Omipalisib Inhibits Esophageal Squamous Cell Carcinoma Growth Through Inactivation of Phosphoinositide 3-Kinase (PI3K)/AKT/ Mammalian Target of Rapamycin (mTOR) and ERK Signaling

Authors' Contribution:
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Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
Manuscript Preparation **E**
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Background: Esophageal squamous cell carcinoma (ESCC) is a life-threatening digestive tract malignancy with no known curative treatment. This study aimed to investigate the antineoplastic effects of omipalisib and its underlying molecular mechanisms in ESCC using a high throughput screen.

Material/Methods: MTT assay and clone formation were used to determine cell viability and proliferation. Flow cytometry was conducted to detect cell cycle distribution and apoptosis. Global gene expression and mRNA expression levels were determined by RNA sequencing and real-time PCR, respectively. Protein expression was evaluated in the 4 ESCC cell lines by Western blot analysis. Finally, a xenograft nude mouse model was used to evaluate the effect of omipalisib on tumor growth *in vivo*.

Results: In the pilot screening of a 1404-compound library, we demonstrated that omipalisib markedly inhibited cell proliferation in a panel of ESCC cell lines. Mechanistically, omipalisib induced G_0/G_1 cell cycle arrest and apoptosis. RNA-seq, KEGG, and GSEA analyses revealed that the PI3K/AKT/mTOR pathway is the prominent target of omipalisib in ESCC cells. Treatment with omipalisib decreased expression of p-AKT, p-4EBP1, p-p70S6K, p-S6, and p-ERK, therefore disrupting the activation of PI3K/AKT/mTOR and ERK signaling. In the nude mouse xenograft model, omipalisib significantly suppressed the tumor growth in ESCC tumor-bearing mice without obvious adverse effects.

Conclusions: Omipalisib inhibited the proliferation and growth of ESCC by disrupting PI3K/AKT/mTOR and ERK signaling. The present study supports the rationale for using omipalisib as a therapeutic approach in ESCC patients. Further clinical studies are needed.

MeSH Keywords: **Apoptosis Regulatory Proteins • Cell Cycle Checkpoints • Esophageal Neoplasms • Phosphatidylinositol 3-Kinases • TOR Serine-Threonine Kinases**

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Background

Esophageal cancer is a major global health issue. In 2018, there were 572 000 new cases of esophageal cancer and 209 000 related deaths worldwide [1]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma are the 2 main histological subtypes of esophageal cancer. A phenotypic shift has occurred in esophageal adenocarcinoma in recent decades, but ESCC remains dominant globally [2]. The standard therapy for esophageal cancer is surgery with adjuvant chemotherapy or radiotherapy. Although some improvements have been made in surgical methods and chemotherapy in recent years, the overall 5-year survival rate for esophageal cancer patients has remained only 15–25% [3]. Because of the poor prognosis of esophageal cancer, new therapeutic strategies are urgently needed.

Over the past decade, there has been unprecedented development of targeted and immunotherapies for ESCC; however, the successful clinical application of these therapies is a long way off. Drug repurposing – the identification of new therapeutic indications for approved drugs – is a reasonable way to accelerate "new" drug discovery and development [4,5]. For example, metformin is an oral drug for type 2 diabetes, which decreases gluconeogenesis and intestinal glucose absorption and enhances insulin sensitivity. Mounting evidence suggests that metformin exerts a suppressive effect on multiple types of tumors by suppressing oxidative phosphorylation and the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways. Clinical trials combining metformin with chemotherapy, tyrosine kinase inhibitors, and radiotherapy are underway [6].

To seek potential drugs for use in ESCC, we screened 1404 FDA-approved compounds from a compound library for bioactivity against ESCC. After excluding chemotherapeutic drugs, we identified 13 compounds that inhibited proliferation in a panel of ESCC cell lines. From the 13 compounds, we focused on omipalisib, a highly selective and potent inhibitor of the PI3K/AKT/mTOR signaling pathway. The PI3K/AKT/mTOR pathway is involved in cell proliferation, survival, and metabolism and has been identified as a potential target for the treatment of multiple types of cancers [7–10]. A previous study demonstrated that the activation of genes in the PI3K pathway was found in 24% of ESCC patients worldwide [11]. Furthermore, mTOR signaling activation was identified as an independent risk factor significantly associated with poor prognosis in ESCC patients [12].

