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Original Article

MEN1 promotes ferroptosis by inhibiting mTOR-SCD1 axis in pancreatic neuroendocrine tumors

Zeng Ye[1,2,3,4,5,†], Haidi Chen[1,2,3,4,5,†], Shunrong Ji[1,2,3,4,5,†], Yuheng Hu[1,2,3,4,5], Xin Lou[1,2,3,4,5], Wuhu Zhang[1,2,3,4,5], Desheng Jing[1,2,3,4,5], Guixiong Fan[1,2,3,4,5], Yue Zhang[6], Xuemin Chen[6], Qifeng Zhuo[1,2,3,4,5], Jie Chen[1,3,4,5], Xiaowu Xu[1,2,3,4,5], Xianjun Yu[1,2,3,4,5], Jin Xu[1,2,3,4,5,*], Yi Qin[1,2,3,4,5,*], and Heli Gao[1,2,3,4,5,*]

[1]Center for Neuroendocrine Tumors, Fudan University Shanghai Cancer Center, Shanghai 200032, China, [2]Department of Pancreatic Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, China, [3]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China, [4]Shanghai Pancreatic Cancer Institute, Shanghai 200032, China, [5]Pancreatic Cancer Institute, Fudan University, Shanghai 200032, China, and [6]Department of Hepatobiliary and Pancreatic Surgery, The Third Affiliated Hospital of Soochow University, Changzhou 213003, China

[†]These authors contributed equally to this work.

*Correspondence address. Tel: +86-21-64175590; E-mail: gaoheli@fudanpci.org (H.G.) / E-mail: qinyi@fudanpci.org (Y.Q.) / E-mail: xujin@fudanpci.org (J.X.)

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Abstract

Pancreatic neuroendocrine tumor (pNET) is the second most common malignant tumors of the pancreas. Multiple endocrine neoplasia 1 (*MEN1*) is the most frequently mutated gene in pNETs and MEN1-encoded protein, menin, is a scaffold protein that interacts with transcription factors and chromatin-modifying proteins to regulate various signaling pathways. However, the role of MEN1 in lipid metabolism has not been studied in pNETs. In this study, we perform targeted metabolomics analysis and find that MEN1 promotes the generation and oxidation of poly-unsaturated fat acids (PUFAs). Meanwhile lipid peroxidation is a hallmark of ferroptosis, and we confirm that MEN1 promotes ferroptosis by inhibiting the activation of mTOR signaling which is the central hub of metabolism. We show that stearoyl-coA desaturase (SCD1) is the downstream of MEN1-mTOR signaling and oleic acid (OA), a metabolite of SCD1, recues the lipid peroxidation caused by MEN1 overexpression. The negative correlation between MEN1 and SCD1 is further verified in clinical specimens. Furthermore, we find that BON-1 and QGP-1 cells with MEN1 overexpression are more sensitive to everolimus, a widely used drug in pNETs that targets mTOR signaling. In addition, combined use everolimus with ferroptosis inducer, RSL3, possesses a more powerful ability to kill cells, which may provide a new strategy for the comprehensive therapy of pNETs.

Key words pancreatic neuroendocrine tumor, MEN1, ferroptosis, mTOR signaling, SCD1

Introduction

Pancreatic neuroendocrine tumors (pNETs) are relatively rare heterogeneous malignancies which originate from neuroendocrine system of pancreas and account for approximately 3% of primary pancreatic malignancies [1,2]. Based on mitotic count and Ki-67 Index, pNETs are histopathologically classified as low-grade (G1), intermediate-grade (G2), and high-grade (G3) tumors [3]. Overall, pNETs have a better prognosis than pancreatic ductal adenocarcinoma. The 5-year survival rates for G1, G2, and G3 pNETs are 75%, 62%, and 7%, respectively [4]. Histopathologic grade and liver metastasis are the main factors affecting prognosis [5]. However, due to the rarity of pNETs, the related molecular mechanisms are not well studied.

According to a whole-genome sequencing of 102 primary pNETs, multiple endocrine neoplasia 1 (*MEN1*) is the most frequently mutated gene in pNETs, which occurs in 37% cases [6]. The *MEN1*-encoded protein menin is a scaffold protein which interacts with transcription factors and chromatin-modifying proteins in respond to the stimulations of transforming growth factor-β (TGF-β), Wnt/β-catenin, Hedgehog and other signaling pathways [7]. *MEN1* was

considered to be a classical tumor suppressor gene and MEN1 inactivation has been confirmed in several cancers [8,9]. However, previous studies have reported that *MEN1* can function as tumor-promoting gene [10,11]. This is not hard to understand because menin, as a transcriptional driver, can interact with different factors to promote the transcription of oncogenes or suppressor genes [12–14]. In terms of biological effects, it has been reported that menin and menin-associated proteins function as transcriptional sensors to balance glycolysis and mitochondrial oxidative phosphorylation in respond to stressful microenvironments [15].

In pNETs, MEN1 is involved in the mTOR signaling, histone modification, altered telomere length and DNA damage repair pathways [6]. mTOR is the central hub of metabolism and determines the fate of cells [16,17]. It has been reported that mTOR is closely associated with ferroptosis, an iron-dependent form of non-apoptotic cell death characterized by lipid peroxidation [18]. Ferroptosis is widely involved in various diseases and cancers, and pharmacological modulation of ferroptosis has shown great potential for the treatment of drug-resistant cancers [19]. Our previous study confirmed that ferroptosis potentiated cytotoxic effect of gemcitabine in pancreatic cancer [20]. However, the role of ferroptosis in pNETs has not been studied. Intriguingly, a large number of studies have confirmed that inhibition or activation of mTOR signaling promotes or inhibits ferroptosis. For instance, everolimus, an inhibitor of mTOR, potentiates erastin and RSL3-induced ferroptosis in renal cell carcinoma [21]. PI3K-AKT-mTOR signaling suppresses ferroptosis by promoting SREBP1-SCD1-mediated monounsaturated fatty acid (MUFAs) metabolism [22]. In addition, mTOR inhibition also decreases GPX4 protein level to sensitize cancer cells to ferroptosis [23]. Both MEN1 and mTOR pathway genes are frequently altered in pNETs, and everolimus plays an important role in the treatment of pNETs [24,25]. It was reported that menin inhibits AKT activation by regulating the cellular localization of AKT [26]. This attracts our attention to whether MEN1 regulates metabolism and ferroptosis via AKT-mTOR signaling.

In the present study, we explored the influence of MEN1 on the metabolism of polyunsaturated fat acids (PUFAs). Our study suggested that MEN1 promoted ferroptosis by increasing the lipid peroxidation. Further exploration showed that MEN1 inhibited mROT-SREBP1-SCD1 signaling to sensitize pancreatic neuroendocrine tumor cells to ferroptosis and everolimus. Targeting ferroptosis might provide new strategies for the treatment of pNETs.

