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ORIGINAL ARTICLE

Inhibition of squalene epoxidase linking with PI3K/AKT signaling pathway suppresses endometrial cancer

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Abstract

Endometrial cancer (EC) is a common malignant tumor that lacks any therapeutic target and, in many cases, recurrence is the leading ca use of morbidity and mortality in women. Widely known EC has a strongly positive correlation with abnormal lipid metabolism. Squalene epoxidase (SQLE), a crucial enzyme in the cholesterol synthesis pathway regulating lipid metabolic processes has been found to be associated with various cancers in recent years. Here, we focused on studying the role of SQLE in EC. Our study revealed that SQLE expression level was upregulated significantly in EC tissues. In vitro experiments showed that SQLE overexpression significantly promoted the proliferation, and inhibited cell apoptosis of EC cells, whereas SQLE knockdown or use of terbinafine showed the opposite results. Furthermore, we found out that the promotional effect of SQLE on the proliferation of EC cells might be achieved by activating the PI3K/AKT pathway. In vivo, studies confirmed that the knockdown of SQLE or terbinafine can observably inhibit tumor growth in nude mice. These results indicate that SQLE may promote the progression of EC by activating the PI3K/AKT pathway. Moreover, SQLE is a potential target for EC treatment and its inhibitor, terbinafine, has the potential to become a targeted drug for EC treatment.

KEYWORDS
endometrial cancer, PI3K/AKT pathway, SQLE, targeted therapy, terbinafine

Abbreviations: IHC, immunohistochemistry; qPCR, quantitative real-time PCR; SQLE, squalene epoxidase; TCGA, The Cancer Genome Atlas; UCEC, Uterine Corpus Endometrial Carcinoma; WB, western blotting.

Liangjian Ma and Wunan Huang are contributed equally to this work.

1 | INTRODUCTION

Endometrial cancer (EC) is a primary epithelial malignant tumor of the endometrium, and has the potential to infiltrate the uterine muscle wall and spread further. EC accounts for 20%–30% of female reproductive tract tumors, with ~320,000 new cases and 76,000 deaths each year globally.[1] The specific pathogenesis of EC remains elusive yet it has been currently reported to be related to estrogen level, metabolic diseases, early age of menarche, fertility, Lynch syndrome, and old age.[2] According to the Bokhman classification, EC can be clinically divided into two types. Type I (estrogen-dependent) is most common, accounting for 80% of EC patients with typically favorable prognosis. Type II (estrogen non-dependent) accounts for less than 10% of EC diagnoses and may have a poor prognosis.[3] In 2013, the classification of EC into four subtypes based on multiple 'omic characteristics and prognostic associations was established by TCGA. This classification is used to evaluate patient prognosis and recurrence. Patients with recurrence after surgical treatment or with severe side effects of chemotherapy more often have a poor prognosis that seriously affects the patient's quality of life.[4] Hence, it is imperative to discover new pathogenic mechanisms of EC to develop novel targeted therapies. Cancer cells undergo metabolic reprogramming to sustain the energy consumption and material required for cell growth, division, and survival. EC also causes many metabolic disorders in patients, such as glucose and lipid metabolism. More recently, studies have reported the dysregulation of lipid metabolism in EC. Lipid metabolism supports tumorigenesis and cancer progression in many ways, including membrane biosynthesis, energy production, and the generation of signal intermediates.[5,6]

In recent decades, numerous studies have shown that cholesterol can promote tumor progression by regulating the proliferation and migration of tumor cells, inhibiting apoptosis, affecting tumor microenvironment, and activating tumor signal pathways, among others.[7,8] This ultimately leads to the progression of malignant tumors including liver cancer, oral squamous cell carcinoma, and cancers of kidney, prostate, and breast.[9–14] Patients with EC often suffer from cholesterol metabolic disorders and abnormal expression of regulatory genes. A high cholesterol level also affects the insulin regulatory axis, thus promoting the progression of EC.[15–17] Fatostatin and BF175, cholesterol level regulator SREBP inhibitors, and commonly used cholesterol-lowering drugs have been proven to inhibit the growth of EC cells.[18–22] Abnormal cholesterol metabolism is mainly manifested by the up-regulation of cholesterol synthesis levels, its increased intake, and the abnormal accumulation of a large number of metabolites.[23] Therefore, we proposed a hypothesis that the key metabolic molecules, such as enzymes, participating in cholesterol synthesis may play a role in the occurrence of EC.

Squalene epoxidase is an important cholesterol synthase encoding gene 8q22.1 in the human genome. It oxidizes squalene to 2,3-epoxysqualene in the endoplasmic reticulum.[24,25] Previous studies have suggested that SQLE was an oncogenic factor and was closely related to the development of malignant tumors such as lung squamous carcinoma, liver cancer, colon cancer, bladder cancer, oral cancer, and prostate cancer.[26,27] SQLE inhibitors, such as terbinafine and NB-598, can significantly inhibit tumor growth.[28-30] However, the biological mechanism of SQLE in EC has not been systematically elucidated. Therefore, the current study aimed to investigate the relationship between SQLE and the occurrence of EC using TCGA, clinical specimen analysis, and validation experiments. The PI3K/AKT pathway is one of the classical tumor signaling pathways, it has been proven in many studies that the activation of this pathway will promote physiological mechanisms such as the proliferation and migration of tumor cells.[31,32] Moreover, the PI3K/AKT pathway has been found to affect the progression of EC. A recent study has found that SQLE can activate PI3K/AKT pathway to promote the occurrence of nasopharyngeal carcinoma.[29] Therefore, we hypothesized that the critical gene, *SQLE*, of the cholesterol pathway, might activate the PI3K/AKT pathway, thereby promoting EC progression.[33,34]

Here, we reported that the expression of SQLE was significantly elevated in EC. SQLE can promote the proliferation, migration, and the inhibition of apoptosis of EC cells by activating the PI3K/AKT signaling pathway. In addition, we found that terbinafine, an inhibitor of SQLE, could significantly inhibit the viability of EC cells and form a tumor-forming ability in nude mice. Therefore, we concluded that SQLE is an oncogenic factor of EC and that terbinafine has the potential to be the targeted therapeutic drug for EC treatment.

2 | MATERIALS AND METHODS

2.1 | Data source

The database was derived from a tumor TCGA SQLE and various tumor gene expressions (https://cancergenome.nih.gov/), downloading mRNA expression data and clinical information for 587 patients, 552 of whom had EC, and 35 had normal endometrium tissues. The BioPortal for Cancer Genomics (https://cbioportal.org) was used to download the gene data for the EC patient group, and the relationship between the SQLE expression level and EC was analyzed using R and Perl software.

2.2 | Clinical samples

All experiments involving patients were approved by the Ethics Committee for Human Research, Lanzhou University (Ethics number: LDYYLL2021-241). Informed consent was obtained from all patients. Women diagnosed with EC were recruited for the study. No local or systemic treatments were administered to these patients before surgery. Matched tumor and its adjacent para-tumor tissues were extracted from eight EC patients at the Department of Gynecology, The First Hospital of Lanzhou University. The tissues were frozen and stored at −80°C.

2.3 | Immunohistochemistry

Paraffin slides of tumors collected from humans were used. SQLE signal was assessed using an anti-SQLE Ab (ab833; Abcam). The

percentage of positive staining cells in the total number of tumor cells was calculated using Image J software.

2.4 | Cell culture and transfection

The Ishikawa and KLE cell lines were purchased from the China Center of the ATCC and cultured in DMEM/F12-DMEM (Service bio) with 10% FBS (Gibco) at 37°C in 5% CO_2.

2.5 | Plasmids and stable cell lines construction

A total of Ishikawa 2×106 cells were added to a 6-well plate, and 2 mL of DMEM was added to each well. After 24 h, plex-EGFP-SQLE and shSQLE lentivirus (Table S2, ObiO Technology) were added to the plate, respectively. After the virus was introduced to the cells for 20 h, the medium was replaced. After 72 h of virus infection, the cells were observed under a microscope to confirm that the lentivirus was successfully transduced into the target cells. After a 3-day puromycin (Gibco) treatment, the uninfected cells were removed. Transduction efficiency was determined using WB.

