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Research article

ACOT7 promotes retinoblastoma resistance to vincristine by regulating fatty acid metabolism reprogramming

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A R T I C L E I N F O

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A B S T R A C T

The rate of vincristine (VCR) resistance in the treatment of retinoblastoma (RB) is relatively high, and the exact role and mechanism of autophagy and fatty acid (FA) metabolism in RB are still unknown. The aim of this study was to elucidate the molecular mechanism by which acyl-CoA thioesterase 7 (ACOT7) regulates FA metabolism and autophagy, which may lead to potential therapeutic strategies for RB. In the present study, the relationship between FA metabolism and cellular drug sensitivity was evaluated through ACOT7 overexpression or inhibition tests in RB-resistant cells. The lipase inhibitor orlistat and the autophagy inhibitor CQ were used to determine the effects of ACOT7 on FA metabolism, autophagy, and cellular drug sensitivity, as well as the therapeutic value of ACOT7 targeting. The results showed that ACOT7 was upregulated in VCR-resistant RB cells, significantly enhancing cell resistance and indicating that ACOT7 may serve as a biomarker for VCR resistance in RB cells. Knockdown of ACOT7 inhibited FA metabolism and reduced cell viability in VCR-resistant RB cells. The effect of ACOT7 overexpression was opposite to that of ACOT7 knockdown, and ACOT7 overexpression promoted autophagy in VCR-resistant RB cells. After treatment with orlistat or CQ, FA metabolism in VCR-resistant RB cells decreased, cell viability and autophagy were inhibited, EMT was inhibited, and the sensitivity of RB cells to VCR was increased. In conclusion, ACOT7 knockdown can mediate FA metabolism to inhibit autophagy and the migration of RB cells, thereby improving the sensitivity of RB cells to VCR.

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1. Introduction

Retinoblastoma (RB) is a serious malignant tumor disease that occurs frequently in children [1]. RB originates from the retina, and the most common symptoms are white pupil syndrome (cat eye reflex) and strabismus [2]. The clinical treatments of RB mainly include chemotherapy, radiotherapy, surgery, and laser therapy [3]. The standard combination of chemical drugs for intraocular RB is carboplatin etoposide and vincristine (VCR) [4]. However, the clinical efficacy of VCR in the treatment of RB is usually limited by drug resistance. VCR is an alkaloid extracted from roses that is commonly used as an antitumor drug and widely used in combination chemotherapy for acute lymphoblastic leukemia, neuroblastoma, and RB [5]. The combination of VCR and cisplatin induces apoptosis in the human RB cell lines Y79 and WERI-Rb1 [6]. In clinical practice, VCR requires high doses to achieve good chemotherapeutic effects, but these doses can increase the resistance of cancer cells and have significant side effects on patients [6,7]. Drug resistance in RB patients often leads to relapse and poor prognosis. Although studies have shown that VCR has anti-tubulin and apoptotic effects on cancer cells, the molecular mechanism by which VCR induces drug resistance in RB cells is still unclear. Therefore, elucidating the mechanism of VCR resistance in RB cells and identifying potential biomarkers of VCR resistance in RB cells are important.

Acyl-CoA thioesterases (ACOTs) are hydrolases of long-chain acyl-CoA in the mammalian cytoplasm [8]. Acyl-CoA thioesterase 7 (ACOT7) is a major subtype of the ACOT family and one of the most widely studied ACOTs. Additionally, ACOT7 plays an important role in fatty acid (FA) metabolism [9]. Research has shown that ACOT7 is highly expressed in RB cell lines and is also expressed in primary RB and the retina [10]. High expression of ACOT7 also affects the FA metabolic pathway, which is involved in lipid metabolic reprogramming [11]. Lipid metabolic reprogramming is a marker of tumor formation and can alter the production of biologically active lipid molecules, such as fatty acids (FAs) [12]. FAs are the basic components of cancer cell membrane biology and can serve as a fuel source under metabolic stress conditions, and reshaping FA metabolism in cancer helps create a microenvironment for tumor progression [13]. After nutrients such as FAs are recovered by cancer cells through oxidation, oxidative phosphorylation (OXPHOS) is used to provide energy for the tricarboxylic acid cycle and biosynthesis, a process that is continuously promoted by autophagy triggered by the tumor system [14]. It is currently unclear how ACOT7 reshapes FA metabolism and plays a role in regulating autophagy in RB. However, research has shown a relationship between autophagy and cancer resistance to chemotherapy [15]. Therefore, we hypothesized that ACOT7 can affect FA metabolism and thus play a role in VCR-resistant RB cells.

Autophagy is one of the metabolic pathways involved in cancer dysregulation and involves the process of feeding tumor cells with intracellular components recovered through both autonomous and nonautonomous mechanisms (from local and distant environments). Even in tumor microenvironments established under nutrient-deprived conditions, an increase in autophagic flux can promote the abnormal proliferation and growth of cancer cells [14]. Autophagy makes tumor cells resistant to drugs; therefore, inhibiting this process can increase the cytotoxicity of chemical drugs [16]. The development of drug resistance after chemotherapy mainly stems from the significant metabolic reprogramming of tumor cells, which shapes a more favorable growth microenvironment by allowing the cells to adapt to chemotherapy-induced stress and protect the surrounding matrix. In tumor cells, the activation of autophagy in drug-resistant RB is also a resistance mechanism against adverse factors [17]. Therefore, inhibiting autophagy can serve as a protective mechanism for mediating chemotherapy resistance in RB [16]. Here, we speculated that ACOT7 might play a role in VCR-resistant RB cells by influencing the autophagy level of RB cells and regulating the metabolic pathway of FAs.

To test the above hypothesis, two VCR-resistant RB cell lines, Y79/VCR and SO–Rb50/VCR, were established in this study, and the effects of ACOT7 overexpression or knockdown were investigated to determine the effects of ACOT7 on the proliferation, autophagy and FA metabolism of VCR-resistant RB cells. The results of this study may provide new insights into RB resistance and help in the exploration of new therapeutic approaches.

2. Materials and methods

2.1. Cell culture

RB cell lines (Y79 and SO–Rb50) were purchased from Haodi Otwo Biotechnology Co., LTD (Shenzhen, China). The cells were cultured in medium supplemented with 10% FBS, 50 U/ml penicillin and 50 μg/ml streptomycin in an incubator at 37 °C with 5% CO_2.

2.2. Establishment of drug-resistant cells

Y79- and SO–Rb50-resistant cells were established by increasing the VCR dose. The initial drug dose was 0.1 μM, and the dose was doubled every 10 days for 100 days. The established drug-resistant cell lines were cultured in drug-free medium (complete medium without VCR) for 2 weeks before index detection. The VCR-resistant cell lines were named Y79/VCR and SO–Rb50/VCR.

