Downregulation of MACC1 facilitates the reversal effect of verapamil on the chemoresistance to active metabolite of irinotecan in human colon cancer cells

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\textbf{ABSTRACT}

The aim of this study is to investigate the reversal effect of verapamil (VER) on chemoresistance to irinotecan (CPT-11) in human colon cancer cells and relevant mechanisms. Cell counting kit-8 (CCK-8) test and colony-forming unit (CFU) experiment results show that VER strengthens the sensitivity of human colon cancer cell line HT29 to CPT-11 but has a small effect on SW480 cells. High-throughput transcriptome sequencing, RT-PCR, and Western blot results show that the inhibition of metastasis-associated in colon cancer-1 (MACC1) expression by VER is the key factor for reversal effect on chemoresistance to CPT-11. Transfection experiments further show that VER can reverse the resistance of human colon cancer cells to SN-38, the active metabolite of CPT-11, when MACC1 is overexpressed. The nude mouse transplantation tumor experiment provides an in vivo proof that VER can strengthen sensitivity to CPT-11 in drug-resistant human colon cancer cells, and the effect might be related to the inhibited expression of MACC1. In summary, VER might strengthen the reversal effect of VER on chemoresistance to CPT-11 in human colon cancer cells and facilitate the apoptosis of human colon cancer cells by downregulating MACC1 expression.

1. Introduction

Colon cancer is one of the most common malignant tumors of the digestive tract and threatens human health (H. Chen, Luo and Guo, 2020; S. Wang, Miao, Yang, Wang and Zhang, 2018). According to the World Health Organization (WHO), the incidence of the disease accounts for about 24.3% of its global incidence, and its mortality accounts for about 22.9% of the global rate. Moreover, the number of patients with colon cancer is increasing gradually, and the age of patients tends to decrease over time (Benson et al., 2018). To date, clinical therapies for colon cancer mainly include excision, postoperative chemotherapy, radiotherapy, and biotherapy (Gosavi et al., 2021; Kanani et al., 2021). Commonly used chemotherapy drugs include 5-Fu, oxaliplatin, and bevacizumab, which considerably increase the 5 year survival rate and survival quality of patients (Bertagnolli et al., 2009; Dienstmann et al., 2015; Gelibter et al., 2019). However, the inhibitory effects of chemotherapy on tumor cells weaken at increasing dosage, and thus drug resistance increases, leading to chemotherapy failure (He et al., 2019; Lei et al., 2021). Colon cancer cells not only develop resistance to chemotherapeutics after long-term administration but also develop cross-resistance to other unused drugs with different structures and action mechanisms (Zhou et al., 2012). Hence, low-toxicity and high-efficiency resistance reversal agents are necessary. Exploring the reverse mechanism of colon cancer chemoresistance has become a major topic of interest in the field of tumor chemotherapy.

Verapamil (VER) is an L-shaped calcium channel inhibitor mainly used to treat cardiovascular and cerebrovascular diseases and can reverse multidrug resistance (MDR) in tumor cells (Han et al., 2021). VER at concentrations of 6.0–10.0 μmol/L exerts effective reversal effect on tumor drug resistance and can improve the clinical efficacy of chemotherapy for hepatocellular cancer, colon cancer, lung cancer, and malignant ascites (Liu et al., 2011). Studies have shown that the reversal effect mechanism of VER in MDR in tumors is likely related to P-glycoprotein (P-gp) (Gao et al., 2021; Hunt et al., 2021). VER resists MDR in tumors by regulating the expression of P-gp and can improve the clinical efficacy of chemotherapy for hepatocellular cancer, colon cancer, lung cancer, and malignant ascites (Liu et al., 2011).
Metastasis-associated in colon cancer-1 (MACC1) is a newly discovered by Stein U and his team in 2009 after analyzing and comparing normal colon tissues, colonic adenoma tissues, primary tumors, and metastasis by using differential displaying qRT-PCR technology (Kopczynska, 2016; Z. Z. Wu et al., 2016). Some studies have suggested that MACC1 is a prognosis marker of cancer progression (Li et al., 2011), a key regulatory factor, and a biomarker in the progression and metastasis of more than 20 cancer types, including bladder, colon, and esophagus cancer (Huang et al., 2020; Radhakrishnan et al., 2018; Zhu et al., 2013). It can participate in MDR and tumor cell invasion and metastasis by regulating the expression of hepatocyte growth factor and its receptor cellular mesenchymal-to-epithelial transition factor (Li et al., 2015; Stein et al., 2009). Notably, high MACC1 level in the circulation indicates increased risk of cancer metastasis, and MACC1 expression is related to MACC1 expression. Additionally, the nude mouse transfection showed that VER-induced enhancement of sensitivity to CPT-11 is effective targets of the antagonizing drug-resistance therapy of VER and CPT-11 has significant antitumor effects.