Materials and Methods

Patient population

A total of 63 patients diagnosed with pNETs and underwent surgical treatment at the Fudan University Shanghai Cancer Center (FUSCC) from June 2012 and March 2020 were enrolled and followed up. With informed consents, their paired cancer and adjacent tissues were collected for tissue microarray analysis. Progression-free survival (PFS) was defined as the time from the date of surgery to the date of progression or the date of last follow-up. This study was approved by the Clinical Research Ethics Committee of FUSCC.

Cell lines

Human pancreatic neuroendocrine tumor cell line BON-1 was provided by Professor Martyn Caplin (Gastroenterology & Gastrointestinal Neuroendocrinology Centre for Gastroenterology, Royal Free Hospital, London, UK) and was maintained in Dulbecco's modified Eagle medium/Nutrient Mixture F-12 (DMEM/F12; BasalMedia Technologies, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; Moregate BioTech, Bulimba, Australia). Human pancreatic neuroendocrine tumor cell line QGP-1 was obtained from Shanghai Zhong Qiao Xin Zhou Biotechnology (Shanghai, China), and was confirmed by short tandem repeat (STR) and was maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (BasalMedia Technologies) supplemented with 10% FBS.

Plasmids

The coding sequences of human *MEN1* were cloned into the lentiviral vector pCDH-CMV-MCS-EF1-puro (SBI, Palo Alto, USA) to generate pCDH-CMV-MEN1-Flag-EF1-puro. Empty vector (pCDH-CMV-MCS-EF1-puro) was used as the control.

Chemicals

RSL3 (S8155), everolimus (S1120), MHY1485 (S7811) and oleic acid (S4707) were purchased from Selleck (Shanghai, China) and dissolved in dimethylsulfoxide (DMSO) before use. CAY10566 (HY-15823) was purchased from MedChemExpress (Shanghai, China) and dissolved in DMSO.

Western blot analysis

BON-1 or QGP-1 cells were washed with pre-cooled 1 × phosphate-buffered saline (PBS) to remove the residual medium and lysed in RIPA buffer for 15 min. Ultrasound was used to further disrupt the cells. Cell debris was removed by centrifugation. BCA Protein Assay kit (Epizyme Biomedical Technology, Shanghai, China) was used to measure the protein concentration. After concentration correction, the same quantity and volume of samples were subject to SDS-PAGE and transferred to PVDF membranes. The primary antibodies used in this study are listed as follows: anti-MEN1 (D262984; Sangon Biotech, Shanghai, China), anti-mTOR (AB32028; Abcam, Cambridge, UK), anti-mTOR (phosphor S2481) (ab137133; Abcam), anti-mTOR (phosphor S2448) (ab109268; Abcam), anti-SREBP1 (ab3259; Abcam), anti-SCD1 (ab236868; Abcam), anti-ACTB (AC038; Abclonal, Wuhan, China), anti-GPX4 (ab125066; Abcam). HRP-conjugated Goat Anti-Rabbit IgG (AS014; Abclonal) was used as the secondary antibody in this study. Finally, omni-ECL enhanced pico light chemiluminescence kit (Epizyme Biomedical Technology) was used to visualize the protein bands, and the band intensities were quantified using β-actin as the loading control.

Tissue microarrays and immunohistochemical staining (IHC)

Tissue microarrays (TMAs) were prepared with paraffin-embedded tissues from patients pathologically diagnosed with pNETs by two experienced gastrointestinal pathologists at FUSCC. In brief, paired tumor area and adjacent normal tissue area were identified and re-embedded into TMA blocks. After deparaffinization, rehydration, heat-mediated antigen retrieval, and suppression of endogenous peroxidase activity, the slides were blocked with 5% normal goat serum and incubated with primary antibodies overnight. Antibodies against MEN1 and SCD1 were used at the dilution of 1:200. After extensive wash, the slides were incubated with secondary antibody and then were stained with DAB solution. The nuclei were stained

with hematoxylin. After dehydration, the slides were sealed with neutral gum and examined under a microscope.

Targeted metabolomics study

Targeted standards used in this study were obtained from Sigma-Aldrich (St Louis, USA) and were dissolved in methanol to obtain 5.0 mg/mL individual stock solution. Then, these were prepared to make stock calibration solutions. Metabolites were extracted, underwent derivatization and sealed for LC-MS analysis according to the manufacturer's instructions. Metabolites were quantified with an ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S; Waters Corp., Milford, USA). To reduce the error, each group of samples was repeated 6 times.

Cell viability assay

Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Tokyo, Japan) was used to detect the cell viability according to the manufacturer's instructions. In brief, 3000 cells were seeded in each well of a 96-well plate. After incubation overnight, the cells were treated with different drugs or DMSO for 48 h. Finally, cells were incubated with Cell Counting Kit-8 reagent for 30 min and the absorbance of each well was measured at 450 nm. Each experiment was repeated more than 6 times and the relative cell viability was calculated by comparing with the control group.

Lipid peroxidation assay

BODIPY 581/591 C11 (D3861; Thermo Fisher, Waltham, USA) was used to detect lipid peroxidation as described previously [27]. Briefly, cells were harvested and incubated with BODIPY 581/591 C11 (2 μM) for 30 min. Then a flow cytometer was used to analyze these samples.

Statistical analysis

All data were analyzed using SPSS 22.0 software (IBM, Armonk, USA) and shown as the mean ± SD. Two-tailed paired Student's *t*-test was used to compare differences between groups and chi-square test was used to analyze the correlation between two categorical variables. Kaplan-Meier method was used to plot the survival curve. $P < 0.05$ was considered to be statistically significant.

Results

MEN1 promotes polyunsaturated fatty acid metabolism and lipid peroxidation

In order to elucidate the role of MEN1 in lipid metabolism, we performed targeted metabolomics analysis to explore the effect of MEN1 on the metabolism of medium-long chain fatty acids (Figure 1). We constructed stable BON-1 cell line that could overexpress flag-labeled MEN1 (Figure 2A). We found that in BON-1 cells with MEN1 overexpression, the most significant increase was polyunsaturated fatty acids (PUFAs) (Figure 2B and Supplementary Table S1). Elevated polyunsaturated fatty acids tend to oxidize to form lipid peroxides, leading to ferroptosis [18]. Thus, we hypothesized that MEN1 may promote lipid peroxidation and ferroptosis. In order to verify this speculation, we used BODIPY 581/591 C11 to detect lipid peroxidation in BON-1 cells overexpressing MEN1 and compared with that in cells transfected with the empty vector. The results showed that MEN1 overexpression promoted the formation of lipid peroxidation in BON-1 cells (Figure 2C).