Omipalisib is a selective and reversible ATP-competitive inhibitor with subnanomolar activity against p110α, p110β, p110γ, p110σ, and the mTOR1 and mTOR2 complexes [13]. Preclinical studies demonstrated that omipalisib suppresses the phosphorylation of AKT, S6, and 4EBP1, exerting antineoplastic activity *in*

vitro and *in vivo* [14,15]. A phase I study showed that omipalisib induces lasting objective responses in multiple advanced solid tumor types, such as in kidney, breast, and endometrial cancers [16]. However, the effect of omipalisib against ESCC remains elusive.

In the current study, we demonstrated the capacity of omipalisib against ESCC and its molecular mechanism *in vitro* and *in vivo*.

Material and Methods

Reagents

The 1404-compound library and cisplatin were purchased from Selleck Chemicals (Shanghai, China) and formulated to a concentration of 10 mM in dimethyl sulfoxide (DMSO). For the animal studies, omipalisib was dissolved in DMSO at 50 mg/mL as a stock solution and further diluted with sterile saline solution.

Cell lines and cell culture

Human ESCC cell lines KYSE150, KYSE70, KYSE180, and KYSE520 were obtained from CoBioer Biosciences Co, Ltd, (Nanjing, China) and authenticated by a third-party short tandem repeat analysis (Genetic Testing Biotechnology, Suzhou, China). All cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and incubated at 37 °C in a humidified atmosphere of 5% CO_2.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The next day, cells were seeded into 96-well plates and treated with various concentrations of compounds (1 µM for compound screening) or vehicle (DMSO). After 72 hours of incubation, 10 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added to each well and incubated for 4 hours. Next, the supernatant was removed and 100 µl DMSO was added to dissolve the crystals. The absorbance was recorded at 570 nm and 630 nm with a spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). The assays were performed in triplicate, and the EC_{50} of omipalisib for each cell line was calculated by Prism software.