2.6 | Cell proliferation assay

The CCK8 kit assay (Yeasen, 40206ES76) was used to measure cell proliferation of the KLE and Ishikawa cells. The cells were seeded onto the 96-well culture plates with a 5×10^3 cells/well density. After incubation for a given time, 10 μL of CCK8 solution was added to each well with 100 μL fresh medium and the cells were cultured for another 1 h. Cell proliferation ability was determined by measuring the absorbance at 450 nm using a microplate reader. The experimental time intervals were 0, 1, 2, 3, and 4 days for the KLE and Ishikawa cells.

2.7 | Colony formation assay

A colony formation assay was performed to assess the dimensions and numbers of colonies. The cells were seeded (1×10^3 cells/well, depending on the cell type) into 6-well plates. After being treated with different concentrations (0, 30, and 60 μM) of terbinafine hydrochloride for 14 days, drug-containing medium was removed, and the cells were washed twice with PBS. The colonies were fixed in 4% paraformaldehyde and stained with 0.5% crystal violet. Only colonies with more than 50 cells per colony were counted.

2.8 | Migration assay

The migration assay was performed using transwell inserts (Costar; 8 mm pore size) in 24-well dishes. After treatment with terbinafine for 24 h, the cells were harvested and seeded into transwell inserts at 2×10^4 cells/well in serum-free medium and then incubated for 24 h at 37°C in 5% CO_2. Five random fields of each membrane were chosen to be photographed and counted using an inverted phase-contrast microscope. All experiments were carried out in triplicates.

2.9 | Cellular ACE inhibitory assay

CCK-8 kit assay was used to determine the cytotoxicity of terbinafine hydrochloride toward the KLE and Ishikawa cells. Terbinafine hydrochloride was dissolved in DMSO to make a 10 mM mother liquid. Cells with a density of 5×10^3 cells/well were counted and seeded onto 96-well plates. Outer wells were left unused to avoid edge effects. After adhering for 24 h, the medium was sterilely aspirated and replaced with 100 μL of fresh medium containing terbinafine or DMSO serially diluted at concentrations ranging from 1 to 400 μM. After 48 h, the medium was carefully removed, and 110 μL of freshly prepared CCK8 solution (10 μL of CCK8 premixed with 100 μL of complete medium) was added to each well. After 1 h, the absorbance of each well was then measured at 450 nm using a microplate reader. IC_{50} values were calculated as the concentration of each compound required for 50% inhibition of cell growth relative to the no compound controls (GraphPad Prism 8.0).

2.10 | Apoptosis assay

Cells were seeded into 12-well plates. After the cells were collected, the cells were strictly protected from light, dyes were added, and the apoptotic ability of the cells was detected by flow cytometry.

2.11 | RNA extraction, quantitative real-time PCR

Total RNA was extracted from the cells using a TIANGEN RNAprep Pure Cell Kit or from EC tissue powders using TRIzol reagent, according to the manufacturer's protocols. Reverse transcription was performed with a PrimeScript™ RT Master Mix using 1 μg total RNA. The qPCR reactions were run with EvaGreen Dye or Hieff qPCR SYBR Green Master Mix in triplicate on an MX3005P cycler. The specific primers used for qPCR were the same as those described previously. Relative mRNA levels were determined using the comparative Ct method and normalized against the β-actin mRNA level. A SYBR green assay was used for mouse genes: *SQLE* (primers: forward 5′-CTCTCCTACCGCTGTCGCC-3′ and reverse 5′-GGTTCCTT TTCTGCGCCTCC-3′) and *β-actin* (primers: forward 5′-GTTGTCGA CGACGAGCG-3′ and reverse 5′-GCACAG AGCCTCGCCTT-3′).

2.12 | Western blotting

The tissue powders and cultured cells were lysed with RIPA lysis buffer supplemented with 10 μL/mL PMSF and cocktail. Protein

concentrations were determined using a Bicinchoninic Acid (BCA) Protein assay kit. Equal amounts of protein were separated by SDS-PAGE and then transferred to PVDF membranes. Then, the membranes were blocked with 5% non-fat milk at room temperature for 1.5 h before being incubated with the appropriate primary antibodies at 4°C overnight. The membranes were subsequently incubated with the appropriate HRP-conjugated secondary antibodies for 1 h at room temperature, then visualized using an Enhanced Chemiluminescence Detection kit and photographed under a Bio-Rad Chemidoc MP imaging system.

2.13 | In vivo tumorigenicity

Ncr-nu/nu-nude mice were provided by Shanghai SLAC Laboratory Animals company. The protocols were performed after approval by the Animal Ethics Committee of Lanzhou University, according to issued guidelines. In total, 5×10^6 cells were injected subcutaneously into the armpit of 6-week-old female Ncr-nu/nu-nude mice. To explore the effect of the inhibitor terbinafine, nude mice were given terbinafine at a dose of 80 mg/kg per day for intragastric administration. Tumor sizes were monitored using digital vernier calipers, and tumor volumes were calculated using the formula (Volume = length × width^2×0.5). Finally, the expression of SQLE and ki67 protein was detected in tumor tissues.

2.14 | Statistical analysis

GraphPad Prism 8 was used for statistical analysis. Data are represented as the mean ± SEM of three independent experiments. Differences between groups were assessed through a one-way analysis of variance. A p-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | SQLE is upregulated in EC tissues

To investigate the expression of SQLE in EC patients, we conducted a comparative analysis of 552 cases of EC tissues and 35 cases of normal endometrial tissues (including 23 pairs of cancer and adjacent normal tissues) from the TCGA database. Our findings revealed that the expression of SQLE mRNA in cancer tissues was significantly higher compared with normal endometrial tissues (Figure 1A). Furthermore, the expression level of SQLE mRNA in cancer tissues was significantly higher than that in the adjacent normal tissues (Figure 1B). Our analysis of SQLE expression in various TCGA molecular subtypes revealed that its expression was the most elevated in the CN-high group, which is associated with poor prognosis (Figure 1C). To validate this finding, we conducted qPCR (Figure 1D), IHC (Figure 1E,F), and WB (Figure 1G,H) to detect SQLE mRNA and protein expression levels in cancer and adjacent normal tissues of EC patients. The results indicated that tumor tissues had significantly higher levels of SQLE expression than adjacent normal tissues, suggesting that SQLE overexpression is a common occurrence in EC.

3.2 | Overexpression of SQLE leads to increased proliferation and migration of EC cells, while simultaneously reducing apoptosis

To investigate the oncogenic effect of SQLE in EC cells, stable SQLE overexpression, and vector cell lines were generated in EC cell lines. The efficiency of stable overexpression was confirmed by qPCR and WB (Figure 2A,B). CCK8 assays revealed a significant increase in cell proliferation in SQLE cells compared with vector cells after 48 h of culture (Figure 2A,B). The colony formation assay showed a significant increase in cell cloning ability in SQLE cells compared with vector cells after 10 days of culture ($p<0.01$) (Figure 2C). Additionally, the transwell experiment showed a significant increase in cell migration proportion in SQLE cells compared with vector cells ($p<0.001$) (Figure 2D). The study conducted showed that overexpression of SQLE led to a significant decrease in the apoptosis rate of EC cells. The western blot results also indicated an increase in the expression of the apoptosis-related protein BCL-2, while the expression of Bax and caspase-3 significantly decreased after SQLE overexpression (as shown in Figure 2E,F). These findings suggest that SQLE may promote the growth and migration of EC cells while simultaneously reducing apoptosis in vitro.