MEN1 affects the regulation of lipid peroxidation by mTOR signaling

It has been reported that MEN1 is involved in the regulation of mTOR signaling, while mTOR signaling is an important regulator of lipid peroxidation and ferroptosis [22]. Thus, we speculated that MEN1 may regulate lipid peroxidation and ferroptosis by affecting the mTOR signaling pathway. To confirm this, we detected the change of mTOR and p-mTOR (Ser2448 and Ser 2481) in BON-1 and QGP-1 cells overexpressing MEN1 and compared with those in cells transfected with the empty vector. The results showed that MEN1 overexpression decreased both mTOR and p-mTOR (Ser2448 and Ser 2481) (Figure 3A), indicating that overexpression of MEN1 inhibited the mTOR signaling pathway. In addition, we found that inhibition of mTOR signaling by everolimus also increased the generation of lipid peroxidation in BON-1 and QGP-1 cells (Figure 3B), confirming that mTOR signaling also regulated lipid peroxidation and ferroptosis in pNETs. Furthermore, we found that activation of mTOR signaling by MHY1485 partly recued the increase of lipid peroxidation caused by MEN1 overexpression in BON-1 and QGP-1 cells (Figure 3C,D). These results indicated that MEN1 promoted lipid peroxidation and ferroptosis partly through the inhibition of mTOR signaling pathway.

MEN1 promotes lipid peroxidation by inhibiting the expression of SCD1

It has been reported that mTOR signaling pathway inhibits ferroptosis by increasing the protein levels of GPX4 or SCD1 [22,23]. In order to explore whether GPX4 or SCD1 played an important role in the regulation of ferroptosis by MEN1, we detected the change of GPX4 or SCD1 protein level in BON-1 and QGP-1 cells with MEN1 overexpression and compared with that in cell transfected with empty vector. It was found that only SCD1 was decreased significantly while GPX4 did not show any significant change (Figure 4A). These results indicated that MEN1 regulated ferroptosis probably through the change of SCD1. Further experiments confirmed that inhibiting SCD1 by CAY10566 promoted lipid peroxidation and ferroptosis in both BON-1 and QGP-1 cells (Figure 4B). In addition, oleic acid (OA), metabolites of SCD1, entirely or partly recued the production of lipid peroxidation caused by MEN1 overexpression in BON-1 and QGP-1 cells (Figure 4C,D). These results indicated that MEN1 promoted lipid peroxidation and ferroptosis through inhibiting of SCD1.

MEN1 promotes lipid peroxidation by inhibiting the mTOR-SCD1 axis

In order to explore whether MEN1 regulates the protein level of SCD1 through mTOR signaling, we pretreated BON-1 and QGP-1 cells with different concentrations of everolimus to inhibit the activation of mTOR signaling. The results showed that SCD1 was decreased with the inhibition mTOR signaling (Figure 5A). In addition, we also pretreated cells with different concentrations of MHY1485 to activate the mTOR signaling in BON-1 and QGP-1 cells with MEN1 overexpression or transfected with the empty vector. The results showed that SCD1 was increased with the activation of mTOR signaling and the increased mTOR signaling rescued the level of SCD1 caused by MEN1 overexpression in both BON-1 and QGP-1 cells (Figure 5B). These results indicated that MEN1 inhibited SCD1 expression by inhibiting the activation of mTOR signaling in pNETs. In order to further explore the function of the MEN1-mTOR-SCD1