2.3. Cell transfection

The siRNA constructs si-NC, si-ACOT7 #1, si-ACOT7 #2, si-ACOT7 #3, OE-NC, and OE-ACOT7 were transfected into Y79 and SO–Rb50 cells and VCR-resistant cells using Lipofectamine 3000 (Thermo Fisher Scientific) following the manufacturer's instructions. After 48 h, the transfected cells were collected for drug treatment. The si-ACOT7 #1, si-ACOT7 #2, si-ACOT7 #3, OE-ACOT7, siRNA-NC (si-NC), and OE-NC genes were designed and synthesized by Sangon Biotech, and their sequences were validated through Sanger sequencing. The transfected cells were screened by purinomycin, and the cells were subsequently collected for drug treatment. According to the experimental requirements, a fixed dose (20 μM) of the autophagy inhibitor (CQ) was added for 24 h, or different doses

(25, 50, 100, 200, 400 μM) of the lipase inhibitor orlistat were added.

2.4. Cell viability determination

Cell viability was assessed using a CCK-8 Cell Counting Kit. After 24 or 48 h of drug treatment, the cells were treated with CCK-8 reagent for 2 h and tested at a wavelength of 450 nm. The calculation of cell viability was based on cells that were not treated as 100% viable. The drug resistance index (RI) was calculated as the ratio of the drug-resistant cell IC50 to the parent cell IC50.

2.5. RT–qPCR

The cells were lysed with RNAiso reagent, and total RNA was extracted with an RNAiso Reagent RNA Kit (Takara, Japan) according to the manufacturer's instructions. The extracted RNA was reverse-transcribed into cDNA using a cDNA synthesis kit. Fluorescence-based quantitative PCR was performed using cDNA, and primer design was performed using Primer Premier 5.0 software. GAPDH was used as an internal control. The primers used are shown in Table 1. The results were obtained using the $2^{-\Delta\Delta Ct}$ method.

2.6. Western blot

Total proteins were extracted with RIPA lysis buffer, and the concentrations of the proteins were determined with a BCA protein concentration detection kit (Solarbio, Beijing, China). The proteins were subsequently separated via SDS–PAGE. The isolated proteins were transferred to PVDF membranes and blocked with 5% nonfat milk powder. The membranes were incubated overnight at 4 °C with the following antibodies (Abcam, 1:1000): ACOT7, ELOVL2, FADS1, E-cadherin, N-cadherin, vimentin, LC3, Beclin1, and p62. Then, the membranes were incubated with secondary antibody (goat anti-rabbit IgG (H + L)) (1:6000). After that, the membranes were stained with an enhanced chemiluminescence (ECL) kit (Millipore, USA). Finally, ImageJ software was used to conduct relative quantitative analysis of the bands.

2.7. Quantification of lipid metabolites

According to the manufacturer's instructions, an EnzyChrom FA kit (BioAssay Systems), glycerol kit (Cayman Chemical, Ann Arbor, MI), and lipogenesis colorimetric/fluorescence kit (BioVision, Milpitas, CA) were used to measure FA, glycerol, triacylglycerol (TAG), diacylglycerol (DAG) and monoacylglycerol (MAG), respectively.

2.8. Observation of intracellular lipid droplets using Nile red staining

The cells were fixed with 4% paraformaldehyde for 30 min and stained with 1 ml of Nile red staining solution (Sigma Aldrich, USA) for 20 min. Finally, the cells were observed using a fluorescence microscope.

2.9. LC3B immunofluorescence detection

An LC3 autophagy antibody kit (Thermo Fisher Scientific, US) was used for LC3 single fluorescence detection. The cells were treated according to the experimental requirements, the liquid was removed, and a 2- to 3-mm layer of 100% cold methanol was used to cover the cells, and they were fixed at −20 °C. Then, the cells were washed 3 times with PBS. The washed cells were then blocked with blocking solution for 60 min, the blocking buffer was removed, diluted primary antibody was added, and the cells were incubated at 4 °C overnight. After incubation, the cells were washed again with PBS three times, incubated with the secondary antibody in the dark for 2 h, and washed again with PBS. Finally, a fluorescence microscope was used to observe the cells and capture images.

2.10. Functional enrichment analysis

First, we analyzed a common dataset of mRNA expression data from retinoblastoma tissues, including RNA-seq data from The Cancer Genome Map (TCGA) and microarray data from the GSE26511 and GSE63514 datasets from the Gene Expression Omnibus (GEO). Pearson χ was used to evaluate the relationship between the expression levels of ACOT7, ELOVL2, and FADS1 and clinicopathological features using the Pearson χ^2 test or Fisher's exact test. To determine the correlations between target genes and fatty acid synthesis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted

Table 1
Primer sequences.

Target	Sequence (F: Forward primer, R: Reverse primer)
ACOT7	*F: 5′-ATGGCGCGGGCCGGGCTCATTATT-3′*
	R: 5′-TGGCACCTGTGAGGATGTTTTT -3′
GAPDH	*F: 5′-GCAACTAGGATGGTGGTGGCT-3′*
	R: 5′-TCCCATCCCACGCTCTCATA-3′

on the fatty acid synthesis-related genes. Finally, visualization was performed using the R software package "ggplot2".

2.11. Statistical analysis

GraphPad Prism 8 was used for all the statistical analyses, and the data are expressed as the mean ± standard deviation (SD). To eliminate experimental errors, all the experiments were repeated three times. Student's *t*-test was used for comparisons between two groups, and one-way analysis of variance (ANOVA) was used for comparisons between multiple groups. All the data were verified to be normally distributed. A p value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Expression of ACOT7 in VCR-resistant RB cells

First, the VCR resistance index (IC50) of the cells was tested, and the results are shown in Table 2. These experimental data indicate the successful establishment of VCR-resistant Y79/VCR and SO–Rb50/VCR cells. In addition, the activities of VCR-resistant cells and parental RB cells were compared, and VCR-resistant RB cells were found to exhibit greater viability after treatment with the same dose of VCR (Fig. 1A and B). To test the effect of ACOT7 on VCR resistance in RB cells, RT−qPCR analysis was performed, and the results revealed significant increases in ACOT7 mRNA levels in Y79/VCR and SO–Rb50/VCR cells compared to parental Y79 and SO–Rb50 cells (Fig. 1C and D). Western blot analysis of VCR-resistant RB cells revealed that the ACOT7 protein levels were significantly greater in the resistant cells than in the parental cells (Fig. 1E and F). These data indicate that ACOT7 is upregulated in Y79/VCR and SO–Rb50/VCR cells.

3.2. Enhanced FA metabolism in VCR-resistant RB cells

Bioinformatics analysis revealed that the expression levels of ACOT7, fatty acid desaturase 1 (FADS1), and extremely long-chain fatty acid extender 2 (ELOVL2) were significantly enhanced in RB, and ACOT7 mRNA enrichment was associated with FA synthesis (Fig. 2A and B). To evaluate the role of RB-resistant cells in FA metabolism, the number of Nile red-stained lipid droplets (LDs) in the cytoplasm was calculated. The accumulation of LDs in RB-resistant cells was obviously greater than that in parental cells, and the atrophy of LDs was more pronounced in parental cells (Fig. 2C). In addition, we examined intracellular indicators to evaluate lipolytic activity (glycerol and free fatty acids). The levels of intracellular glycerol and free fatty acids were significantly greater in the RB-resistant cells than in the parental cells (Fig. 2D and E). LDs can store TAG, DAG and MAG, which are neutral oils. Our data showed that Y79/VCR and SO–Rb50/VCR cells had significantly greater amounts of neutral lipids (Fig. 2F). The expression levels of the ELOVL2 and FADS1 proteins were significantly greater in the Y79/VCR and SO–Rb50/VCR groups than in the Y79 and SO–Rb50 groups (Fig. 2G). These data suggest that ACOT7 is involved in FA metabolic reprogramming in RB and that FA metabolism is enhanced in VCR-resistant RB cells.