2. Materials and methods

2.1. Experimental materials

SN-38 and VER were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. CCK-8 was purchased from Tongren Institute of Chemistry (Japan). RNA extraction and reverse transcription kits were purchased from TIANGEN Company. Anti-human MACC1 and D-glyceraldehyde-3-phosphate dehydrogenase antibodies were purchased from Sigma Company. Antarit sheep HRP-marked second antibodies were purchased from Guizhou Jinqiao Biological Company. High-throughput sequencing was authorized by Guangzhou Ruibo Company. siRNA used in gene transfection was bought from Guangzhou Ruibo Company. The overexpression plasmid and empty carrier was TrueORF GOLD (ORIGENE). The annexin V-FITC dual-staining kit was bought from Beijing Beibo Company. Primer design and synthesis kits were bought from Shanghai ShineGene Molecular Biotechnology Co., Ltd. Human colon cancer cells SW480, HT29 and HCT116 were purchased from the cell library of Chinese Academy of Sciences.

2.2. CCK-8 test

SN-38 was diluted with DMEM, and the final concentrations were 50, 25, 12.5, 6.25 and 3.125 nM. The final concentration of VER was 10 μM. Human colon cancer cells at the logarithmic growth stage were inoculated into a 96-hole plate (density: 5000 cells/hole) and cultured in the incubator at 37 °C with 5% CO2 24 h. Different concentrations of SN-38 or VER diluted with high-glucose DMEM were added. Each dose had three repeated holes, and the culture time was 24 h. Next, CCK-8 (10 μL/hole) was added. The PBS group was cultured for 1 h and used as the control group. Absorbance at 450 nm was measured by a microplate reader. The survival rates of cells were calculated (IC50 value) by SPSS 21 software.

2.3. Clone formation assay

HT29 or SW480 cells were inoculated into six-hole plates and cultured overnight. The plates were taken out, and 0 and 10 nM SN-38 were added successively. PBS and 10 μM VER were then added. Cell holes with drugs were placed in an incubator at 37 °C with 5% CO2 for 9 days. Cell holes were collected, rinsed with PBS, and then fixed with methyl alcohol at room temperature for 10 min. After cell fixation with methyl alcohol, crystal violet staining buffer was added for dark staining, and cells were incubated at 37 °C for 30 min. Next, the crystal violet staining buffer was removed, and the cells were rinsed with deionized water and then dried at room temperature. Clone formation images were acquired under a microscope at 200 magnification. Image J software was used to directly read the images of clone formation and count the clones after taking pictures through the microscope and saving the images.

2.4. High-throughput transcriptome sequencing with an illumina technology sequencing platform

High-throughput sequencing was performed, and HT29 cells were used as the objects. The cells were divided into the normal (NC), SN-38 (10 nM), and SN-38 (10 nM) + VER (10 μM) groups. A Hiseq whole genome deep sequencing technology was applied. The differentially expressed genes (DEGs) of HT29 cell strains between the SN-38 and SN-38 + VER groups were screened through bioinformatics analysis and comparison with reference genes.

2.5. qRT-PCR

Total RNA was extracted, and quantitative analysis was carried out according to the specification of the kit’s manufacturer. RNA was inversely transcribed into cDNA with PrimeScript™RT kit. Primers were designed using Primer 5.0 software (Table 1). A reaction system was prepared as follows: 12.5 μL of 2 × SYBR Green Universal qPCR Master Mix, 1.5 μL of each primer, and 3 μL of cDNA mixed, and distilled water was added to a final volume of 25 μL. PCR degeneration was implemented for 1 min at 95 °C, and 40 cycles of amplification were performed with a StepOne™RT-qPCR system. Each cycle was
performed for 15 s at 95 °C, 20 s at 56 °C, and 40 s at 72 °C. The mRNA levels of the candidate genes were standardized into GAPDH and then evaluated with the 2^–ΔΔCt method. Each experiment was repeated three times, and three cycles were performed.