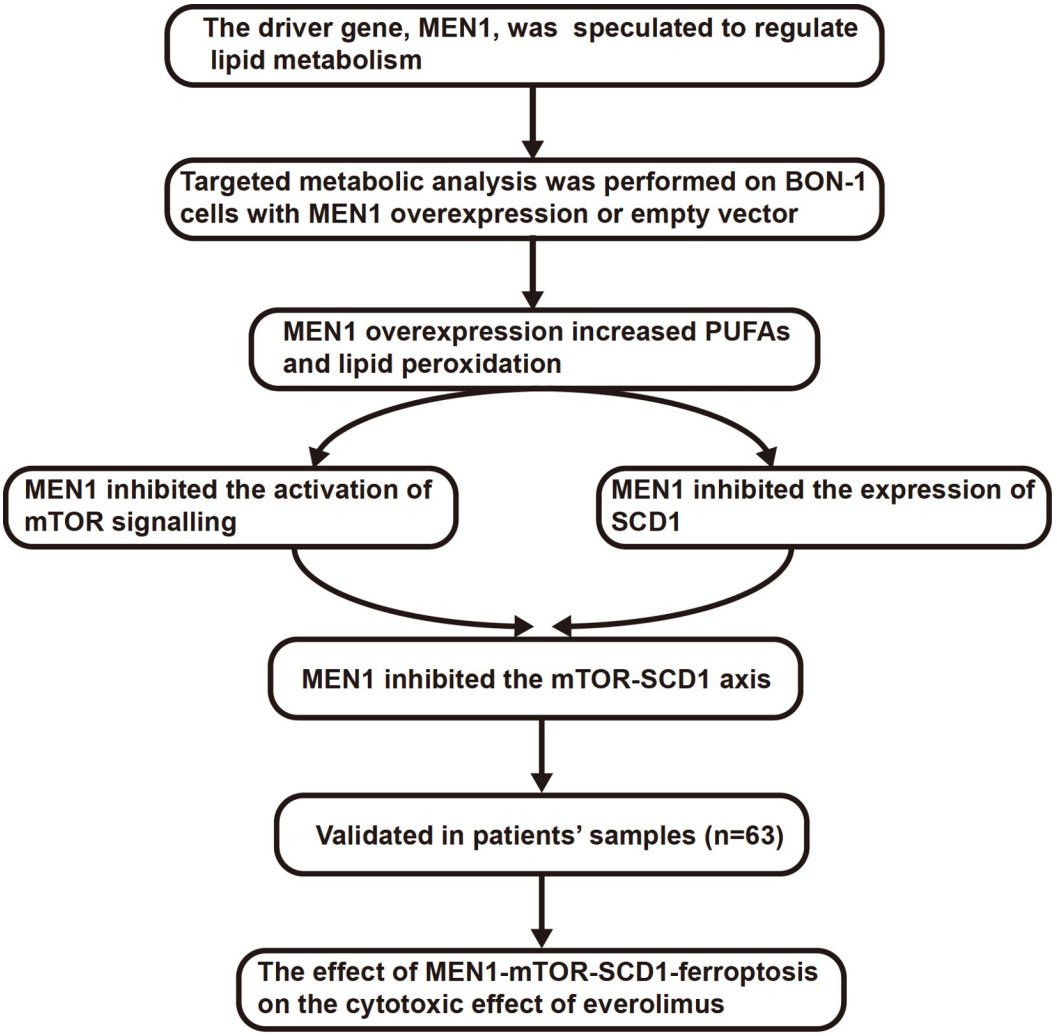

Figure 1. **Flowchart showing the analytical process of this study**

axis in regulating ferroptosis in pNETs, we treated cells with MHY1485 or Oleic acid (OA) together with RSL3 in BON-1 and QGP-1 cells with MEN1 overexpression or transfected with the empty vector. Coincidently, BON-1 cells with MEN1 overexpression were more sensitive to ferroptosis inducer, RSL3, and RSL3 or MEN1 overexpression induced a decrease of cell viability, which could be partly rescued by MHY1485 or OA in BON-1 cells (Figure 5C). The same result was obtained in QGP-1 cells (Figure 5D). These results indicated that MEN1 promoted ferroptosis by inhibiting the mTOR-SCD1 axis.

MEN1 is negatively related with SCD1 in pNETs samples

In order to explore the connection between MEN1 and SCD1 in clinical samples, we performed immunohistochemical staining to detect the expressions of MEN1 and SCD1 in tissue microarray containing paired tumor and paratumor tissue of 63 patients. We found that SCD1 was highly expressed in 19 patients (Figure 6A). Further PFS survival analysis showed that, though the difference was not significant, high SCD1 expression might suggest a worse prognosis (Figure 6B). We further divided the samples into MEN1 or SCD1 high or low expression group, which showed two typical samples: MEN1 high expression with SCD1 low expression or MEN1 low expression with SCD1 high expression (Figure 6C). Further statistical analysis showed that SCD1 was more likely to be expressed in patients with low MEN1 expression (Figure 6D). These results indicated that SCD1 is negatively related with MEN1 in clinical samples.

MEN1 potentiates cytotoxic effect of everolimus

Previous experiments have confirmed that both MEN1 overexpression and everolimus promote the generation of lipid peroxidation and ferroptosis. Therefore, it is reasonable to speculate that everolimus may be more powerful to kill cells with MEN1 overexpression. To confirm this, we detected the lipid peroxidation in BON-1 and QGP-1 cells pretreated with everolimus or DMSO. We found that the lipid peroxidation was significantly increased in BON-1 and QGP-1 cells with MEN1 overexpression compared with that in their corresponding control cells (Figure 7A,B). The results of cell viability also showed that everolimus caused more cell death in BON1 cells with MEN1 overexpression ($P=0.001$) and in QGP-1 cells with MEN1 overexpression ($P=0.018$). Furthermore, the combination of everolimus with RSL3 showed stronger lethal effects in BON-1 and QGP-1 cells. The effects were even more significant in MEN1-overexpressing BON-1 ($P<0.001$) and QGP-1

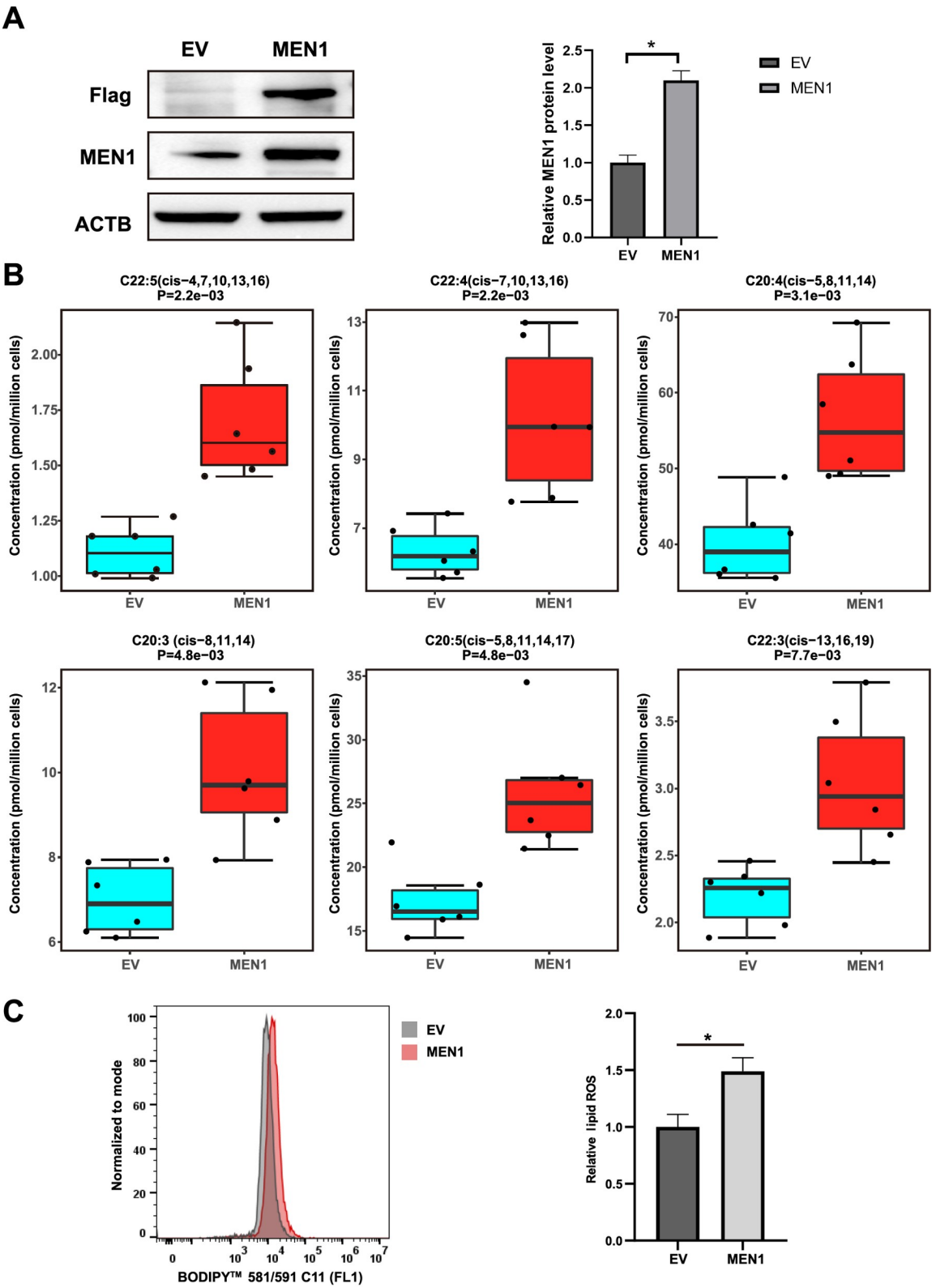

Figure 2. MEN1 promotes polyunsaturated fatty acid metabolism and lipid peroxidation (A) Western blot analysis of the change of MEN1 and flag-labeled MEN1 in BON-1 cells with MEN1 overexpression or transfected with empty vector. (B) Targeted metabolomics analysis of BON-1 cells with MEN1 overexpression or transfected with empty vector. Six fatty acids with the most significant changes were shown. (B) BODIPY 581/591 C11 (2 μM) was used to detect the change of lipid peroxidation in BON-1 cells with MEN1 overexpression or transfected with empty vector. *$P < 0.05$.