3.3 | Knockdown of SQLE expression resulted in decreased proliferation and migration of EC cells while increasing apoptosis

To investigate the impact of SQLE knockdown on EC development, we created stable SQLE knockdown (shSQLE) and vector cell lines within EC cell lines. qPCR and WB confirmed the stable knockdown efficiency (Figure 3A,B). CCK8 assays revealed a significant decrease in cell proliferation in shSQLE cells compared with vector cells after 48 h of culture ($p<0.05$) (Figure 3A,B). Additionally, colony formation assays showed a significant reduction in cell cloning ability in shSQLE cells compared to vector cells after 10 days of culture (Ishikawa, $p<0.001$; KLE, $p<0.01$) (Figure 3C). The transwell experiment also demonstrated a significantly reduced migration cell proportion in SQLE-KD cells compared with in SQLE-vector cells (Ishikawa, $p<0.001$; KLE, $p<0.01$) (Figure 3D). Moreover, the results of flow cytometry indicated that the knockdown of SQLE significantly increased EC cell apoptosis (Figure 3E). Western blot analysis revealed that the expression levels of apoptosis-related proteins Bax, Bcl-2, and cleaved caspase3 were affected by SQLE downregulation, with Bax and cleaved-caspase3 expression increasing and Bcl-2 expression decreasing (Figure 3F). Overall, our findings suggest that the downregulation of SQLE inhibits the proliferation and migration of EC cells while promoting apoptosis.

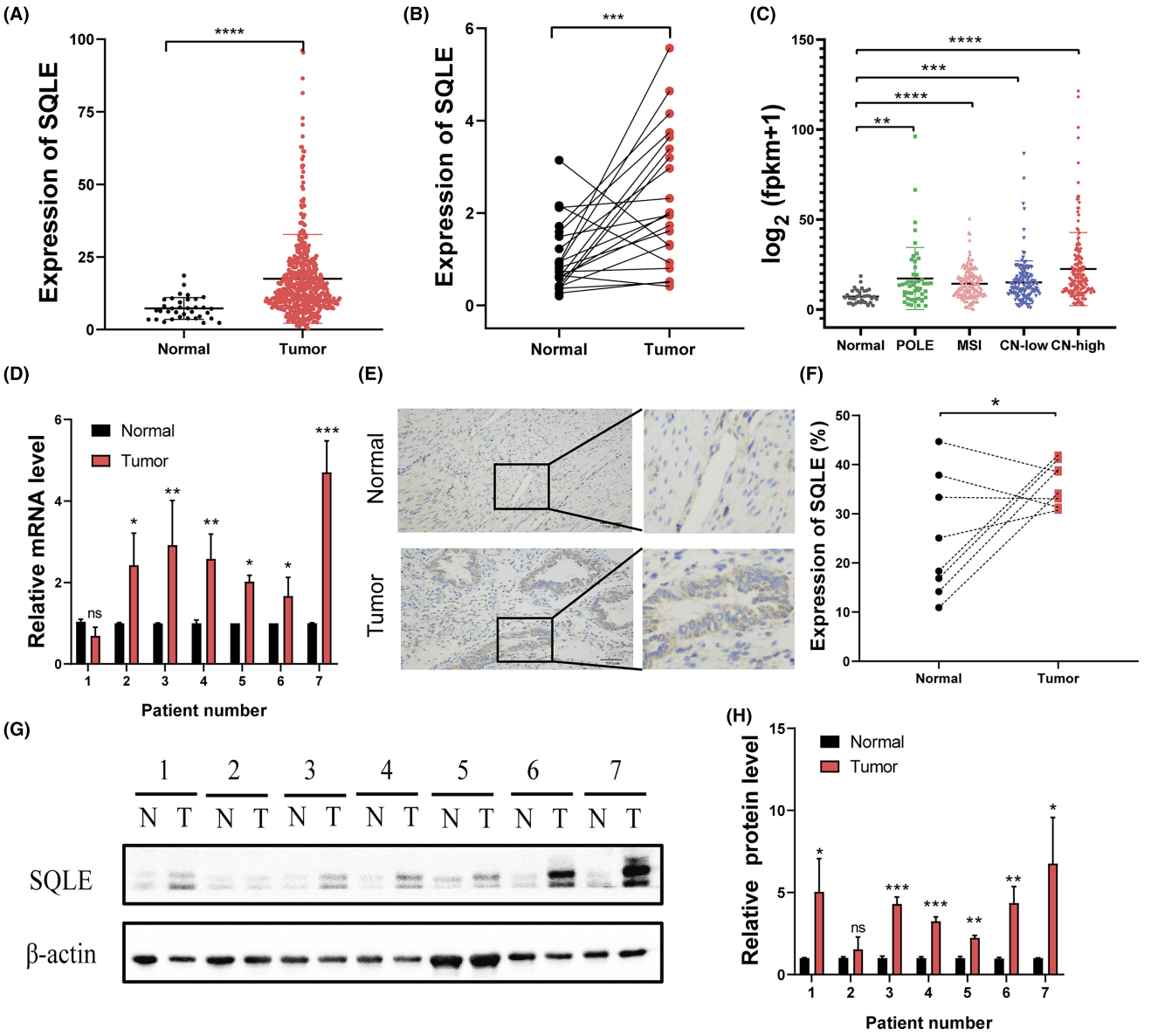

FIGURE 1 SQLE is upregulated in EC tissues. (A) SQLE mRNA expression in 552 EC patients and 35 normal tissues from TCGA data. (B) SQLE protein expression in 23 pairs of cancer and adjacent normal tissues from TCGA data. (C) SQLE mRNA expression in different TCGA molecular types. (D) SQLE mRNA expression in an EC sample. (E) IHC results of SQLE protein expression in cancer and adjacent tissues of EC patients (×200 and ×400 magnification). (F) Statistical diagram of IHC results. (G) Western blot results of SQLE protein expression in EC samples. (H) Statistical diagram of WB results. N, normal tissue; T, tumor tissue. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.001$.

3.4 | Pharmacological inhibitor of SQLE inhibits EC cell proliferation and migration while increasing apoptosis

To further support the significance of SQLE as a carcinogen in EC, we conducted an investigation on the impact of the SQLE enzyme inhibitor terbinafine on EC cells. Initially, we determined the IC_{50} of terbinafine in EC cells, which was found to be 70.15 μM in the Ishikawa cells and 73.72 μM in the KLE cells, as demonstrated by CCK8 results (Figure 4A). The CCK8 assay also showed that the proliferation of EC cell lines was significantly inhibited in a dose-dependent manner by terbinafine (0, 30, and 60 μM), with a noticeable increase in concentration ($p<0.001$) (Figure 4B), Meanwhile, the inhibitory effect on hEEC cells was significantly lower than that of EC cells (Figure S1). Furthermore, we evaluated the activity and migration of EC cells through clone formation and transwell assays. We observed that the activity and migration of EC cells were significantly suppressed at 30 μM, with almost 100% inhibition at 60 μM ($p<0.001$) (Figure 4C,D). Additionally, we utilized the flow cytometry assay to identify apoptosis and observed that terbinafine could induce the apoptosis of EC cells (Figure 4E). Additionally, western blot analysis of apoptosis-related proteins revealed a decrease in Bcl-2 expression after terbinafine treatment, whereas the expression of Bax and cleaved caspase 3 increased (Figure 4F). Our findings suggest that SQLE is a viable target in EC and that terbinafine can effectively inhibit the activity of EC cells.