### 2.6. Western blot (WB)

HT29 or SW480 cells were inoculated into six-hole pieces (5.0 × 10^5 cells/hole). The grouping was the same as that in Section 1.3. After drug treatment, the cells were collected and split with RIPA lysate for 30 min. After centrifugation at 12,000 rpm for 30 min at 4 °C, the supernatant was collected, and the protein was quantified using the BCA protein detection kit (Beyotime, Shanghai, China). After protein separation through SDS-PAGE, the proteins were transferred to a PVDF membrane (Millipore, Sigma) and blocked with TBST containing 5% skim milk for 2 h, and the membranes were washed three times with TBST (15 min in each). Then, protein antibodies (GAPAH = 1:2000, MACCI = 1:1000) with the membrane were incubated overnight at 4 °C. Next, the membrane was washed three times (15 min in each) with TBST, and secondary antibodies (1:5000) were added before incubation at room temperature for 2 h. The blots were visualized by a chemiluminescence imager (Tanon 5200, Shanghai, China).

### 2.7. Cell transfection

MACC1 siRNA lentivirus transduction particles (MACC1 siRNA sequence: forward, 5'-AAGAGGGGACGGGGACACGGCTT-3' and reverse, 5'-TTGGGCAACCGGACAGGGGAC-3') and negative siRNA control were acquired from Sigma-Aldrich. MACC1-knockdown plasmid (PCMV6-AC-GFP) and empty carrier (PCMV6-Entry) were bought from Origene Inc. Colon cancer cells were inoculated into six-hole plates and grew overnight to 80% and then mixed. Subsequently, the cells were transfected with siRNA-MACC1 or PCMV-MACC1 by using AmaxaNucleofector (Amaza, Kölner) according to the specification of the manufacturer. Downregulation and overexpression efficiency was determined with Western blot (WB). Cell toxicity experiment was performed using CCK-8, and the grouping and drug concentration were the same as those in Section 1.2.

### 2.8. Hepatoma cell apoptosis detection by annexin V-PI dual-staining method

HT29, HT29 siRNA-MACC1, SW480 and SW480 shRNA-MACC1 cells were employed. Drugs were divided into SN-38 (10 nM) and SN-38 (10 nM) + VER (10 μM) groups. The cells were cultured in six-hole plates with 10% FBS DMEM for 24 h until the degree of convergence reached about 70%. Pancreatin action was terminated with 10% FBS medium after trypsinization. The cells were centrifuged for 5 min at 1000 rpm and 4 °C, rinsed twice with precooled PBS, resuspended in 100 μL of binding buffer, mixed evenly with 2 μL of annexin V-FITC, and finally placed in ice for 15 min in the dark. Then, 400 μL of PBS was added. Another 1 μL of PI solution was added to each sample, which was loaded on a flow cytometer. The cells were blown uniformly, and then cell apoptosis was tested quickly.

### 2.9. Nude mouse transplantation tumor experiments

The experiments were performed according to the animal nursing standards approved by the Ministry of Science and Technology of the People's Republic of China and ARRIME guideline (animal preclinical study). All animal experimental procedures were approved by the Animal Experimental Ethics Committee of Shandong University (Jinan, China). Female BALB/c nude mice weighing approximately 20 g (n = 12), 8–12 weeks old, were kept in mini-isolator cages (20 cm × 15 cm × 13 cm). The animals were maintained under positive pressure at 22 °C on 14 h light/10 h dark cycle, with appropriate water and food. They were randomly divided into four groups: (i) NC, (ii) VER (20 mg/kg), (iii) SN-38 (20 mg/kg) and (iv) SN-38 (20 mg/kg) + VER (20 mg/kg). HT29 cells (2 × 10^6) were suspended in PBS containing Matrigel (1:1), and 200 μL of suspension was injected into the right axilla of each mouse. After tumors grew to 100 mm3, drug was administered to the mice every 3 days. The tumor volumes of mice were measured and recorded every 3 days. After 30 days of treatment, the mice were killed, tumor weight was recorded, and tumor tissues were collected. For the extraction of protein samples, after the tumor tissue was sheared into fine pieces, 1 mL of RIPA lysis buffer was added per 100 mg of tumor tissue. The lysed sample was transferred to a 1.5 mL centrifuge tube and centrifuged at 12,000 rpm for 5 min at 4 °C. The supernatant was used for subsequent electrophoresis and WB analysis.