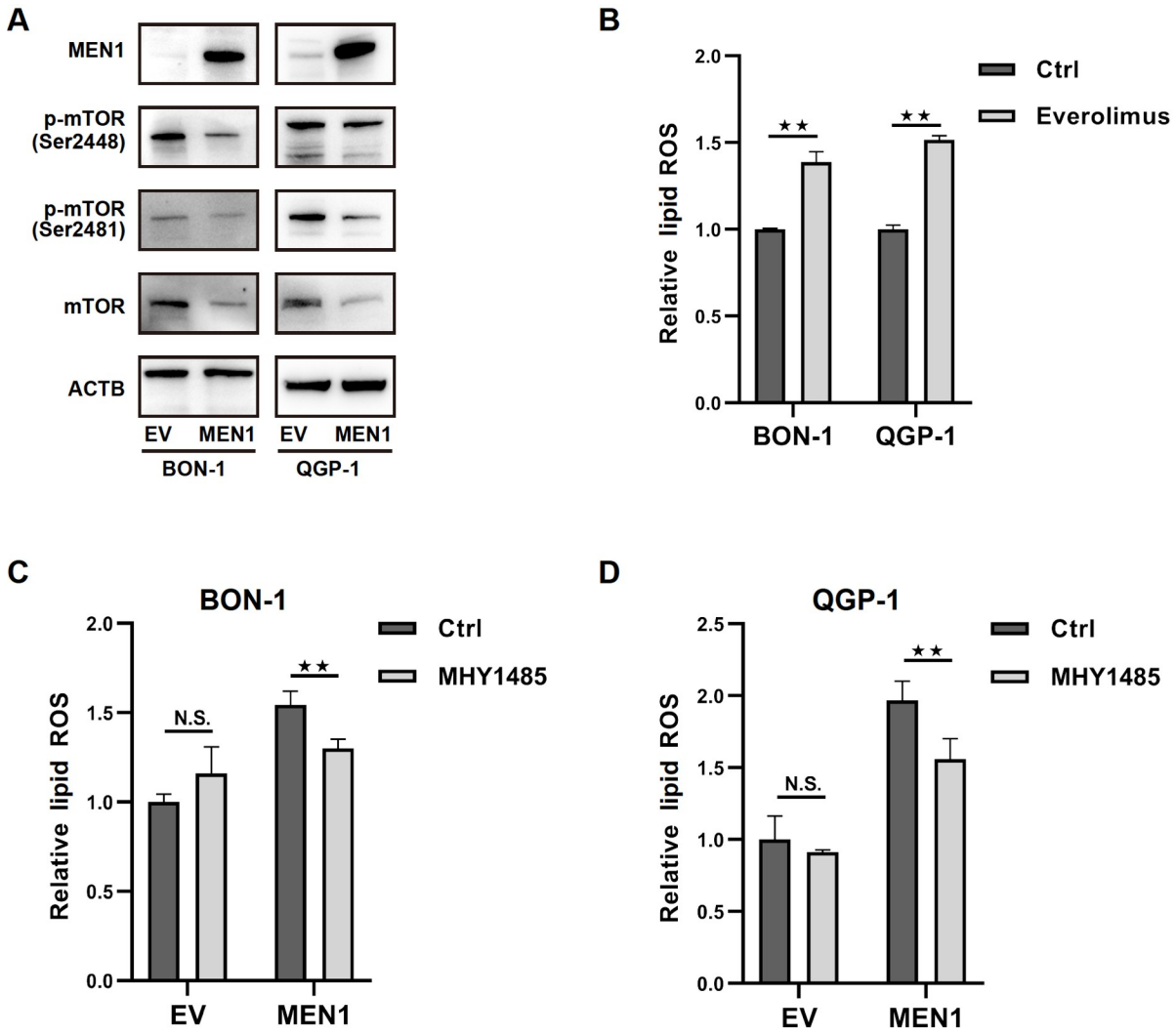

Figure 3. mTOR signaling affects the regulation of lipid peroxidation by MEN1 (A) Western blot analysis of the protein levels of mTOR signaling in BON-1 and QGP-1 cells overexpressing MEN1 or transfected with empty vector. (B) BON-1 or QGP-1 cells were treated with everolimus (10 μg/mL) or DMSO for 48 h and then lipid peroxidation was detected. (C) BON-1 cells were treated with MHY1485 (10 μM) for 48 h and then lipid peroxidation was detected. (D) QGP-1 cells were treated with MHY1485 (10 μM) for 48 h and then lipid peroxidation was detected. $^{**}P < 0.01$.

cells ($P = 0.006$; Figure 7C,D). These results indicated that MEN1 and ferroptosis inducer potentiate the cytotoxic effect of everolimus (Figure 7E).

Discussion

pNETs are heterogeneous tumors arising in pancreatic neuroendocrine system, characterized by a relatively indolent rate of growth [1]. Surgical excision is the main treatment and the 5-year survival following resection is 65% [28]. However, 70% patients suffer with metastasis [29]. *MEN1* mutations are the major driving factor in the occurrence and development of pNETs, because more than 40% of sporadic pNETs and all MEN1 patients possess somatic mutations of the *MEN1* gene [6,30,31]. Current treatments towards to pNETs often do not make a big difference in outcomes [32]. Thus, more attention should be paid to the major driver gene, *MEN1*, and its encoded protein, menin, to better understand the biology characters of pNETs. *MEN1* gene replacement therapy has shown potential use *in vivo* [33,34].