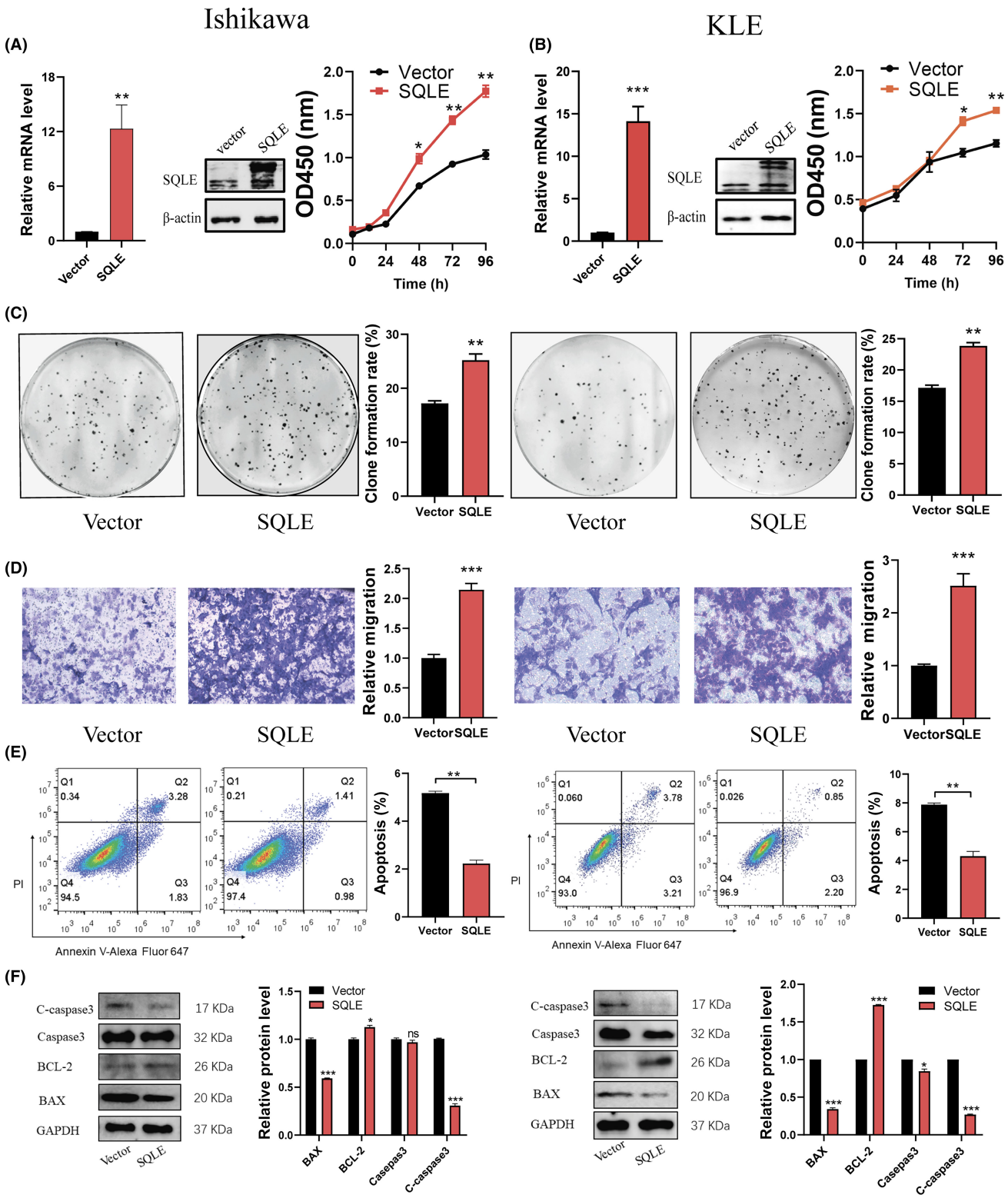

FIGURE 2 Overexpression of SQLE promotes EC cell proliferation, and migration and reduces cell apoptosis. (A–C) qPCR and WB successfully confirmed the overexpression of SQLE in Ishikawa and KLE cells, and overexpression of SQLE promotes EC cell growth. (D) Overexpression of SQLE promotes EC cell migration. (E, F) Overexpression of SQLE inhibits EC cell apoptosis and quantitative grayscale analysis of all western blot bands using ImageJ software. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

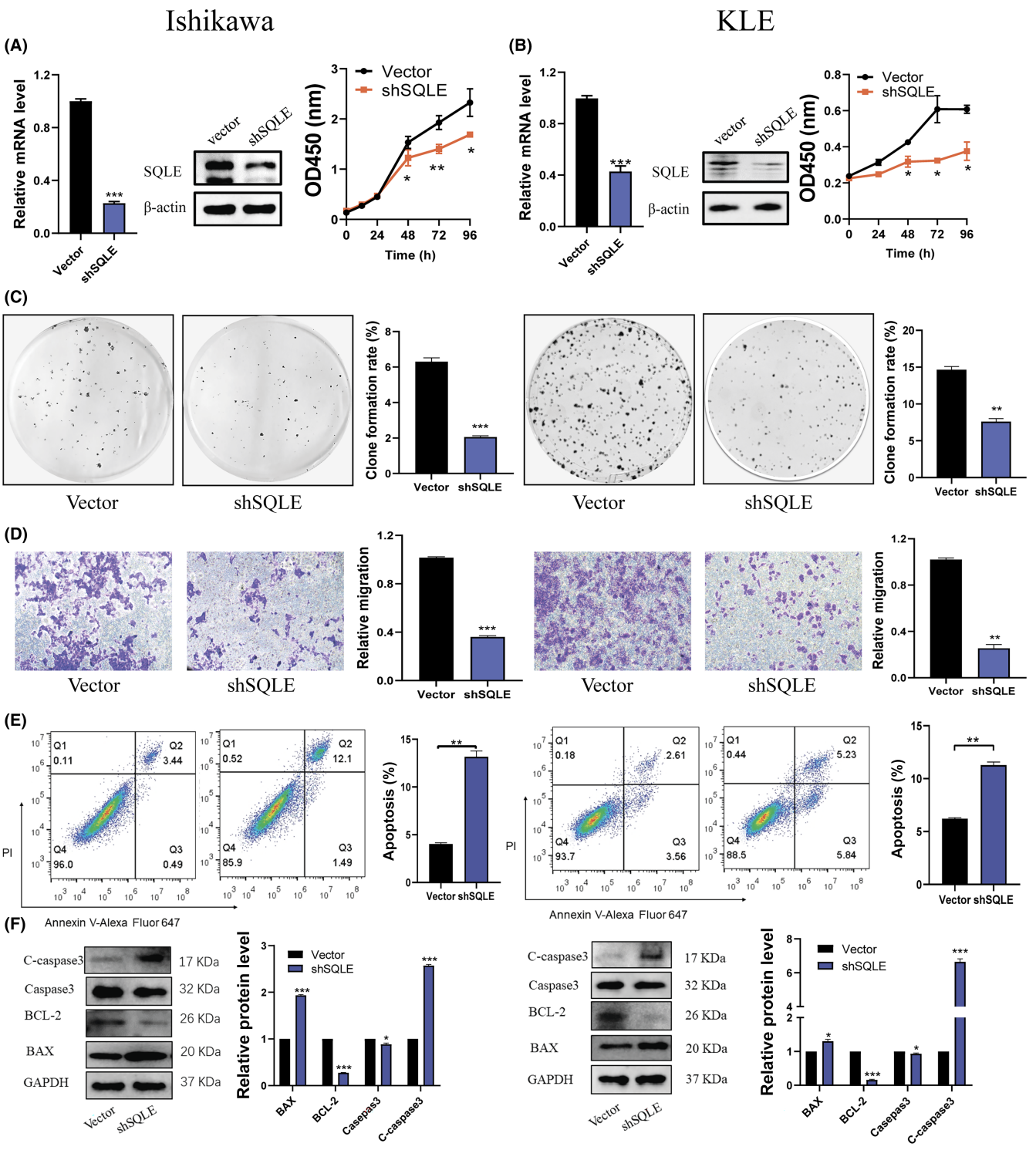

FIGURE 3 Knockdown of SQLE significantly reduces EC cell proliferation, and migration and promotes cell apoptosis. (A–C) qPCR and WB successfully confirmed the knockdown of SQLE in Ishikawa and KLE cells, and knockdown of SQLE suppresses EC cell growth. (D) Knockdown of SQLE suppresses EC cell migration. (E, F) Knockdown of SQLE promotes EC cell apoptosis and quantitative grayscale analysis of all western blot bands using ImageJ software. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

3.5 | SQLE may promote EC cell viability and migration by activating the PI3K/AKT pathway