### 2.10. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Statistical significance was analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc tests using GraphPad Prism 5 software.

### 3. Results

#### 3.1. Drug resistance of VER + SN-38 in three colon cell lines (SW480, HT29 and HCT116)

CPT-11 is a derivative of camptothecin, and its active metabolite is SN-38 (Goldenberg and Sharkey, 2019; Jensen et al., 2016). In this study, three colon cell lines (SW480, HT29 and HCT116) were used in discussing the collaborative effects of VER and SN-38 on colon cells with different genetic characteristics (Figure 1A). First, the sensitivity of the three colon cell lines to VER was evaluated with CCK-8 test (Figure 1B). After VER acted for 48 h, VER (10 μM) had no influence on the proliferation of the cell lines (inhibition rate of cells was <10%). Hence, the collaborative administration dosage of VER was set at 10 μM. Next, the sensitivity of the cell lines to SN-38 under independent administration and collaborative administration with VER was evaluated (Figure 1C). Compared with the independent administration of SN-38, the collaborative administration of VER and SN-38 can decrease the IC50 (from 0.6 μM to 0.3 μM). Comparing the sensitivity of the HT29 cells, the highest, whereas the sensitivity of the SW480 cells was the lowest. Therefore, in follow-up studies, HT29 cells were selected as sensitive cells for investigating the reversal effect of VER on tumor drug resistance, whereas SW480 cells were selected as reversal drug-resistance cells.

#### 3.2. Effects of VER + SN-38 on the proliferation of HT29 and SW480 cells

Clone formation is related to the independent proliferation ability of cells and group dependence. When cell environment changes, clone formation in cells may change accordingly (Dobson et al., 2020; Park et al., 2016). To further verify the collaborative influences of VER and SN-38 on the proliferation of colon cancer cells, differences in the clone formation of cells after SN-38 and VER + SN-38 administration were analyzed with a panel clone formation experiment (Figure 2A). Compared with the NC group, the SN-38 group significantly inhibited the proliferation of two cell lines. However, the clone formation of HT29 cells was significantly inhibited after the collaborative administration of VER and SN-38 compared with that after the independent administration of SN-38, but the clone formation of SW480 cells was only influenced...
slightly (Figure 2B). This result indicated that HT29 was more sensitive to the collaborative administration of VER and SN-38.

3.3. Mechanism of VER in strengthening the sensitivity of colon cancer cell lines to SN-38

VER strengthens the sensitivity of HT29 cells to SN-38 significantly but influences the inhibitory effect of SN-38 on SW480 proliferation slightly. In this study, HT29 cells were selected as research objects. DEGs that might mediate VER activity strengthening sensitivity to SN-38 were screened through transcriptome sequencing on the Illumina technology sequencing platform and expression detection of DEGs.

Clustering analyses of DEGs in HT29 cells after independent administration of SN-38 and collaborative administration of VER and SN-38 were carried out. The results showed that the gene expression profiles of HT29 cells differed significantly (Figure 3A and B). In this study, upregulated and downregulated genes related to drug resistance were selected as candidate genes, including MCM4, CLN6, MACC1, TXNDC5, YES1, TGOLN2, ZFP36, APC and NRIP1. qRT-PCR verification reflected that MACC1 presented the highest degree of differential expression in HT29 cells after VER intervention (Figure 3C). In WB assay, MACC1 expression in the HT29 and SW480 cells was further tested. MACC1 had high expression levels in HT29 cells but extremely low expression levels in SW480 cells (Figure 4A and B). This result agreed with previous reports. Additionally, the expression of MACC1 in HT29 cells declined significantly after VER intervention, but its expression in the SW480 cells did not show obvious changes (Figure 4C). Hence, this gene was further verified.

3.4. Changes in VER effects strengthening sensitivity to SN-38 after the downregulated expression and overexpression of MACC1 in colon cancer cell lines

We transfected siRNA and shRNA of target MACC1 into HT29 and SW480 cells to further verify whether it is the key gene that strengthen the ability of VER to increase sensitivity to SN-38. The downregulated expression of MACC1 in HT29 cells was realized effectively by siRNA-MACC1 (Figure 5A), and the sensitivity of HT29 cells to SN-38 was strengthened significantly. Sensitivity did not change obviously after VER addition (Figure 5B). In SW480 cells, shRNA-MACC1 strengthened the expression of MACC1 effectively (Figure 5C), and SW480 cells developed significant drug resistance to SN-38 after overexpression, but this effect was reversed by VER (Figure 5D).