Clone formation assay

A clone formation assay was performed as previously described [17]. Briefly, cells were cultured overnight in 12-well plates, and vehicle (DMSO) and a series of concentrations of omipalisib were added to the cells. After 72 hours of incubation, the drug was replaced with fresh medium and cultured for an additional 5 to 10 days. Next, the cells were fixed with methanol
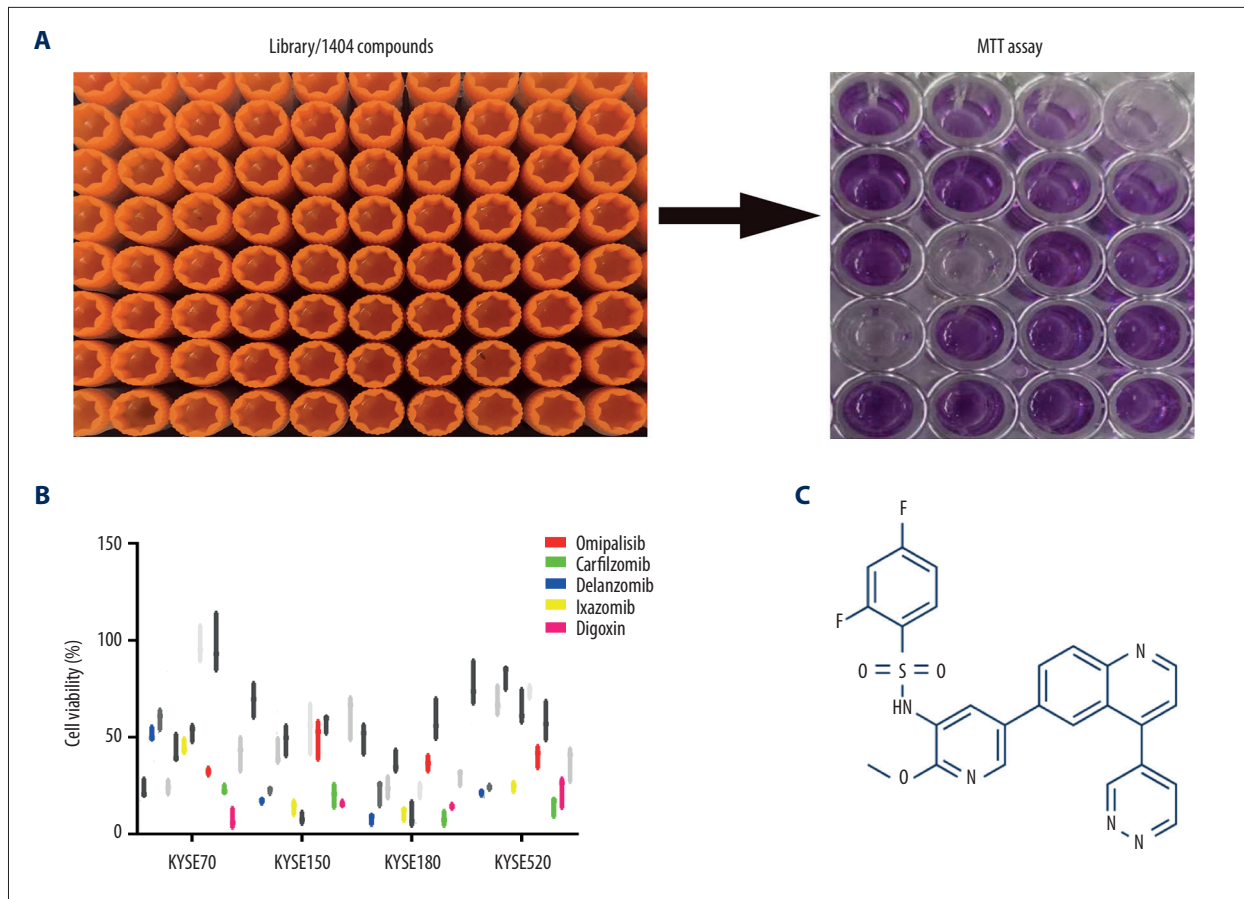
Figure 1. Omipalisib was identified as a potential agent against Esophageal squamous cell carcinoma (ESCC). (**A**) MTT assay was used to screen compounds in a 1404-compound library. (**B**) In total, 13 compounds showed inhibitory effects on 4 ESCC cell lines. (**C**) Chemical structure of omipalisib.

Table 1. EC_{50} of 5 compounds for growth inhibition in Esophageal squamous cell carcinoma (ESCC) cell lines.

Compound	Target	EC_{50} for 4 OSCC cells (nM, SD)			
		KYSE70	KYSE150	KYSE180	KYSE520
Carfilzomib	Proteasome	22.78±7.99	8.20±1.26	15.74±1.32	14.61±4.60
Delanzomib	Proteasome	32.85±3.44	12.90±1.43	33.53±5.58	44.22±14.24
Ixazomib	20S proteasome	253.97±132.90	355.09±195.70	237.03±92.29	163.21±67.37
Digoxin	Na$^+$, K$^+$-ATPase	121.98±24.37	294.85±43.17	304.27±36.29	185.17±36.09
Omipalisib	PI3K/mTOR	138.87±10.18	163.80±21.34	160.17±30.14	122.59±26.03

EC_{50} – concentration for 50% of maximal effect; SD – standard deviation.

for 15 minutes at room temperature and were then stained with Giemsa solution for 15 minutes at room temperature. The colonies containing more than 50 cells were counted manually.