3.3. ACOT7 knockdown enhanced the sensitivity of resistant cells to VCR

To explore the potential role of ACOT7 in RB cell resistance, specific siRNAs were used to knock down ACOT7 in VCR-resistant Y79/VCR and SO–Rb50/VCR cells. There are many protein subtypes of ACOT7 [18]. Therefore, we tested the knockdown efficiencies of three common subtypes of ACOT7 using the siRNA constructs si-ACOT7 #1, si-ACOT7 #2, and si-ACOT7 #3, in RB cells. Among them, the expression level of ACOT7 was the lowest in the si-ACOT7 #3 group, indicating that the knockdown efficiency of si-ACOT7 #3 was the strongest (Fig. 3A and B). The results of Western blot analysis also confirmed that the knockdown efficiency of the si-ACOT7 #3 gene was most significant in RB-resistant cells (Fig. 3C, D, E); thus, we selected si-ACOT7 #3 as the subsequent research object. Cell viability was determined through CCK-8 assays, and the results showed that, compared to the levels in the Y79/VCR and SO–Rb50/VCR groups, cell viability was significantly lower in the ACOT7 knockdown group (Fig. 3F and G). The above data indicate that ACOT7 expression can enhance VCR resistance in RB cells.

3.4. Knocking down ACOT7 inhibits FA metabolism in VCR-resistant RB cells

To investigate the effect of ACOT7 on FA metabolism in RB cells, we first detected the LDs contents in the cells. The results showed that LDs accumulation decreased after ACOT7 was knocked down (Fig. 4A and B). Glycerol release and free fatty acid levels were

Table 2
IC50 and resistance index of VCR-resistant and parental RB cells.

	IC50 (μM)	Resistance index (RI)
Y79	1.99	–
Y79/VCR	21.00	10.58
SO–Rb50	11.00	–
SO–Rb50/VCR	70.82	6.44

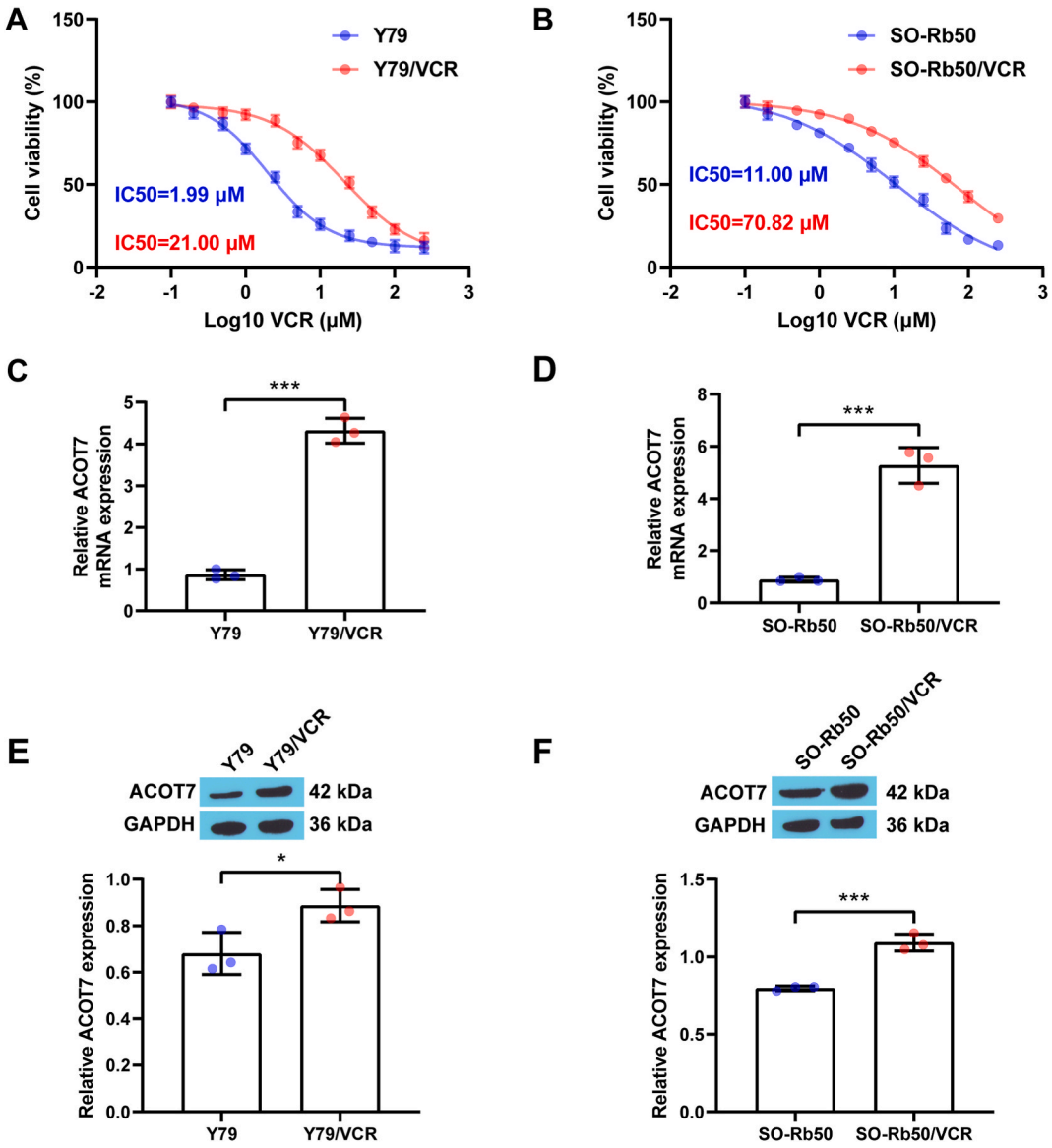

Fig. 1. Expression of ACOT7 in VCR-resistant RB cells.
(A) CCK-8 was used to evaluate the viability of Y79/VCR and Y79 cells, (B) SO–Rb50/VCR cells and their parent cells, SO–Rb50 cells, treated with different concentrations of VCR (n = 3). Cells that were not treated with VCR were used as controls and were considered to be 100% viable. The mRNA and protein levels of ACOT7 in Y79/VCR, SO–Rb50/VCR, and their parental cells were detected using (C–D) RT−qPCR and (E–F) Western blotting. Parental Y79 and SO–Rb50 cells were used as controls; *p < 0.05, ***p < 0.001. Uncropped images of blots were provided in the supplementary material.

measured, and the results showed that, compared with those in the Y79/VCR and SO–Rb50/VCR groups, the intracellular glycerol release and free fatty acid levels in the ACOT7 knockdown group were significantly lower (Fig. 4C, D, E, F). Subsequently, after testing the neutral lipid levels in the cells, it was found that the knockdown of ACOT7 reduced the levels of TAG, DAG and MAG (Fig. 4G and H). These results indicate that knocking down ACOT7 can inhibit FA metabolism in VCR-resistant RB cells.