3.5. Detection of apoptosis in colon cancer cell lines with annexin V-PI dual-staining method

Figure 6A and B shows that in HT29 cells, the apoptosis rates of the SN-38 and SN-38 + VER groups were 11.16% and 68.89%, respectively, showing significant differences. After the downregulated expression of MACC1, the apoptosis rates of the SN-38 and SN-38 + VER groups increased significantly to 65.73% and 66.04%, respectively, compared with those before downregulated expression (Figure 6C and D). However, no significant difference in apoptosis rate was observed between the two groups. In SW480 cells, the apoptosis rates of the SN-38 and SN-38 + VER groups were 58.40% and 66.27%, respectively, showing no significant differences (Figure 6E and F). After the overexpression of
MACC1, the apoptosis rate of SN-38 was inhibited significantly and decreased to 8.85% (Figure 6G). After co-incubation with VER, the apoptosis rate of the SN-38 + VER group declined significantly (60.59%, Figure 6H). This result further indicated that VER increased the pro-apoptotic ability of SN-38 by inhibiting the expression level of MACC1.

3.6. Nude mouse transplantation tumor experiment

Nude mouse transplantation tumor model of HT29 cells was used, and the NC group was used as the negative control. For the real-time detection of tumor growth conditions, tumor volume was measured every 3 days in the whole experiment (Figure 7). Tumor volume in different groups increased continuously over time. However, tumor volume in the SN-38 + VER group at different measuring points after 9 days was significantly smaller than the tumor volumes of the SN-38 and NC groups (Figure 7B). Meanwhile, compared with the SN-38 group (inhibitory rates of 58.34%), the SN-38 + VER group showed 31.27% reduction in tumor weight every 3 days, which was more potent than SN-38 alone (Figure 7A and C). Moreover, consistent with the in vitro model, the expression levels of MACC1 obviously decreased in the VER and SN-38 + SN-38 groups in the tumor model (Figure 7D and E). This result further indicated that VER strengthened the sensitivity of HT29 cells to CPT-11 by downregulating the expression of MACC1.

4. Discussions

MDR is one of major constraints of the clinical chemotherapeutic effect of colon cancer. P-gp is a protein encoded by ABCB1, which can induce the hydrolysis of ATP to produce ADP and release energy (Hennessy and Spiers, 2007). In addition, calcium ions facilitate the binding of P-gp to chemotherapeutic drugs in cells and pump them out of the cells (H. Zhang et al., 2021). Therefore, drug concentration in cells decreases, the toxicity of drugs to tumor is weakened, and MDR occurs. VER is a calcium channel antagonist and ABCB1 inhibitor. It can inhibit the expression ABCB1 and synthesis of P-gp, thus increasing the concentration of chemotherapeutics in tumor cells to overcome drug resistance. Chiu et al. showed that VER can reverse the drug resistance of the drug-resistant subfamilies of lung cancer, which are not associated with ABCB1 expression (Chiu et al., 2010). Zhang et al. showed that the expression level of P-gp and effect of VER have no direct relationships with reversal of MDR in hepatoma cell lines (Tengyue et al., 2017). However, the involvement of P-gp still cannot be ignored. VER can inhibit P-gp efflux (Baek and Cho, 2015). Moreover, the combination of VER and a drug can increase the expression of P-gp in lung cancer cells. This finding provides evidence of reduced drug resistance (Jaferian et al., 2018). Moreover, VER can enhance multidrug resistance to chemotherapeutics by targeting the transport function of P-gp (Zhao et al., 2016). VER has several reverse abilities in different cancer cells, but the
Figure 3. A. High-throughput transcriptome sequencing identifies differentially expressed genes related to the reversal effect of VER on chemoresistance to SN-38 in HT29 cells treated with SN-38 or VER. B. Volcano diagram shows DEGs between the SN-38 + VER and SN-38 groups. C. The expression of candidate genes in HT29 cells was validated through qRT-PCR. *p < 0.05, **p < 0.01 versus the SN-38 group.