In order to investigate the molecular mechanism of the carcinogenic effect of SQLE, we analyzed the protein expression of the PI3K/AKT pathway in tumor tissues. Our WB results showed that, compared with adjacent normal tissues, the expression level of p-AKT was higher in the tumor tissues of four patients (Figure 5A). Additionally, we treated EC cells with the AKT activator SC79 after SQLE knockdown and observed that SC79 reversed the effect of

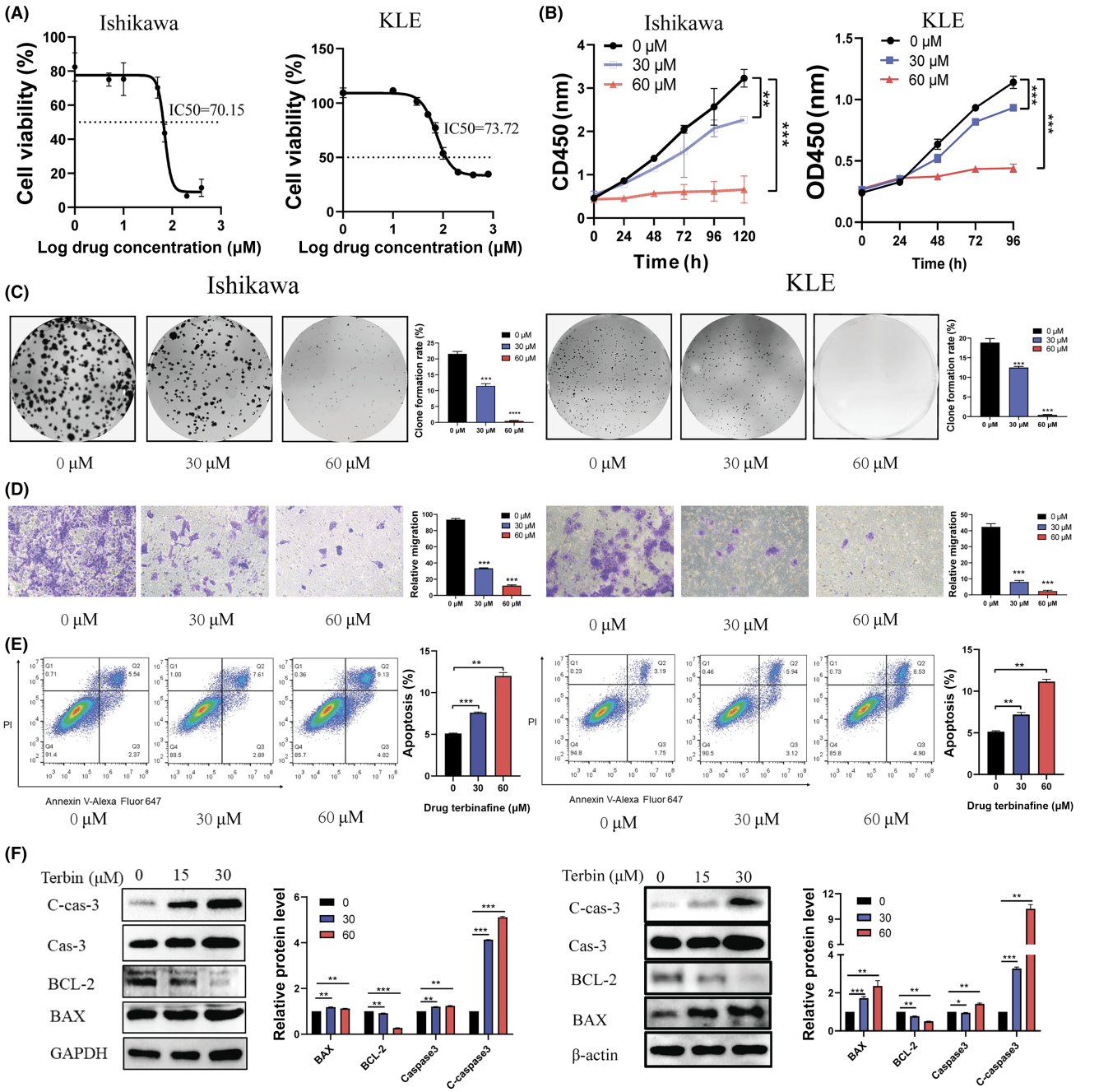

FIGURE 4 Terbinafine significantly inhibited EC cell proliferation, and migration and promotes cell apoptosis. (A) The results of CCK8 reflected the IC50 of terbinafine in EC cells. (B) The results showed that terbinafine significantly inhibited the proliferation of EC cells. (C) Clone formation experiment showed that the activity of EC cells was significantly inhibited by terbinafine. (D) Transwell experiment showed that the migration of EC cells was significantly inhibited by terbinafine. (E) Flow cytometry analysis found that terbinafine can promote the apoptosis of EC cells. (F) SQLE inhibitor terbinafine can alter the expression of apoptosis-related proteins. Quantitative grayscale analysis of all western blot bands using ImageJ software. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

shSQLE on EC cell viability (Figure 5B, Figure S2A). Conversely, the addition of the PI3K inhibitor LY294002 to SQLE overexpressed EC cells significantly inhibited the enhanced effect of SQLE on cell proliferation (Figure 5C, Figure S2B). Next, we examined the impact of SQLE overexpression on PI3K/AKT pathway protein expression in vitro. Our findings indicated an increase in p-AKT levels following SQLE overexpression, while the knockdown of SQLE resulted in a decrease in p-AKT levels (Figure 5D,E). Additionally, we evaluated the effects of terbinafine treatment on PI3K/AKT pathway protein levels in EC cells, which revealed a significant reduction in the expression of P-PI3K and P-AKT after terbinafine treatment (Figure 5F,G). These results suggested that SQLE may play a role in promoting EC progression through the activation of the PI3K/AKT pathway.