Flow cytometry

For apoptosis analysis, cells were treated with 160 nM omipalisib or vehicle (DMSO) for 72 hours, washed with PBS and binding buffer, and stained with Annexin V (BD Pharmingen) at room temperature in the dark for 15 minutes. Then, the cells were counterstained with propidium iodide (PI) and analyzed immediately by flow cytometry (BD LSRFortessa Cell Analyzer, San Diego, CA, USA). The data were analyzed using FlowJo software.
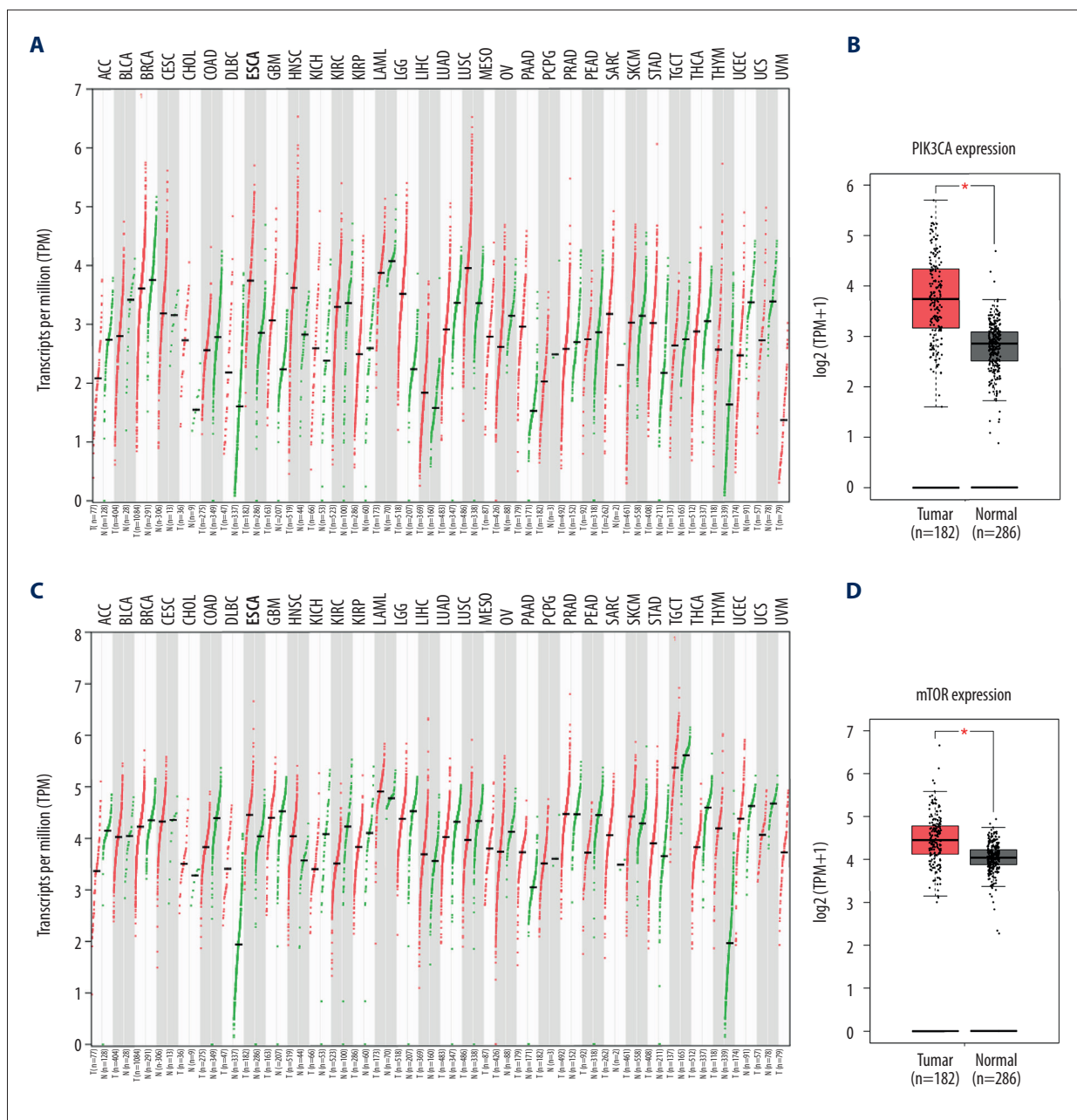
Figure 2. PIK3CA and mTOR were highly expressed in Esophageal squamous cell carcinoma (ESCC) patients. (**A, B**) The PIK3CA and mTOR expression levels in all tumor samples. (**C, D**) The PIK3CA and mTOR mRNA expression in esophageal cancer samples and normal tissues (* $P<0.05$).

For cell cycle analysis, cells were treated with various doses of omipalisib (20, 40, 80, and 160 nM) or vehicle (DMSO) for 24 hours. Then, the cells were harvested by trypsinization, followed by fixation with cold 75% ethanol at −20°C overnight. The fixed cells were washed with PBS and incubated with 50 μg4L PI and 100 μg/mL RNAse for 30 minutes at room temperature. The cells were run through a flow cytometer, and the data were analyzed using ModFit LT software.