Figure 4. Protein expression of MACC1 in HT29 (A) and SW480 cells (B) treated with SN-38 or VER as detected by WB assay. C. Quantification of relative protein level were displayed. **p < 0.01 versus the control group.
potential mechanism remains unknown. We discussed the influences of three colon cancer cell lines on chemoresistance to CPT-11 to explore novel targets that can mediate the reversal effect of VER on chemoresistance in human colon cancer. Reversal effects were observed in all three cell lines. However, the optimal reversal effect was observed in the HT29 cells, but only a mild effect was detected in the SW480 cells. This difference may be related to the source of cell lines, genes and other factors (Khamas et al., 2012; Lauzier et al., 2019; van den Berg et al., 2020). Subsequently, we performed high-throughput transcriptome sequencing on the HT29 cells, which were sensitive to the reversal effect of VER, to screen key genes that may mediate such effect. Compared with the negative control group, PIGT, CLN6, MACC1, TXNDC5, YES1, TGOLN2, ZFP36, APC, and NRIP1 all had significantly different expression levels in HT29 cells after VER treatment. qRT-PCR showed that differences between the MACC1 expression levels before and after VER treatment were the most obvious. According to WB assay, MACC1 had high expression levels in the HT29 cells but low expression levels in the SW480 cells. Therefore, MACC1 might be a key gene that mediates the reversal effect of VER on HT29 resistance to CPT-11.

Tight junction molecules are related to the incidence and metastasis of tumors. In closely bonded molecules, MACC1 is the major transmembrane protein and composed of 24 gene families (Arlt and Stein, 2009; Katarzyna, Jacek, Leszek, 2009). It serves as a semitransparent barrier against the transportation of ions and solutes, divided into the top

Figure 5. Changes in the ability of VER to strengthen the sensitivity of colon cancer cell lines to SN-38 after the downregulated expression or overexpression of MACC1. A and B. Effects of VER on the expression of HT29 siR-MACC1 cells and their sensitivity to SN-38 after the downregulated expression of MACC1. C and D. Effects of VER on the expression of SW480 shRNA-MACC1 cells and their sensitivity to SN-38 after the overexpression of MACC1. **p < 0.01 versus the control group.

Figure 6. Detection of the collaborative effects of VER (10 μM) and SN-38 (10 nM) on apoptosis of colon cancer cells based on the annexin V-PI dual-staining method. Results were shown as representative dot plots of HT29 cells (A–D) and SW480 cells (E–H).
end of a separation membrane and fence of external structural domain of a substrate (Sawada, 2013). Tight junction refers to the top component of a compound that connects epithelial cells of mammals (Liang and Weber, 2014; Otani and Furuse, 2020), coordinately the differentiation, proliferation, and polarity of regulatory cells in various signal paths and are multifunctional compound (Garrido-Urbani et al., 2014). Malignant cells often present tight junction molecules and loss of cell polarity. CLNDs, especially CLND3 and MACC1, are overexpressed in breast cancer, ovarian cancer, and pancreatic cancer (Shang et al., 2013; Sueta et al., 2015). Docetaxel is the first-line chemotherapeutic of several tumors (L. Chen et al., 2019; Leveque and Becker, 2017). The downregulated expression of MACC1 can influence the sensitivity of gastric cancer to docetaxel. Hu et al. pointed out that a specific receptor at the C-terminal of clostridium perfringens enterotoxin might destroy the tightly connected barriers among cells and strengthen the absorption ability of cells by regulating the expression level of MACC1, thus improving sensitivity of breast cancer cells to cisplatin and paclitaxel (Hu et al., 2021). To further explore the role of MACC1 in mediating drug resistance of human colon cancer, its expression in human colon cancer cells is controlled through the transfection of siRNA and overexpression carrier of MACC1 (K. Zhang et al., 2014). After the downregulated expression of MACC1 in HT29 cells, the reversal effect of VER disappears basically. By contrast, the overexpression of MACC1 increases the reversal effect of VER on the chemoresistance of SW480 cells to CPT-11. The nude mouse transplantation experimental results showed that VER can strengthen the sensitivity of HT29 cells with high expression of MACC1 to CPT-11 in vivo. These results prove that CPT-11 participates in controlling the reversal effect of VER on chemoresistance in human colon cells, and downregulating expression of MACC1 can improve such reversal effect.

In summary, the combined administration of VER and CPT-11 can strengthen the sensitivity of HT29 cells to CPT-11 and promote apoptosis. The mechanism might be realized by downregulating the expression of MACC1. This study can provide experimental references to the comprehensive treatment of colon cancer.