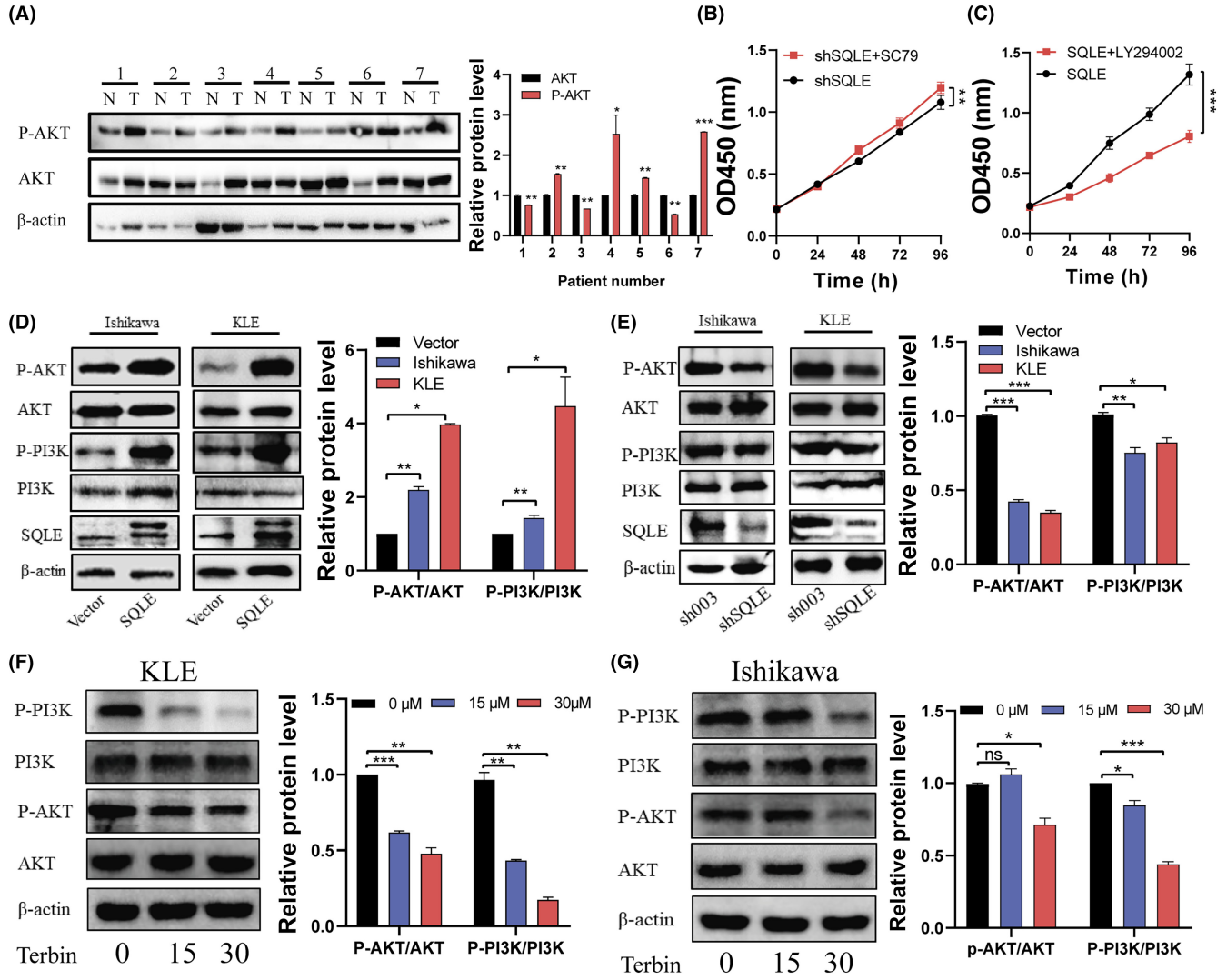

FIGURE 5 Mechanism study of SQLE-mediated EC cell proliferation. (A) WB results of AKT and P-AKT protein expression in tumor tissue. N, normal tissue; T, tumor tissue. (B) Activation of PI3K/AKT promotes the proliferation of EC cells. (C) Inhibition of the PI3K/AKT pathway inhibits the proliferation of EC cells. (D) WB results of the AKT/PI3K pathway protein expression in SQLE overexpression EC cells. (E) WB results of the AKT/PI3K pathway protein expression in SQLE knockdown EC cells. (F, G) Effect of terbinafine on the PI3K /AKT pathway. Quantitative grayscale analysis of all western blot bands using ImageJ software. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

3.6 | Knocking down SQLE significantly inhibits tumor growth in nude mice

Tumors were cultured in nude mice using Ishikawa cells stably transfected with shSQLE or vector for a period of 30 days. The change in tumor volume was recorded and, after 30 days, the mice were sacrificed and the weights of the tumors were measured. The results showed that tumor sizes and weights of mice injected with shSQLE were smaller, and the growth rate was slower than the vector-injected mice (Figure 6A–C). Immunohistochemistry (IHC) indicated that SQLE protein expression in mice injected with shSQLE was lower when compared with mice injected with vector (Figure 6D,E). Additionally, Ki-67 staining showed that the tumors in the mice injected with shSQLE had significantly lower levels of Ki-67-positive cells compared with vector-injected mice (Figure 6D,E). Next, to evaluate the efficacy of terbinafine, the xenograft model was utilized and it was found that it significantly inhibited tumor growth (Figure 6F–H). Further analysis of the tumor showed that terbinafine reduced the expression of SQLE and ki67, but there was no statistically significant difference in ki67 reduction (Figure 6I,J). These findings suggest that SQLE could be a potential target for EC treatment.

4 | DISCUSSION

As a malignant disease, EC is a leading cause of death in women, yet its pathogenesis is largely unknown. In this study, we examined the underlying mechanisms of SQLE in the pathogenesis of EC. We analyzed the expression of SQLE in TCGA data and clinical patient samples. Our study found that, compared with POLE, MSI, and CN-low groups, the CN-high group with the worst prognosis had the highest SQLE expression (Figure 1). In our in vitro study, we observed that

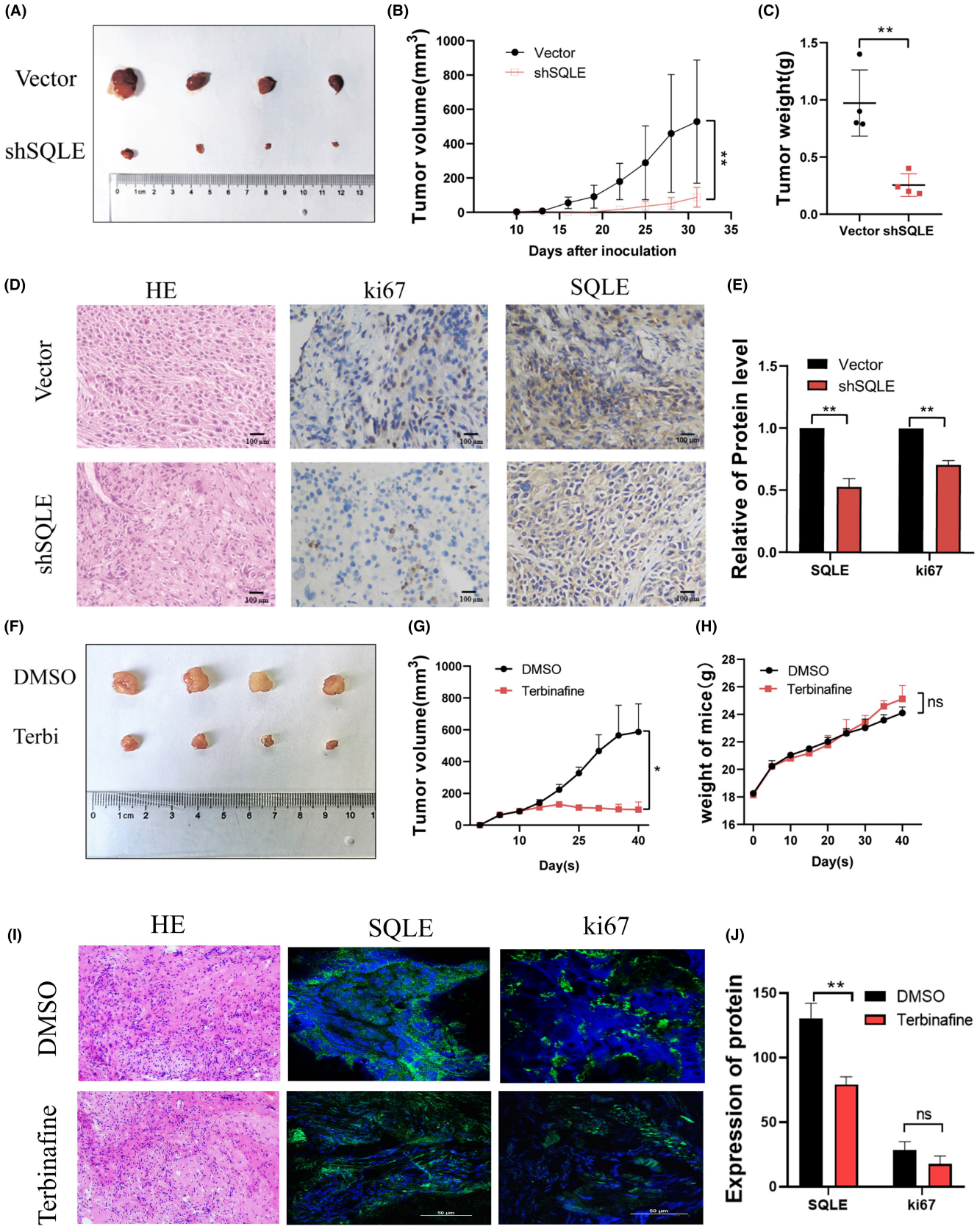

FIGURE 6 Knocking down SQLE significantly inhibits tumor growth in nude mice. (A–C) Tumor growth in the SQLE knockdown group was significantly slower than that in the vector group. (D, E) Expression of ki67 and SQLE proteins in the vector group or the shSQLE group. Bars, 100 μm. (F–H) Terbinafine significantly inhibits xenograft tumor growth in nude mice. (I, J) Expression of ki67 and SQLE proteins in the DMSO group or the terbinafine group. Bars, 50 μm. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