RNA sequencing and bioinformatics analysis

KYSE150 cells were seeded in 6-cm dishes at $8.5×10^5$/dish. The next day, vehicle or 1 μM omipalisib was added to the cells and incubated for 8 hours. Then, the cells were harvested by trypsinization and sent to BGI Genomics (Shenzhen, China) on dry ice for RNA sequencing (RNA-seq) analysis. After sequencing, the raw data were filtered via quality control into clean reads, and these were subjected to alignment with the reference
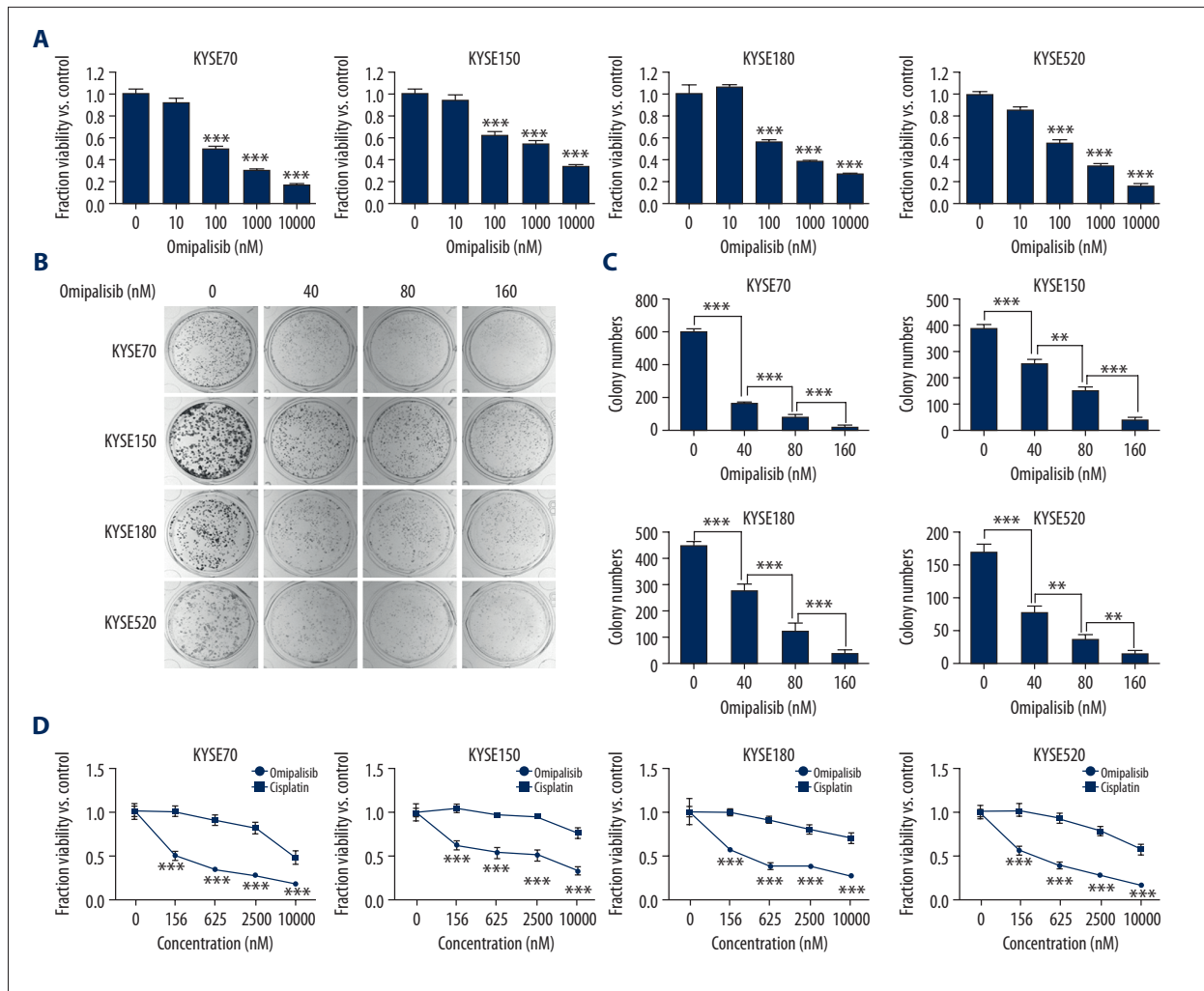
Figure 3. Omipalisib inhibited the proliferation of Esophageal squamous cell carcinoma (ESCC) cells. (**A**) Representative data of 3 independent experiments in cell viability of ESCC exposed to a series of concentrations of omipalisib for 72 hours. (**B, C**) Representative image of clone formation assay for ESCC cells treated with omipalisib for 72 hours. Clone numbers were calculated. (**D**) ESCC cells were treated with a series of omipalisib and cisplatin concentrations for 72 hours, and cell viability was measured by MTT assay. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