5. Lead contact

Further information should be requested from the Lead Contact, Pingsheng Fan (Fanpingshengcsco@163.com).
Declarations

Author contribution statement

Xiaofeng Qian and Yongxin Zhao: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tengyue Zhang and Ning Ge: Analyzed and interpreted the data. Pingsheng Fan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

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References

Ali, D.A., El-Quindy, D.M., Elshahdy, M.A., Sabry, N.M., Kabel, A.M., Gaber, R.A., et al., 2021. The prognostic significance of MACC1 expression in breast cancer and its relationship to immune cells in the tumor microenvironment and patient survival. Medicina 57 (9).

Arii, F., Sein, U., 2009. Colon cancer metastasis: MACC1 and Met as metastatic pacemakers. Int. J. Biochem. Cell Biol. 41 (12), 2356–2359.

Bæk, J.S., Cho, C.W., 2015. Controlled release and reseal of multidrug resistance by co-encapsulation of paclitaxel and verapamil in solid lipid nanoparticles. Int. J. Pharm. 478 (2), 617–624.

Benson, A.B., Venook, A.P., Al-Hawary, M.M., Cederquist, L., Chen, Y.J., Ciombor, K.K., et al., 2016. Effect of verapamil on the pharmacokinetics of pasireotide in healthy volunteers. J. Clin. Pharmacol. 56 (11), 1263–1271.

Lauzier, A., Normandeau-Guimond, J., Vaillancourt-Lavigne, V., Boivin, V., Charbonneau, M., Rocard, N., et al., 2019. Colonctal cancer cells respond differentially to autophagy inhibition in vivo. Sci. Rep. 9 (1), 11316.

Lei, G., Liu, S., Yang, X., He, C., 2021. TRIM29 reverses oxaliplatin resistance of P53 mutant colon cancer cell. Chin. J. Gastroenterol. Hepatol. 2021, 8079097.

Leveque, D., Becker, G., 2017. Generic docetaxel. J. Clin. Pharmacol. 57 (7), 935–945.

Li, H., Zhang, H., Zhao, S., Shi, Y., Yao, J., Zhang, Y., et al., 2015. Overexpression of MACC1 and the association with hepatocyte growth factor/c-Met in epithelial ovarian cancer. Oncol. Lett. 9 (5), 1899–1906.

Li, H., Cheng, Y.X., Weng, J.A., Bae, P., 2017. Metastasis-associated colon cancer-1 in a promising biomarker for the metastasis and prognosis of colorectal cancer. Oncol. Lett. 14 (4), 3899–3908.

Liang, G.H., Weber, C.R., 2014. Molecular aspects of tight junction barrier function. Curr. Opin. Pharmacol. 19, 84–89.

Liu, Y., Lu, Z., Fan, P., Duan, Q., Li, Y., Tong, S., et al., 2011. Clinical efficacy of chemotherapy combined with verapamil in metastatic colorectal patients. Cell Biochem. Biophys. 61 (2), 393–398.

Mahe, E., Hays, J.L., Stover, D.G., Chen, J.L., 2018. The omics revolution continues: the maturation of high-throughput biological data sources. Yearb Med Inform 27 (1), 211–222.

Otani, T., Furuse, M., 2020. Tight junction structure and function revisited. Trends Cell Biol. 30 (10), 855–871.

Park, H.B., Son, W., Chae, D.H., Lee, J., Kim, I.W., Yang, W., et al., 2016. Cell cloning-on-the-spot by using an attachable silicone cylinder. Biochem. Biophys. Res. Commun. 474 (4), 768–772.

Qiu, J., Huang, P., Liu, Q., Hong, J., Li, B., Lu, C., et al., 2011. Identification of MACC1 as a novel prognostic marker in hepatocellular carcinoma. J. Transl. Med. 9 (1), 820.

Radhakrishnan, H., Walther, W., Zincke, F., Kobelt, D., Imbabi, F., Eldem, M., et al., 2018. MACC1—the first decade of a key metastasis molecule from gene discovery to clinical translation. Cancer Metastasis Rev. 37 (4), 805–826.

Sawada, N., 2013. Tight junction-related human diseases. Pathol. Int. 63 (3), 1–12.

Shang, X., Lin, X., Manorek, G., Howell, S.B., 2013. Claudin-3 and claudin-4 regulate transport of copper ions and cisplatin in ovarian cancer. Oncogene 32 (38), 4637–4645.