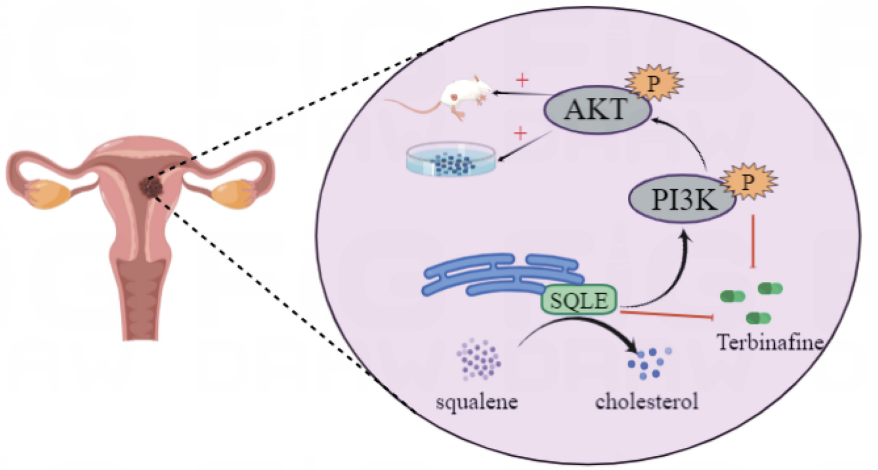

FIGURE 7 Schematic of the function and mechanism of SQLE in EC.

overexpression of SQLE had a significant impact on the proliferation, migration, and apoptosis of EC cells. Our findings also suggest that SQLE activates the PI3K/AKT pathway, leading to increased p-AKT levels. This indicates that SQLE may influence the viability of EC cells through this pathway, thereby promoting the development of EC. Furthermore, our experiments with shSQLE EC cells implanted in nude mice revealed that the knockdown of SQLE resulted in significant inhibition of tumor growth and a reduction in ki67 expression. These results suggest that SQLE plays a crucial role in the physiological activity of EC cells and may have an impact on the progression of tumors in EC patients. Therefore, to investigate whether SQLE can be a potential target for treating EC, we used the SQLE inhibitor terbinafine in cell experiments and found that it significantly inhibited EC cell proliferation and migration, while increasing apoptosis. Additionally, terbinafine was able to inhibit tumor growth in nude mice. Terbinafine is a clinical drug used to treat fungal infections and is reasonably priced with few adverse effects, making it a safe option for EC treatment.[35,36] Moreover, previous studies have shown that terbinafine may also be effective in treating liver, prostate, and nasopharyngeal carcinoma.[37–39] In a word, terbinafine could be a potential drug for treating EC.

Previous studies have highlighted the significance of cholesterol as a crucial component of the cell membrane and a precursor for steroid hormone synthesis. Any disorder in cholesterol metabolism or an increase in its levels can potentially impact the production of downstream products.[40,41] Malignant tumors are known for their high-energy consumption and elevated signal transduction levels, which often lead to metabolic disorders, including abnormal cholesterol metabolism. This dysregulation can affect the activation of proto-oncogenes and lead to abnormalities in the synthesis of downstream steroid hormones.[42] The incidence and development of EC are closely related to persistent estrogen exposure, making it important to consider the patient's cholesterol metabolism. Research has shown that taking cholesterol-lowering drugs or using cholesterol pathway gene inhibitors can significantly inhibit the progress of EC. However, the molecular mechanism behind this remains unclear. SQLE plays a crucial role in the cholesterol synthesis pathway by oxidizing squalene to 2,3-epoxysqualene and is primarily regulated by SREBP2. SQLE has become a molecule of particular interest in cancer research in recent years. For instance, researchers have discovered that SQLE can promote cancer cell metastasis and proliferation, and is a regulator of Ferroptosis in breast cancer.[43,44] Additionally, high expression of SQLE in colon cancer is significantly correlated with lymphatic vessel invasion, tumor budding, invasion depth, regional lymph node metastasis, and TNM pathological stage.[28] SQLE has also been found to promote invasion, metastasis, and drug resistance of PA in prostate cancer.[37,45] Our research has shown that SQLE plays an essential role in EC, providing further evidence of cholesterol's cancer-promoting effect.

The incidence rate in the European Community is increasing at an alarming rate. Women who are not diagnosed early or relapse after treatment often have a poor prognosis, which seriously affects their quality of life.[46]28 Cancer patients often seek medical help after their symptoms are clear, which often misses the best window of time for early detection and treatment.[47] 37Our study showed that SQLE accelerates EC progression by activating the PI3K/AKT pathway, and its inhibitor terbinafine significantly inhibits this effect (Figure 7). At the same time, we also found that SQLE promotes EC progression not through cholesterol or 2,3-epoxysqualene. In future studies, we plan to explore downstream mechanisms to validate SQLE as a therapeutic target for EC patients.

AUTHOR CONTRIBUTIONS

Liangjian Ma: Conceptualization; methodology; investigation; writing – original draft. **Wunan Huang:** Methodology; investigation; writing – original draft. **Xiaolei Liang:** Investigation; validation; writing – review and editing. **Guannan Bai:** Writing – review and editing. **Xiaochen Wang:** Resources. **Hua Jiang:** Writing – review and editing. **Yang Xin:** Writing – review and editing. **Lidan Hu:** Conceptualization; validation; writing – review and editing. **Xiangjun Chen:** Conceptualization; resources; writing – review and editing; supervision. **Chang Liu:** Conceptualization; resources; writing – review and editing; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENTS

Approval of the research protocol by an Institutional Review Board: N/A.

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: LDYYLL2021-241.