MEN1 encoded protein, menin, is a key scaffold protein, which interacts various partners to regulate gene transcription and interplays with multiple signaling pathways [35]. For example, menin directly interacts with JunD and represses JunD-activated transcription [36]. Menin also directly binds to the promoters of p27 and p18 to increase the methylation of lysine 4 in histone H3 and then promotes the expressions of p27 and p18 to inhibit cell growth [37]. In addition, menin regulates multiple signaling pathways, such as, TGF-β signaling pathway [14], Wnt signaling pathway [38], nuclear receptor signaling pathway [39], Ras signaling pathway [40], Akt and FOXO signaling pathway [26], and hedgehog signaling pathway [41]. Thus, the function of menin is extremely complicated and further studies on menin should be carried out in pNETs.

mTOR signaling is the hub of metabolism regulation, which regulates amino acid, glucose, nucleotide, fatty acid and lipid metabolism [42]. Drugs targeted at mTOR signaling have been

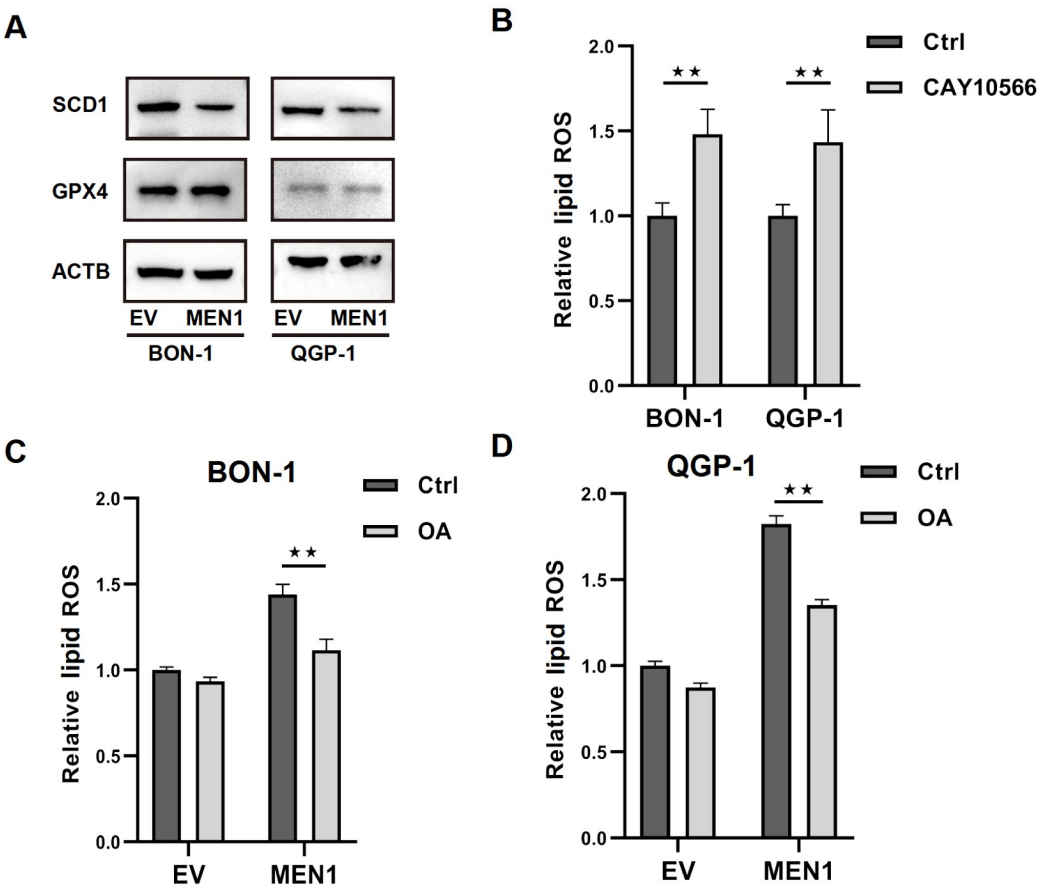

Figure 4. MEN1 promotes lipid peroxidation by inhibiting the expression of SCD1 (A) Western blot analysis of the protein levels of SCD1 and GPX4 in BON-1 and QGP-1 cells overexpressing MEN1 or not. (B) BON-1 or QGP-1 cells were treated with CAY10566 (5 μM) or DMSO for 48 h and then lipid peroxidation was detected. (C) BON-1 cells were treated with OA (80 μM) for 48 h and then lipid peroxidation was detected. (D) QGP-1 cells were treated with OA (80 μM) for 48 h and then lipid peroxidation was detected. $^{**}P < 0.01$.

widely used in cancers as well as pNETs [1,43,44]. It has been reported that MEN1 regulates the mTOR signaling [6]. Thus, we speculated that MEN1 may also affect the metabolism regulated by mTOR signaling. In this study, we focused on the effect of MEN1 on medium and long chain fatty acid metabolism and performed targeted metabolomics analysis to detect the changes. We found that MEN1 increased the level of PUFAs. Increased PUFAs are easy to be attacked by reactive oxygen species to form lipid peroxidation, a prerequisite for ferroptosis. Ferroptosis is a kind of cell death that is characterized by increased intracellular iron and lipid peroxidation [18]. Ferroptosis is now thought to play a bidirectional role in promoting and inhibiting cancer. For example, triggering ferroptosis can promote cancer cell death and inhibit cancer cell growth on the one hand, and promote inflammation-associated immunosuppression in the tumor microenvironment on the other hand [45]. Ferroptosis inducers exhibit great potential in cancer therapy [46]. Thus, in this study, we intended to explore the therapeutic potential of ferroptosis inducer in pNETs. We found that the mTOR-SCD1 signaling play a critical role in the regulation of ferroptosis caused by MEN1. Metabolites of SCD1 as well as activator of mTOR signaling partially rescued MEN1-induced ferroptosis. The negative correlation between MEN1 and SCD1 was further verified in clinical specimens.