Walther, W., Steinhuber, A., Schwabe, H., Smith, J., Fichtner, I., et al., 2009. MACC1, a newly identified key regulator of HGF-MET signaling, predicts cancer colon metastasis. Nat. Med. 15 (1), 57–60.

Subramaniam, I., Verma, S., Kumar, S., Jere, A., Anamika, K., 2020. Multi-omics data integration, interpretation, and its application. Bioinf. Biol. Insights 14, 67–92.
Sueta, A., Yamamoto, Y., Yamamoto-Ibusuki, M., Hayashi, M., Takeshita, T., Yamamoto, S., et al., 2015. Differential role of MACC1 expression and its regulation of the HGF/cMet pathway between breast and colorectal cancer. Int. J. Oncol. 46 (5), 2143–2153.

Tengyue, Z., Kelong, M., Jin, H., Shitang, W., Yabei, L., Gaofei, F., et al., 2017. CDKN2B is critical for verapamil-mediated reversal of doxorubicin resistance in hepatocellular carcinoma. Oncotarget 8 (66).

van den Berg, J., Castricum, K.C.M., Meel, M.H., Goedegebuure, R.S.A., Lagerwaard, F.J., Slotman, B.J., et al., 2020. Development of transient radioresistance during fractionated irradiation in vitro. Radiother. Oncol. 148, 107–114.

Wang, L., Sun, Y., 2020. Efflux mechanism and pathway of verapamil pumping by human P-glycoprotein. Arch. Biochem. Biophys. 696, 108675.

Wang, G., Kang, M.X., Lu, W.J., Chen, Y., Zhang, B., Wu, Y.L., 2012. MACC1: a potential molecule associated with pancreatic cancer metastasis and chemoresistance. Oncol. Lett. 4 (4), 783–791.

Wang, Z., Li, Z., Wu, C., Wang, Y., Xia, Y., Chen, L., et al., 2014. MACC1 overexpression predicts a poor prognosis for non-small cell lung cancer. Med. Oncol. 31 (1), 790.

Wang, S., Miao, Z., Yang, Q., Wang, Y., Zhang, J., 2018. The dynamic roles of mesenchymal stem cells in colon cancer. Chin. J. Gastroenterol. Hepatol. 2018, 7628763.

Wu, Z., Zhou, R., Su, Y., Sun, L., Liao, Y., Liao, W., 2015. Prognostic value of MACC1 in digestive system neoplasms: a systematic review and meta-analysis. BioMed Res. Int. 2015, 252045.

Wu, Z.Z., Chen, L.S., Zhou, R., Bin, J.P., Liao, Y.L., Liao, W.J., 2016. Metastasis-associated in colorectal cancer-1 in gastric cancer: beyond metastasis. World J. Gastroenterol. 22 (29), 6629–6637.

Zhang, K., Tian, F., Zhang, Y., Zhu, Q., Xue, N., Zhu, H., et al., 2014. MACC1 is involved in the regulation of proliferation, colony formation, invasion ability, cell cycle distribution, apoptosis and tumorigenicity by altering Akt signaling pathway in human osteosarcoma. Tumour Biol. 35 (3), 2537–2548.

Zhang, H., Xu, H., Ashby Jr., C.R., Assaraf, Y.G., Chen, Z.S., Liu, H.M., 2021. Chemical molecular-based approach to overcome multidrug resistance in cancer by targeting P-glycoprotein (P-gp). Med. Res. Rev. 41 (1), 525–555.

Zhao, L., Zhao, Y., Schwarz, B., Mysliwietz, J., Hartig, R., Camaj, P., et al., 2016. Verapamil inhibits tumor progression of chemotherapy-resistant pancreatic cancer side population cells. Int. J. Oncol. 49 (1), 99–110.

Zhou, Y., Touzi, F., Chen, J., Fan, F., Xia, L., Wang, J., et al., 2012. Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells. Cancer Res. 72 (1), 304–314.

Zhu, M., Xu, Y., Mao, X., Gao, Y., Shao, L., Yan, F., 2013. Overexpression of metastasis-associated in colon cancer-1 associated with poor prognosis in patients with esophageal cancer. Pathol. Oncol. Res. 19 (4), 749–753.

Zielinski, J.M., Luke, J.J., Gaglietta, S., Krieg, C., 2021. High throughput multi-omics approaches for clinical trial evaluation and drug discovery. Front. Immunol. 12, 590742.