3.5. ACOT7 promotes RB cell resistance to VCR through FA metabolism

The promotion of FA metabolism in VCR-resistant RB cells by ACOT7 has been validated. We speculate that ACOT7 promotes the resistance of VCR-resistant RB cells through FA metabolism. First, the transfection efficiency of ACOT7 overexpression in Y79/VCR and SO–Rb50/VCR cells was detected by RT−qPCR and Western blotting. Compared with that in the OE-NC group, the overexpression of ACOT7 resulted in significant upregulation of intracellular ACOT7 mRNA (Fig. 5A) and protein (Fig. 5B) expression, suggesting that

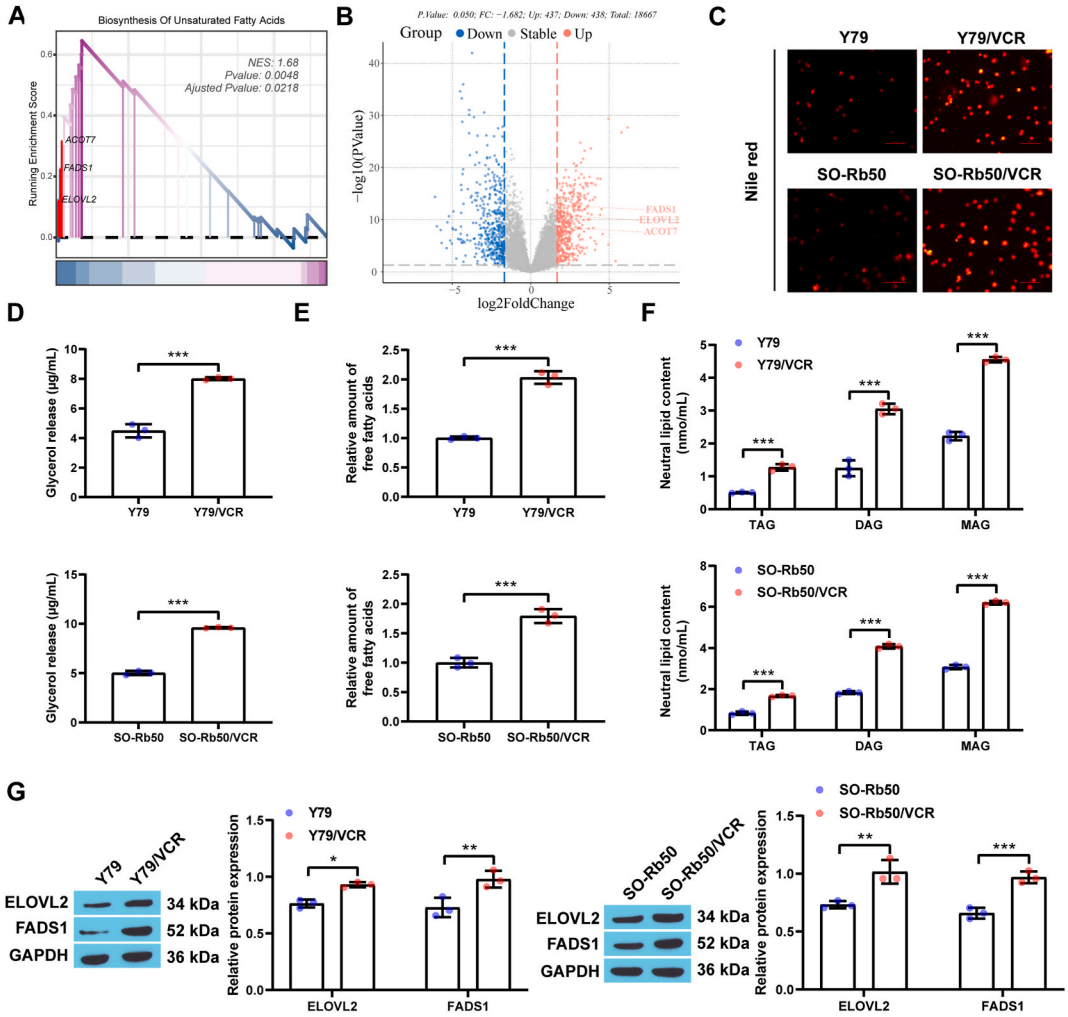

Fig. 2. FA metabolism in VCR-resistant RB cells.
(A) The expression levels of ACOT7, ELOVL2, and FADS1 in RB. (B) The expression levels of ACOT7, ELOVL2, and FADS1 were analyzed in the GEO dataset. (C) Nile red staining was used to detect intracellular lipid droplets. (D) The glycerol contents were measured using a kit. (E) Free fatty acid levels were measured using a kit. (F) Intracellular neutral lipid (TAG, DAG, and MAG) levels were assessed using a kit. (G) Western blot analysis of the expression levels of ELOVL2 and FADS1. *p < 0.05, * *p < 0.01, and ***p < 0.001. Uncropped images of blots were provided in the supplementary material.

the OE-ACOT7 transfection was successful. Subsequently, to evaluate whether FA metabolism has an effect on ACOT7-induced drug resistance in RB cells, the lipase and fatty acid synthase (FASN) inhibitor orlistat was used. The appropriate concentration of orlistat was determined through a CCK-8 assay, and the data showed that orlistat had no significant effect on cell viability at concentrations of 25, 50, and 100 μM, but cell viability was significantly decreased at concentrations of 200 and 400 μM (Fig. 5C). Therefore, we chose 50 μM as the dose for orlistat administration. Kits were used to measure the levels of glycerol and free atty acid, and the results showed that, compared to those in the Y79/VCR and SO–Rb50/VCR groups, the glycerol release and free fatty acid levels were increased in the ACOT7 overexpression group, while orlistat inhibited these upregulations (Fig. 5D and E). Moreover, overexpression of ACOT7 promoted the viability of Y79/VCR and SO–Rb50/VCR cells, while orlistat treatment weakened the effects of ACOT7 overexpression (Fig. 5F). In addition, we examined the effects of ACOT7 overexpression on the viability and FA metabolism of the parental RB cell lines Y79 and SO–Rb50. CCK-8 assay results showed that ACOT7 overexpression promoted the viability of Y79 and SO–Rb50 cells (Fig. S1A). Detection of FA metabolism by kits showed that ACOT7 overexpression increased glycerol release and free fatty acid levels (Figs. S1B and C). These results indicate that ACOT7 promotes the resistance of RB cells to VCR through FA metabolism.