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REFERENCES

1. Lu K, Broaddus R. Endometrial cancer. *N Engl J Med*. 2020;383(21):2053-2064.
2. Concin N, Matias-Guiu X, Vergote I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Radiother Oncol*. 2021;154:327-353.
3. Brooks R, Fleming G, Lastra R, et al. Current recommendations and recent progress in endometrial cancer. *CA Cancer J Clin*. 2019;69(4):258-279.
4. Urick M, Bell D. Clinical actionability of molecular targets in endometrial cancer. *Nat Rev Cancer*. 2019;19(9):510-521.
5. Thieffry C, Van Wynendaele M, Aynaci A, et al. AG-205 upregulates enzymes involved in cholesterol biosynthesis and steroidogenesis in human endometrial cells independently of PGRMC1 and related MAPR proteins. *Biomolecules*. 2021;11(10):1472.
6. Zhao T, Sun R, Ma X, et al. Overexpression of LPCAT1 enhances endometrial cancer stemness and metastasis by changing lipid components and activating the TGF/β-Smad2/3 signaling pathway. *Acta Biochim Biophys Sin*. 2022;54(7):904-916.
7. Kober D, Xu S, Li S, et al. Identification of a degradation signal at the carboxy terminus of SREBP2: a new role for this domain in cholesterol homeostasis. *Proc Natl Acad Sci USA*. 2020;117(45):28080-28091.
8. Kuzu O, Gowda R, Noory M, Robertson G. Modulating cancer cell survival by targeting intracellular cholesterol transport. *Br J Cancer*. 2017;117(4):513-524.
9. He M, Zhang W, Dong Y, et al. Pro-inflammation NF-κB signaling triggers positive feedback via enhancing cholesterol accumulation in liver cancer cells. *J Exp Clin Cancer Res*. 2017;36(1):15.
10. Kim J, Thompson B, Han S, Lotan Y, McDonald J, Ye J. Uptake of HDL-cholesterol contributes to lipid accumulation in clear cell renal cell carcinoma. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2019;1864(12):158525.
11. Lethongsavarn V, Pinault M, Diedhiou A, et al. Tissue cholesterol metabolism and prostate cancer aggressiveness: ethno-geographic variations. *Prostate*. 2021;81(16):1365-1373.
12. Xu X, Chen J, Li Y, et al. Targeting epigenetic modulation of cholesterol synthesis as a therapeutic strategy for head and neck squamous cell carcinoma. *Cell Death Dis*. 2021;12(5):482.
13. Zhang K, Zhu W, Wang S, et al. Organ-specific cholesterol metabolic aberration fuels liver metastasis of colorectal cancer. *Theranostics*. 2021;11(13):6560-6572.
14. Jiang H, Tang E, Chen Y, et al. Squalene synthase predicts poor prognosis in stage I-III colon adenocarcinoma and synergizes squalene epoxidase to promote tumor progression. *Cancer Sci*. 2022;113(3):971-985.
15. Zhan Y, Wang J, Ma Y, et al. Serum insulin-like, growth factor binding protein-related protein 1 (IGFBP-rP1) and endometrial cancer risk in Chinese women. *Int J Cancer*. 2013;132(2):411-416.
16. Kho P, Amant F, Annibali D, et al. Mendelian randomization analyses suggest a role for cholesterol in the development of endometrial cancer. *Int J Cancer*. 2021;148(2):307-319.
17. Dai M, Zhu XL, Liu F, et al. Cholesterol synthetase DHCR24 induced by insulin aggravates cancer invasion and progesterone resistance in endometrial carcinoma. *Sci Rep*. 2017;7:41404.
18. Goossens P, Rodriguez-Vita J, Etzerodt A, et al. Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression. *Cell Metab*. 2019;29(6):1376-1389.e4.
19. Shim JS, Li RJ, Lv J, Head SA, Yang EJ, Liu JO. Inhibition of angiogenesis by selective estrogen receptor modulators through blockade of cholesterol trafficking rather than estrogen receptor antagonism. *Cancer Lett*. 2015;362(1):106-115.
20. Shi Z, Zhou Q, Gao S, et al. Silibinin inhibits endometrial carcinoma via blocking pathways of STAT3 activation and SREBP1-mediated lipid accumulation. *Life Sci*. 2019;217:70-80.
21. Wang Q, Hu R, Li W, et al. BF175 inhibits endometrial carcinoma through SREBP-regulated metabolic pathways in vitro. *Mol Cell Endocrinol*. 2021;523:111135.
22. Yao L, Chen S, Li W. Fatostatin inhibits the development of endometrial carcinoma in endometrial carcinoma cells and a xenograft model by targeting lipid metabolism. *Arch Biochem Biophys*. 2020;684:108327.
23. Xu H, Zhou S, Tang Q, Xia H, Bi F. Cholesterol metabolism: new functions and therapeutic approaches in cancer. *Biochim Biophys Acta Rev Cancer*. 2020;1874(1):188394.
24. Howe V, Sharpe L, Prabhu A, Brown A. New insights into cellular cholesterol acquisition: promoter analysis of human HMGCR and SQLE, two key control enzymes in cholesterol synthesis. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2017;1862(7):647-657.
25. Liu D, Wong C, Zhou Y, et al. Squalene epoxidase induces nonalcoholic steatohepatitis via binding to carbonic anhydrase III and is a therapeutic target. *Gastroenterology*. 2021;160(7):2467-2482.e3.
26. Kim NI, Park MH, Kweon SS, Cho N, Lee JS. Squalene epoxidase expression is associated with breast tumor progression and with a poor prognosis in breast cancer. *Oncol Lett*. 2021;21(4):259.
27. Shen T, Lu Y, Zhang Q. High squalene epoxidase in tumors predicts worse survival in patients with hepatocellular carcinoma: integrated bioinformatic analysis on NAFLD and HCC. *Cancer Control*. 2020;27(1):1073274820914663.
28. Jun S, Brown A, Chua N, et al. Reduction of squalene epoxidase by cholesterol accumulation accelerates colorectal cancer progression and metastasis. *Gastroenterology*. 2021;160(4):1194-1207.e28.
29. Li L, Zhang Q, Wang X, Li Y, Xie H, Chen X. Squalene epoxidase-induced cholesteryl ester accumulation promotes nasopharyngeal

carcinoma development by activating PI3K/AKT signaling. *Cancer Sci*. 2020;111(7):2275-2283.
30. Liu D, Wong C, Fu L, et al. Squalene epoxidase drives NAFLD-induced hepatocellular carcinoma and is a pharmaceutical target. *Sci Transl Med*. 2018;10(437):eaap9840.
31. Li J, Yang T, Wang Q, et al. Upregulation of SQLE contributes to poor survival in head and neck squamous cell carcinoma. *Int J Biol Sci*. 2022;18(9):3576-3591.
32. Shangguan X, Ma Z, Yu M, Ding J, Xue W, Qi J. Squalene epoxidase metabolic dependency is a targetable vulnerability in castration-resistant prostate cancer. *Cancer Res*. 2022;82:3032-3044.
33. Yu L, Wei J, Liu P. Attacking the PI3K/Akt/mTOR signaling pathway for targeted therapeutic treatment in human cancer. *Semin Cancer Biol*. 2021;85:69-94.
34. Ediriweera M, Tennekoon K, Samarakoon S. Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: biological and therapeutic significance. *Semin Cancer Biol*. 2019;59:147-160.
35. Yamada T, Maeda M, Alshahni M, et al. Terbinafine resistance of trichophyton clinical isolates caused by specific point mutations in the squalene epoxidase gene. *Antimicrob Agents Chemother*. 2017;61(7):e00115-17.
36. Zhang E, Zhang X, Wang K, et al. Antifungal agent terbinafine restrains tumor growth in preclinical models of hepatocellular carcinoma via AMPK-mTOR axis. *Oncogene*. 2021;40(34):5302-5313.
37. Xu Z, Huang L, Dai T, et al. SQLE mediates metabolic reprogramming to promote LN metastasis in castration-resistant prostate cancer. *Onco Targets Ther*. 2021;14:4285-4295.
38. Sun H, Li L, Li W, et al. p53 transcriptionally regulates SQLE to repress cholesterol synthesis and tumor growth. *EMBO Rep*. 2021;22(10):e52537.
39. Kalogirou C, Linxweiler J, Schmucker P, et al. MiR-205-driven downregulation of cholesterol biosynthesis through SQLE-inhibition identifies therapeutic vulnerability in aggressive prostate cancer. *Nat Commun*. 2021;12(1):5066.
40. Luo J, Yang H, Song B. Mechanisms and regulation of cholesterol homeostasis. *Nat Rev Mol Cell Biol*. 2020;21(4):225-245.
41. Javitt N. Bile acid synthesis from cholesterol: regulatory and auxiliary pathways. *FASEB J*. 1994;8(15):1308-1311.
42. Yang F, Kou J, Liu Z, Li W, Du W. Corrigendum: MYC enhances cholesterol biosynthesis and supports cell proliferation through SQLE. *Front Cell Dev Biol*. 2021;9:705769.
43. Tang W, Xu F, Zhao M, Zhang S. Ferroptosis regulators, especially SQLE, play an important role in prognosis, progression and immune environment of breast cancer. *BMC Cancer*. 2021;21(1):1160.
44. Yu Z, He Q, Xu G. Screening of prognostic factors in early-onset breast cancer. *Technol Cancer Res Treat*. 2020;19:1533033819893670.
45. Pudova EA, Krasnov GS, Kobelyatskaya AA, et al. Gene expression changes and associated pathways involved in the progression of prostate cancer advanced stages. *Front Genet*. 2020;11:613162.
46. Reijntjes B, van Suijlichem M, Woolderink J, et al. Recurrence and survival after laparoscopy versus laparotomy without lymphadenectomy in early-stage endometrial cancer: long-term outcomes of a randomised trial. *Gynecol Oncol*. 2022;164(2):265-270.
47. van den Helder R, Wever B, van Trommel N, et al. Non-invasive detection of endometrial cancer by DNA methylation analysis in urine. *Clin Epigenetics*. 2020;12(1):165.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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