mTOR inhibitor everolimus is widely used in the treatment of neuroendocrine tumors. However, the use of everolimus is limited due to the development of resistance [47–49]. In this study, we confirmed that MEN1-overexpressing BON-1 and QGP-1 cells were more sensitive to everolimus, and the combination of everolimus with ferroptosis inducer, RSL3, was more powerful to kill cells. Thus, this study may provide a new strategy for the comprehensive therapy of pNETs. The limitation of this study is that we did not illuminate the regulation mechanism of MEN1 on mTOR signaling and did not perform animal experiments for further verification. We will focus on these issues in the follow-up study.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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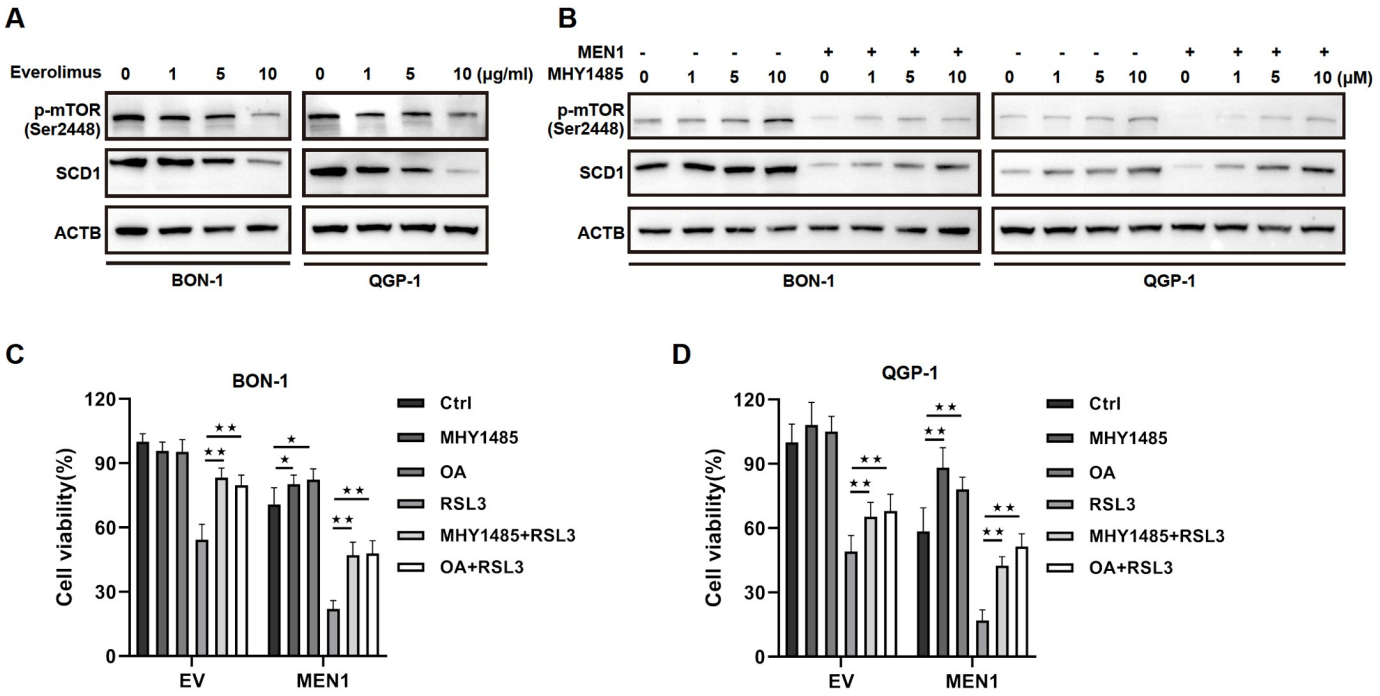

Figure 5. MEN1 promotes lipid peroxidation by inhibiting the mTOR-SCD1 axis (A) Western blot analysis of the protein levels of p-mTOR and SCD1 in BON-1 and QGP-1 cells treated different concentrations of everolimus for 48 h. (B) Western blot analysis of the protein levels of p-mTOR and SCD1 in BON-1 and QGP-1 cells, with MEN1 overexpression or transfected with empty vector, treated with different concentrations of MHY1485 for 48 h. (C) Cell viabilities were detected in BON-1 cells with MEN1 overexpression or transfected with empty vector, which were pretreated with DMSO, MHY1485 (10 μM), OA (80 μM), RSL3 (0.5 μM), both MHY1485 and RSL3, both OA and RSL3, respectively. (D) Cell viabilities were detected in QGP-1 cells with MEN1 overexpression or transfected with empty vector, which were pretreated with DMSO, MHY1485 (10 μM), OA (80 μM), RSL3 (0.5 μM), both MHY1485 and RSL3, both OA and RSL3, respectively. $*P<0.05$, $**P<0.01$.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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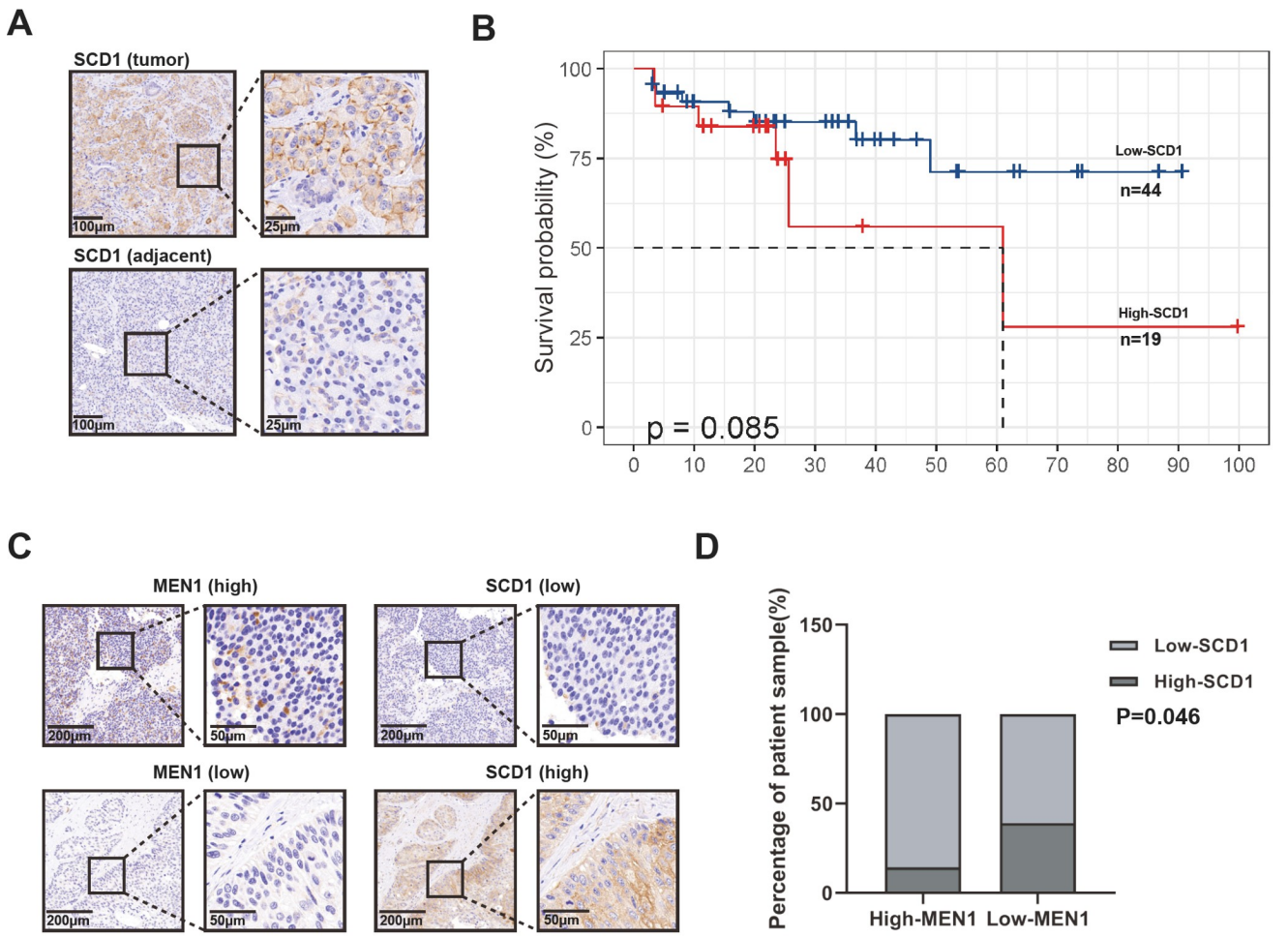