3.6. ACOT7 promotes autophagy and malignant biological behavior in RB cells

We then investigated whether ACOT7 promotes autophagy and migration in VCR-resistant RB cells through reprogramming FA metabolism. First, we detected the levels of proteins associated with autophagy (LC3, Beclin1, and p62) in drug-resistant RB cells. Our

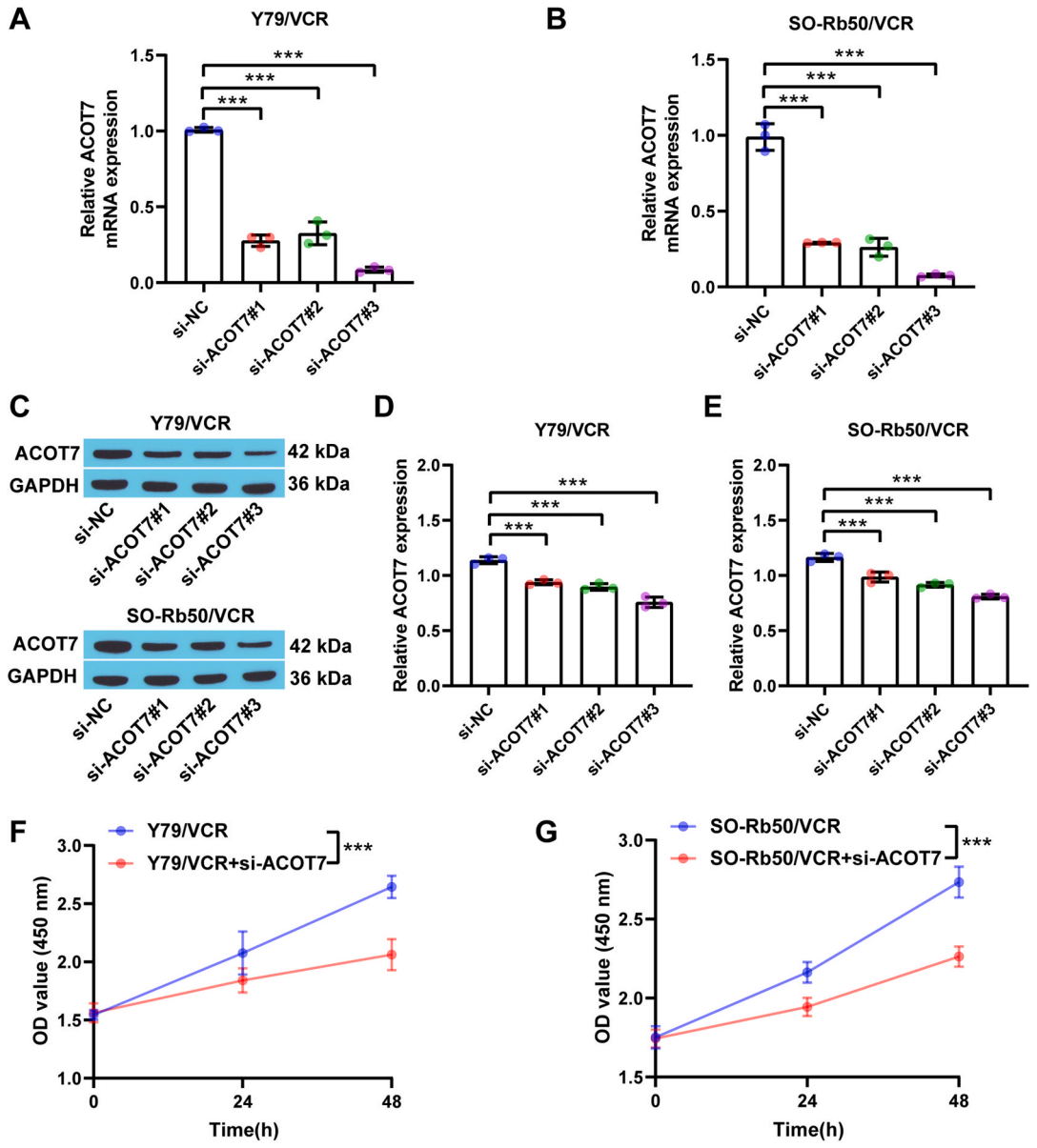

Fig. 3. Knockdown using si-ACOT7 constructs increased the sensitivity of drug-resistant RB cells to VCR.
(A–B) RT−qPCR and (C–E) Western blotting detection of the mRNA and protein levels of ACOT7 in cells. (F–G) CCK-8 detection of cell viability (n = 3). * * *p < 0.001. Uncropped images of blots were provided in the supplementary material.

data showed that OE-ACOT7 promoted autophagy in VCR-resistant RB cells, while orlistat reversed the effect of OE-ACOT7 in drug-resistant RB cells (Fig. 6A and B). Second, OE-ACOT7 promoted LC3B expression in drug-resistant RB cells, while orlistat significantly reduced the ability of OE-ACOT7 to promote LC3B expression in drug-resistant RB cells (Fig. 6C and D). As epithelial–mesenchymal transformation (EMT) is an important factor in cancer cell migration and is related to drug resistance [19,20], further exploration was conducted to investigate the expression of EMT-related proteins (E-cadherin, N-cadherin and vimentin) in drug-resistant RB cells. The data showed that OE-ACOT7 promoted the EMT of VCR-resistant RB cells, thereby promoting their migration, while orlistat reversed the promoting effect of OE-ACOT7 on the EMT of VCR-resistant RB cells (Fig. 6E and F). These results indicate that ACOT7 promotes autophagy and malignant biological behavior in RB cells.

3.7. Inhibition of autophagy weakens the ability of ACOT7 to promote VCR resistance in RB cells

Next, we evaluated the effects of an autophagy inhibitor (CQ) on VCR-resistant RB cells. Cell viability was measured by a CCK-8 assay. The experimental data showed that CQ significantly weakened the ability of OE-ACOT7 to promote the viability of VCR-resistant