sequences (GCF_000001405.38_GRCh38.p12, Homo sapiens, NCBI) using hierarchical indexing for spliced alignment of transcripts software. The gene expression levels were calculated using the FPKM method. The DEGseq method was used to obtain differentially expressed genes (DEGs) [18]. The criteria for screening DEGs were as follows: fold change ≥2 and adjusted P value ≤0.001. The enrichment of DEGs was analysed by Gene Set Enrichment Analysis (GSEA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed as previously described [19]. Complementary DNA was made by a reverse transcription system from 1 μg

total RNA. GAPDH was used as an internal control for each transcript. In accordance with the manufacturer's protocol, qRT-PCR assays were carried out with an Mx3005P RT-PCR system (Agilent Technologies Stratagene) using SYBR Premix Ex Taq (TransGen Biotech, Beijing, China). The primer sequences were as follows:
GAPDH, 5'-GTGAAGGTCGGAGTCAACGG -3' and
5'-CCTGGAAGATGGTGATGGGA-3';
DDIT4, 5'-TTTAGCTCCGCCAACTCTCC-3' and
5'-TCAATGAGCAGCTGTTCCGA-3'; and
CCND1, 5'-GCTTCCTCTCCAGAGTGATC-3' and
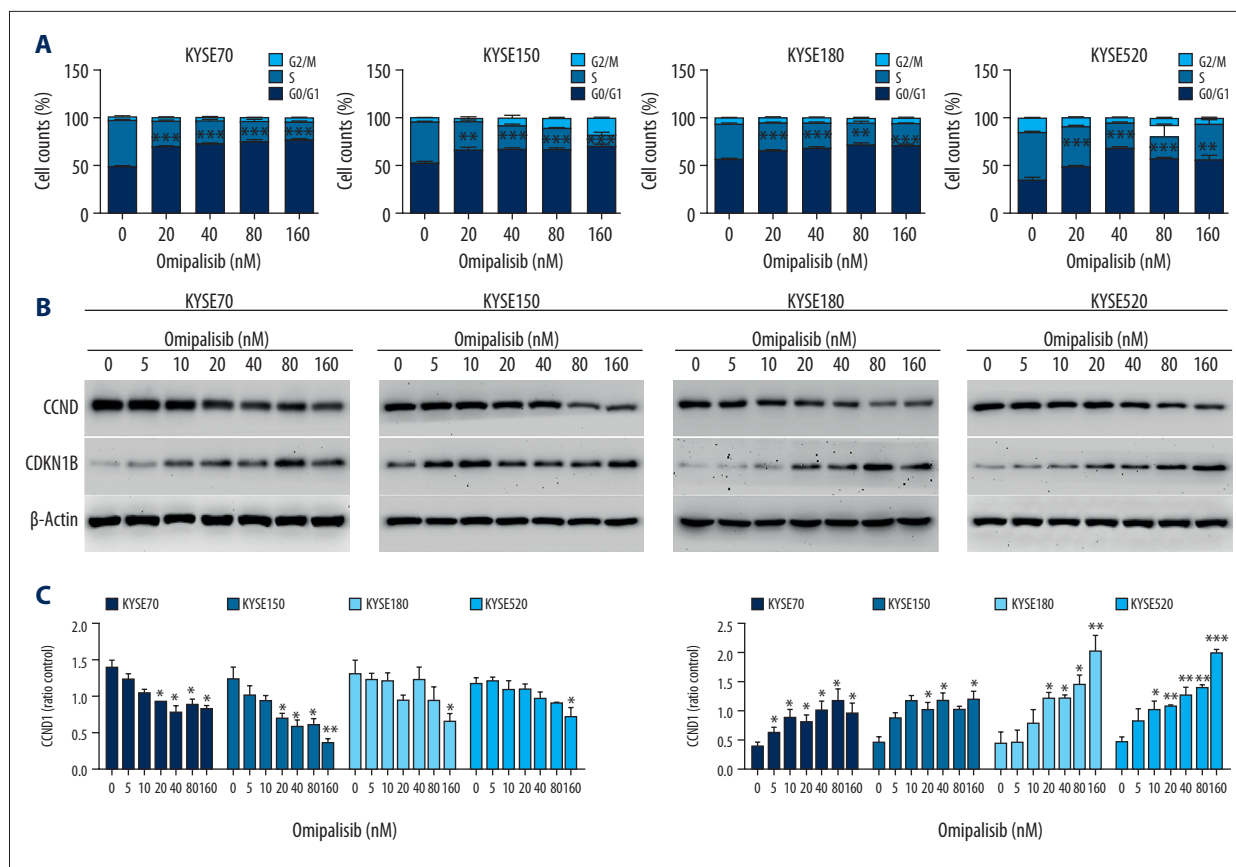5'-GTCCATGTTCTGCTGGGCCT -3'.

