and mesenchymal epithelial transition, cancer, and various diseases. Antioxid Redox Signal 2024; 41: 910-926.

[16] Rim EY, Clevers H and Nusse R. The Wnt pathway: from signaling mechanisms to synthetic modulators. Annu Rev Biochem 2022; 91: 571-598.

[17] de Almeida LGN, Thode H, Eslambolchi Y, Chopra S, Young D, Gill S, Devel L and Dufour A. Matrix metalloproteinases: from molecular mechanisms to physiology, pathophysiology, and pharmacology. Pharmacol Rev 2022; 74: 712-768.

[18] Lin B, Fan Y, Yang X, Pathak JL and Zhong M. MMP-12 and periodontitis: unraveling the mo-

lecular pathways of periodontal tissue destruction. J Inflamm Res 2024; 17: 7793-7806.
[19] He L, Kang Q, Chan KI, Zhang Y, Zhong Z and Tan W. The immunomodulatory role of matrix metalloproteinases in colitis-associated cancer. Front Immunol 2023; 13: 1093990.
[20] Korbozova NK, Kudrina NO, Zhukova NA, Grazhdannikov AE, Blavachinskaya IV, Seitimova GA, Kulmanov TE, Tolstikova TG and Terletskaya NV. Antihypothyroid effect of salidroside. Molecules 2022; 27: 7487.
[21] Wojdasiewicz P, Brodacki S, Cieślicka E, Turczyn P, Poniatowski ŁA, Ławniczak W, Olczak M, Stolarczyk EU, Wróbel E, Mikulska A, Lach-Gruba A, Żuk B, Romanowska-Próchnicka K and Szukiewicz D. Salidroside: a promising agent in bone metabolism modulation. Nutrients 2024; 16: 2387.
[22] Fei SF, Tong DB and Jia F. Antiatherosclerotic effect and molecular mechanism of salidroside. Rev Cardiovasc Med 2023; 24: 97.
[23] Liu RH, Ma TF, Yang Q, Xiao WC, Yin L, Yin M, Zhang JS and Wang CH. Salidroside suppresses proliferation and migration in prostate cancer via the PI3K/AKT pathway. Cancer Biomark 2023; 38: 321-332.
[24] Michelli M, Zougros A, Chatziandreou I, Michalopoulos NV, Lazaris AC and Saetta AA. Concurrent Wnt pathway component expression in breast and colorectal cancer. Pathol Res Pract 2020; 216: 153005.
[25] Zougros A, Michelli M, Chatziandreou I, Nonni A, Gakiopoulou H, Michalopoulos NV, Lazaris AC and Saetta AA. mRNA coexpression patterns of Wnt pathway components and their clinicopathological associations in breast and colorectal cancer. Pathol Res Pract 2021; 227: 153649.
[26] Telang NT. Stem cell models for breast and colon cancer: experimental approach for drug discovery. Int J Mol Sci 2022; 23: 9223.
[27] Bassiouni W, Ali MAM and Schulz R. Multifunctional intracellular matrix metalloproteinases: implications in disease. FEBS J 2021; 288: 7162-7182.
[28] Gobin E, Bagwell K, Wagner J, Mysona D, Sandirasegarane S, Smith N, Bai S, Sharma A, Schleifer R and She JX. A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential. BMC Cancer 2019; 19: 581.
[29] Fan L, Wang Q, Qian Q, Wang Q, Liu D, Gong Y and Xiong Z. Single-cell RNA sequencing revealing that MMP12+ macrophages are associated with cancer liver metastasis. Curr Med Chem 2024; [Epub ahead of print].
[30] Dong Z, Ojha A, Barlow L, Luo L, Liu JY and Zhang JT. The eIF3a translational control axis in the Wnt/β-catenin signaling pathway and colon tumorigenesis. Cancer Lett 2024; 605: 217303.
[31] Nie K, He ZJ and Kong LJ. NR3C2 affects the proliferation and invasiveness of colon cancer cells through the Wnt/β-Catenin signaling pathway. J Cancer Res Clin Oncol 2024; 150: 411.
[32] Song P, Gao Z, Bao Y, Chen L, Huang Y, Liu Y, Dong Q and Wei X. Wnt/β-catenin signaling pathway in carcinogenesis and cancer therapy. J Hematol Oncol 2024; 17: 46.
[33] Zhou Y, Xu J, Luo H, Meng X, Chen M and Zhu D. Wnt signaling pathway in cancer immunotherapy. Cancer Lett 2022; 525: 84-96.
[34] Tejeda-Muñoz N and Mei KC. Wnt signaling in cell adhesion, development, and colon cancer. IUBMB Life 2024; 76: 383-396.

Table S1. Primer sequence

Gene	Upstream primers	Downstream primers
MMP-12	GATGCTGTCACTACCGTGGGAA	CAATGCCAGATGGCAAGGTTGG
GAPDH	GATTCCACCCATGGCAAATTC	CTGGAAGATGGTGATGGGATT