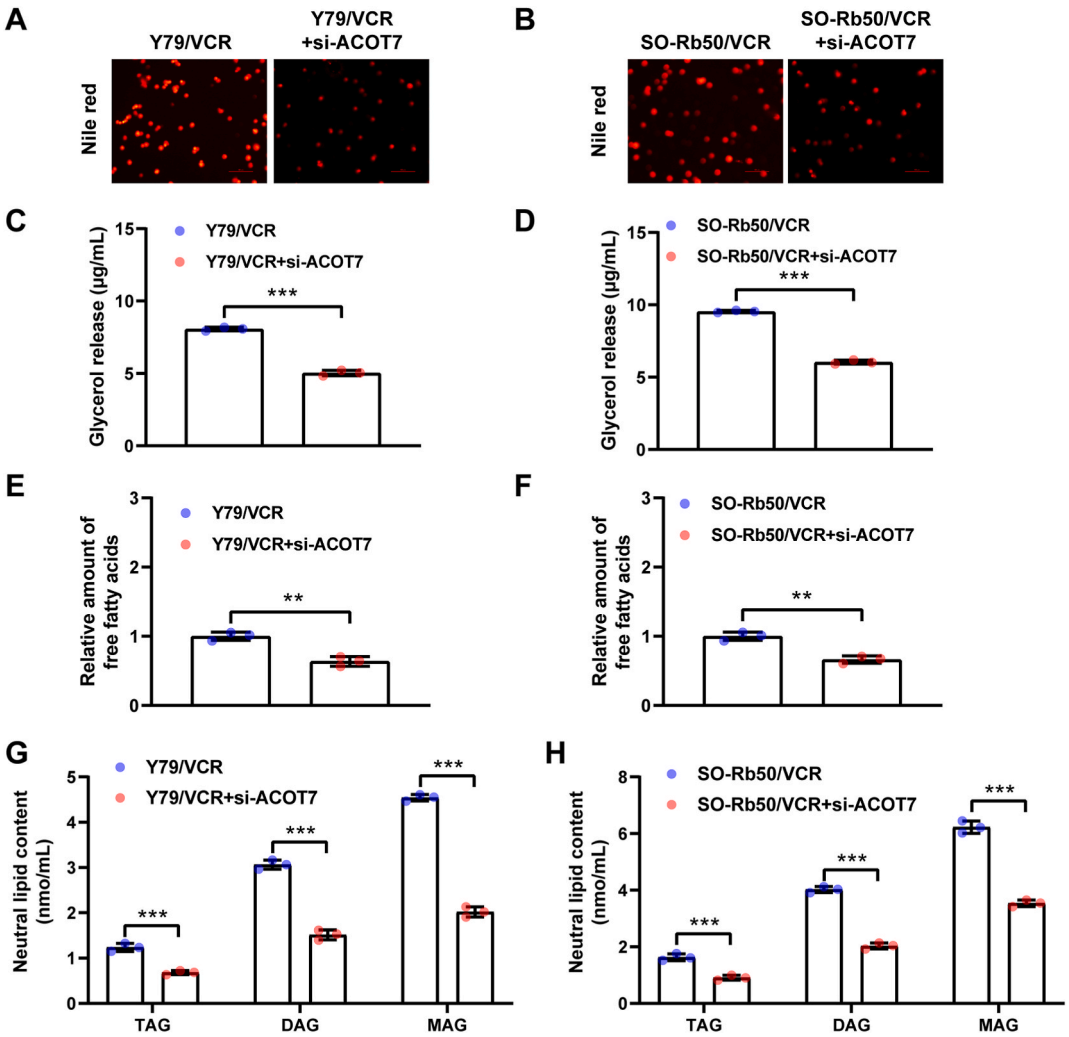

Fig. 4. FA metabolism in VCR-resistant RB cells inhibited by ACOT7 knockdown.
(A–B) Nile red staining for the detection of lipid droplet accumulation. (C–D) Glycerol contents were detected using a kit. (E–F) The free fatty acids were measured using a kit. (G–H) Detection of intracellular neutral lipid (TAG, DAG, and MAG) contents. * *p < 0.01, ***p < 0.001.

RB cells (Fig. 7A and B). In addition, OE-ACOT7 promoted the expression of LC3B in VCR-resistant RB cells, while CQ significantly reduced the ability of OE-ACOT7 to promote LC3B expression in drug-resistant RB cells (Fig. 7C and D). Western blotting was used to detect the expression levels of EMT-related proteins in VCR-resistant RB cells. Experimental data showed that CQ reversed the effect of OE-ACOT7 on the migration of drug-resistant RB cells (Fig. 7E and F). These results suggest that inhibiting autophagy can weaken the effect of ACOT7, inhibit the migration of RB cells, and increase the sensitivity of RB cells to VCR.

4. Discussion

Although treating RB with chemotherapeutic agents plays an important role in improving the disease course, drug resistance is still an important constraint of RB chemotherapy [21]. Therefore, understanding the underlying mechanisms of RB resistance is essential for developing new treatment strategies. In addition, FAs have been found to enhance cancer cell proliferation by providing intermediates essential for maintaining cell membrane structure and function, energy storage, and signal transduction [22]. In this study, we established two VCR-resistant RB cell lines, Y79/VCR and SO–Rb50/VCR, and showed that the FA metabolism of VCR-resistant RB cells was enhanced, which increased the resistance of the cells to VCR treatment. In addition, high doses of VCR also increase the resistance of RB cells and cause side effects on the organism. Moreover, ACOT7 was upregulated in drug-resistant RB cells, providing not only a potential target for VCR resistance therapy but also a potential biomarker for RB resistance diagnosis.

A major mechanism of resistance is the overexpression of certain proteins that eliminate drugs from cancer cells [23]. Importantly, ACOT7 was highly expressed in VCR-resistant RB cells in this study. The HotDog folding structure present in ACOT7 is responsible for catalyzing the conversion of fatty acyl-CoA to free fatty acids and CoA-SH [24]. ACOT7 plays a role in regulating neuronal FA