Figure 6. SCD1 is negatively correlated with MEN1 in pNETs samples (A) IHC was performed in tumors and adjacent tissues to detect the level of SCD1. Typical images showing that SCD1 was highly expressed in tumors tissues compared with that in adjacent tissues. (B) Survival analysis was performed according to the expression of SCD1 in tumor samples. (C) IHC was performed in tumor tissues to detect the levels of SCD1 and MEN1. Typical images showing that MEN1 was highly expressed with low SCD1 expression or SCD was highly expressed with low MEN1 expression in tumor tissues. (D) Chi-square test was used to analyze the correlation between SCD1 and MEN1.

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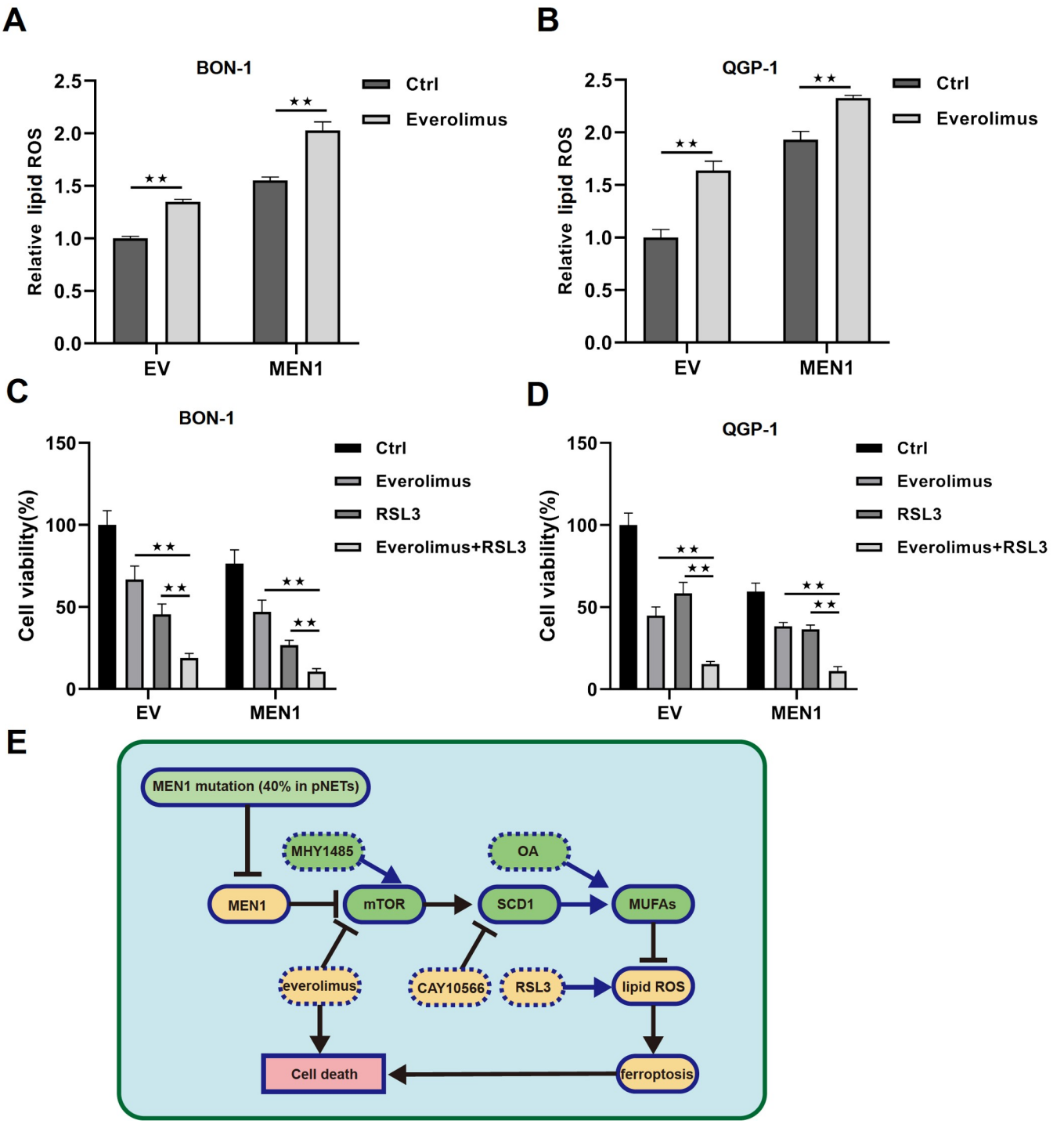

Figure 7. MEN1 potentiates the cytotoxic effect of everolimus (A) Lipid peroxidation was detected in BON-1 cells with MEN1 overexpression or transfected with empty vector, which were pretreated with DMSO or everolimus (10 μg/mL). (B) Lipid peroxidation was detected in QGP-1 cells with MEN1 overexpression or transfected with empty vector, which were pretreated with DMSO or everolimus (10 μg/mL). (C) Cell viabilities were detected in BON-1 cells with MEN1 overexpression or transfected with empty vector, which were pretreated with DMSO, everolimus (10 μg/mL), RSL3 (0.5 μM) or both everolimus and RSL3. (D) Cell viabilities were detected in QGP-1 cells with MEN1 overexpression or transfected with empty vector, which were pretreated with DMSO, everolimus (10 μg/mL), RSL3 (0.5 μM) or both everolimus and RSL3. (E) A comprehensive mechanism pattern showing that MEN1 promotes ferroptosis by inhibiting mTOR-SCD1 axis in pancreatic neuroendocrine tumors and that the MEN1-mTOR-SCD1 axis affects the cytotoxic effect of everolimus. $^{**}P < 0.01$.

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