Figure 4. Omipalisib induced cell cycle arrest of Esophageal squamous cell carcinoma (ESCC) cells. (**A**) Representative data of 3 independent experiments in cell cycle distribution determined by flow cytometry in ESCC cells exposed to omipalisib for 24 hours. (**B**) Representative image of protein expression of 3 independent experiments in 4 ESCC cells exposed to omipalisib for 24 hours. (**C**) Density analysis of protein bands performed with ImageJ software. Plot is the average of 3 independent experiments. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

Western blot analysis

Western blot protein assays were conducted as previously reported [20]. Cells were treated with omipalisib and harvested by trypsinization, followed by resuspension in lysis buffer (containing 1 mM PMSF and 0.5 mM PhosSTOP) with brief sonication. The protein concentrations were determined with a protein assay kit according to the manufacturer's instructions (Beyotime, P0010, China). Cell lysates were separated by SDS-PAGE and transferred onto PVDF membranes (GE Healthcare). After blocking with blocking buffer containing 5% nonfat milk, membranes were incubated with primary antibodies overnight at 4°C, followed by incubation with horseradish peroxidase–conjugated secondary antibody. Blots were imaged with an imaging system (MYECL, Thermo scientific). Primary antibodies, AKT (#9272), ERK (#4695), P70S6K (#9202), S6 (#2217), 4EBP1 (#9644), p-AKT (Ser473, #4060), p-ERK (Thr202/Tyr204, #4376), p-P70S6K (Ser371, #9208), p-S6 (Ser235/236, #2211), p-4EBP1 (Thr37/46, #2855), and CDKN1B (#3688) were purchased from Cell Signaling Technology. CCND1 (ab134175) was obtained from Abcam.