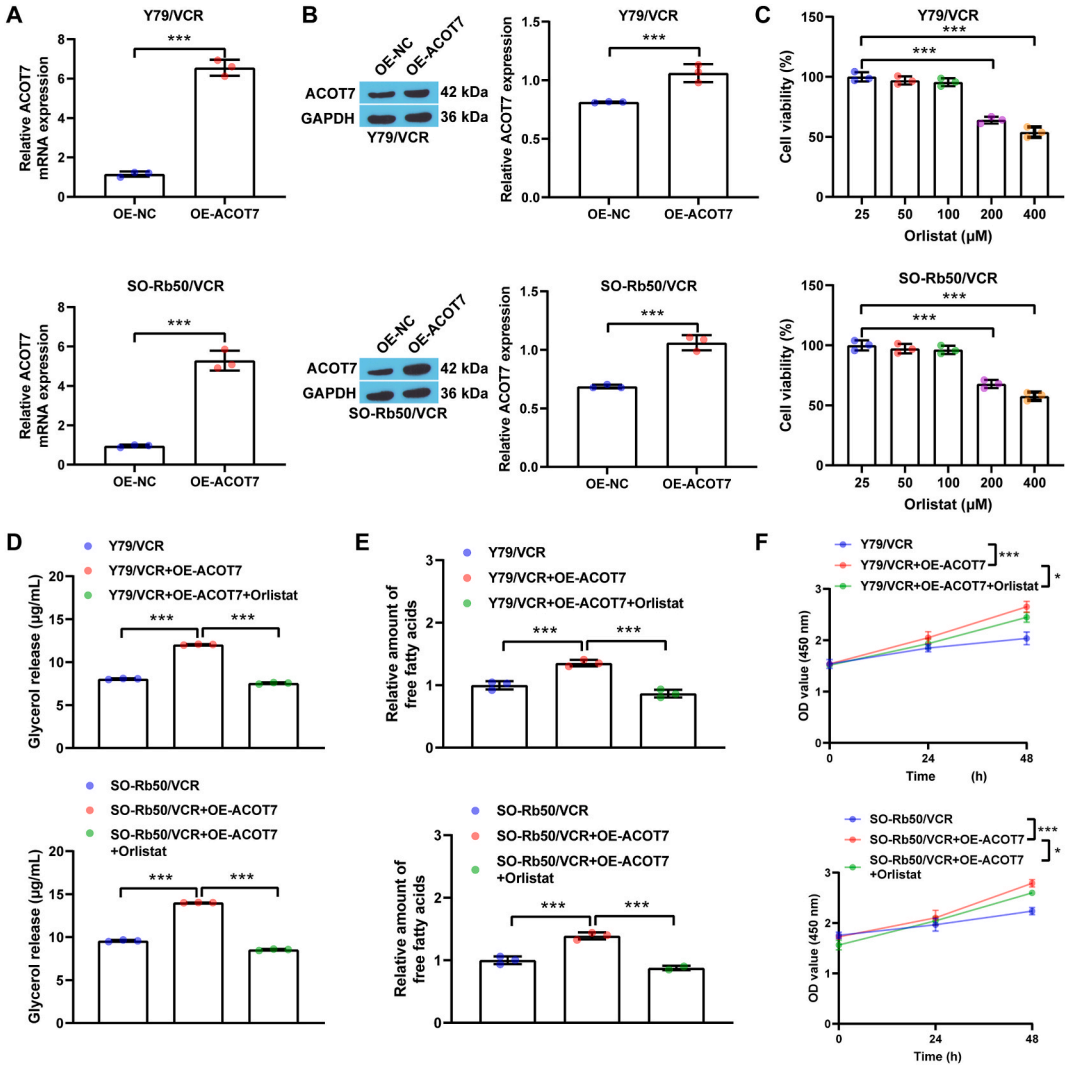

Fig. 5. ACOT7 regulates FA metabolism and promotes the resistance of RB cells to VCR.
(A) RT−qPCR detection of the transfection efficiency of ACOT7. (B) Western blot analysis of the transfection efficiency of ACOT7. (C) The CCK-8 assay was used to determine the optimal concentration of orlistat for each cell line. (D) The glycerol contents were measured using a kit. (E) The free fatty acids were measured using a kit. (F) CCK-8 evaluation of cell viability (n = 3). *P < 0.05, * * *p < 0.001. Uncropped images of blots were provided in the supplementary material.

metabolism to prevent neurotoxicity [25]. FA synthesis and oxidation play crucial roles in tumor occurrence, development, progression, and metastasis [26]. In our study, ACOT7 was found to be abnormally expressed in various cancers through bioinformatics analysis. High ACOT7 expression is significantly associated with poor prognosis in RB patients. ACOT7 expression is also significantly correlated with the release of glycerol and the contents of free fatty acids in cells. Functional experiments in this study showed that ACOT7 knockdown inhibited FA metabolism and cell viability in VCR-resistant RB cells, while overexpression of ACOT7 significantly promoted the resistance of RB cells to VCR, and treatment with the lipase inhibitor orlistat alleviated the effect of ACOT7 overexpression and inhibited FA metabolism in VCR-resistant RB cells. These findings suggested that an imbalance in FA metabolism plays an important role in RB progression and that ACOT7 can regulate FA metabolism and promote the resistance of RB cells to VCR. Therefore, the development of inhibitors targeting FA metabolism in drug-resistant RB cells may lead to new ideas for RB treatment.

Tumor cells are usually associated with increased biosynthesis of FAs, and the accumulation of lipids in cancer cells may be combined with lipolysis [27]. Lipid droplets (indicators of fat autophagy) are selectively targeted to specific organelles by autophagy [28]. Fat autophagy promotes mitochondrial metabolism, providing metabolic fuel for tumor cells, and the synergistic effect of autophagy and mitochondrial metabolism in cancer cells can maintain their survival and proliferation [17,26]. Therefore, autophagy in cancer cells is necessary for lipid droplet breakdown, during which free fatty acids are released at storage sites. Thus, inhibiting autophagic flux can hinder lipid metabolism in cancer cells. Autophagy promotes gene damage and mutation, thereby inducing the formation of retinal cell tumor cells. Further genetic variation leads to malignant transformation and ultimately the formation of

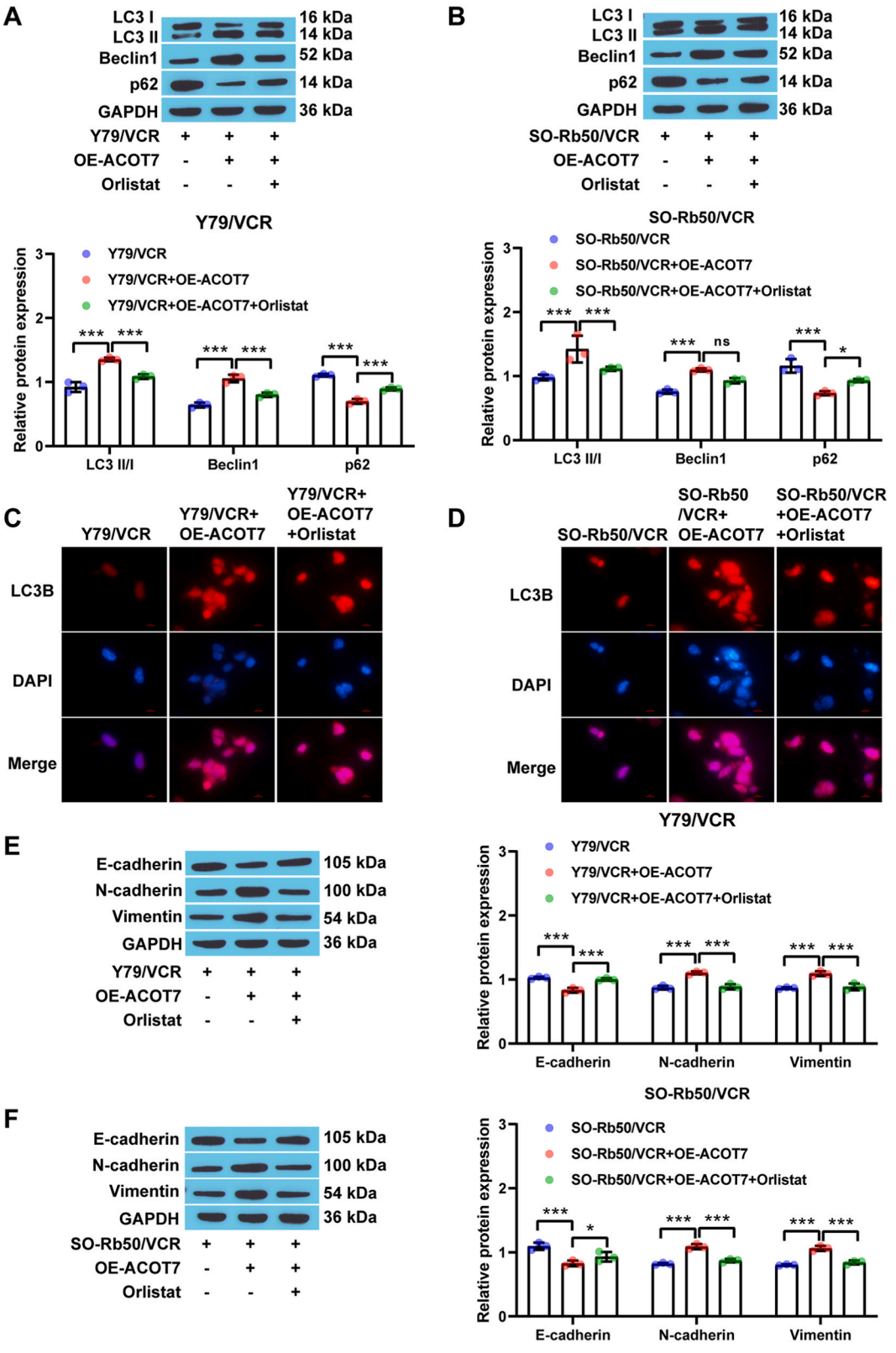

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Fig. 6. ACOT7 regulates FA metabolism and promotes autophagy and EMT in RB cells.
(A–B) Western blot detection of the protein levels of LC3, Beclin1, and p62 in RB cells. (C–D) Immunofluorescence detection of the expression of LC3B. (E–F) Western blotting analysis of the expression levels of E-cadherin, N-cadherin, and vimentin. *p < 0.05, * * *p < 0.001. Uncropped images of blots were provided in the supplementary material.

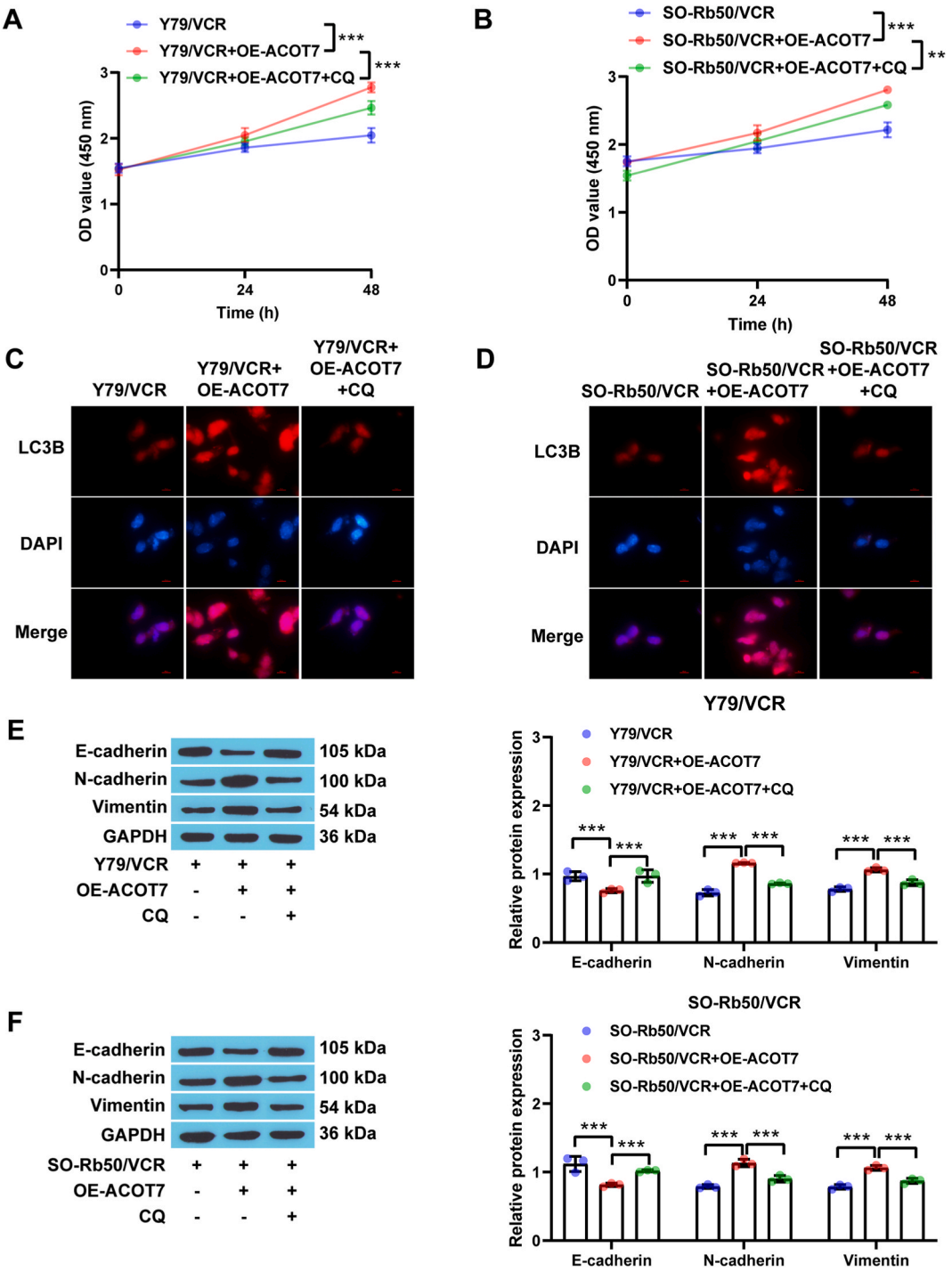

Fig. 7. Inhibition of autophagy weakens the ability of ACOT7 to promote VCR resistance in RB cells.
(A–B) CCK-8 detection of cell viability (n = 3). (C–D) Immunofluorescence detection of the expression of LC3B in cells. (E–F) Western blot analysis of the expression of EMT-related proteins. * *p < 0.01, ***p < 0.001. Uncropped images of blots were provided in the supplementary material.

malignant tumors [29]. Studies have shown that autophagy can regulate the drug sensitivity of tumor cells. Autophagy is an important mechanism of drug resistance and invasion in RB and may be an important cause of poor prognosis [30]. In the present study, overexpression of ACOT7 promoted autophagy in VCR-resistant RB cells, while treatment with the lipase inhibitor orlistat or the autophagy inhibitor CQ promoted the sensitivity of RB cells to VCR, strongly suggesting that autophagy plays a crucial role in the sensitivity of RB cells to VCR therapy.

In summary, our study suggested that ACOT7 can mediate FA metabolism and autophagy in VCR-resistant RB cells and promote VCR resistance in RB cells. Therefore, inhibiting autophagy and reducing the FA metabolic pathway can promote the sensitivity of RB cells to VCR. These studies provide a new basis for the application of chemotherapy in RB treatment. This study has several limitations, as it explains the role of ACOT7 in VCR-resistant RB cells only through cell experiments. In vivo animal experiments are still needed to confirm our in vitro results in the future.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because it included only cell-based experiments.

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CRediT authorship contribution statement

Cairui Li: Data curation, Conceptualization. **Kaiye Dong:** Funding acquisition, Formal analysis. **Yanmei Zhuang:** Methodology, Investigation. **Zhaokui Luo:** Resources, Project administration. **Dong Qiu:** Supervision, Software. **Yingjie Luo:** Validation, Supervision. **Juan Li:** Visualization, Validation. **Dongxia Xing:** Visualization, Resources. **Maicong Ma:** Writing – review & editing, Validation. **Weigang Wu:** Writing – review & editing, Software. **Shuguang Sun:** Writing – review & editing, Writing – original draft, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27156.

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