Xenograft studies

All experimental personnel conducted experiments under the supervision and guidance of the Laboratory Animal Ethics Committee of the First Hospital of Jilin University. All procedures complied with the statute on the Administration of Laboratory Animals approved by China's Council. Twenty-four 6- to 8-week-old female BALB/c nude mice were obtained from Vital River Laboratory Animal Technology Co, Ltd. The animals were housed under pathogen-free conditions. A total of 2.5×10^5 KYSE150 cells in 50 µl PBS was injected subcutaneously into the right dorsal area of the nude mice. When the average tumor volumes reached approximately 100 mm³, the mice were assigned randomly into 4 groups (n=6 per group). Vehicle and various doses of omipalisib (1, 2, and 3 mg/kg) were administered once daily, 5 days per week by gavage for 4 weeks. Tumor volumes and body weights were measured every 2 days. At the end of the experiment, the mice were killed, and the tumor tissues were harvested and stored at −80°C for further Western blot analysis.
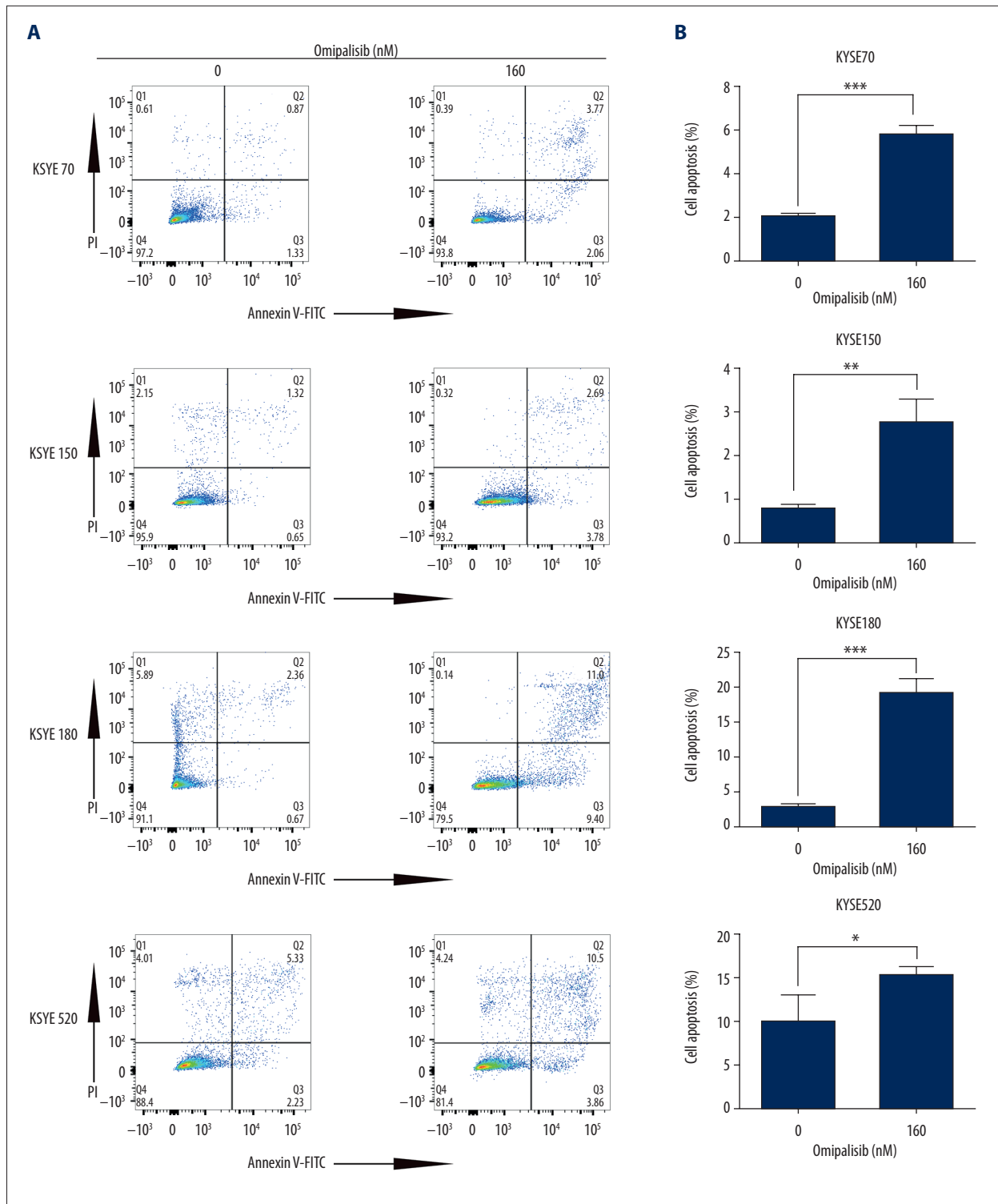
Figure 5. Omipalisib induced apoptosis in Esophageal squamous cell carcinoma (ESCC) cells. (**A**) Representative image of apoptosis in ESCC cells. (**B**) Apoptotic cells in ESCC cells was quantified. (* *P*<0.05, ** *P*<0.01, *** *P*<0.001).
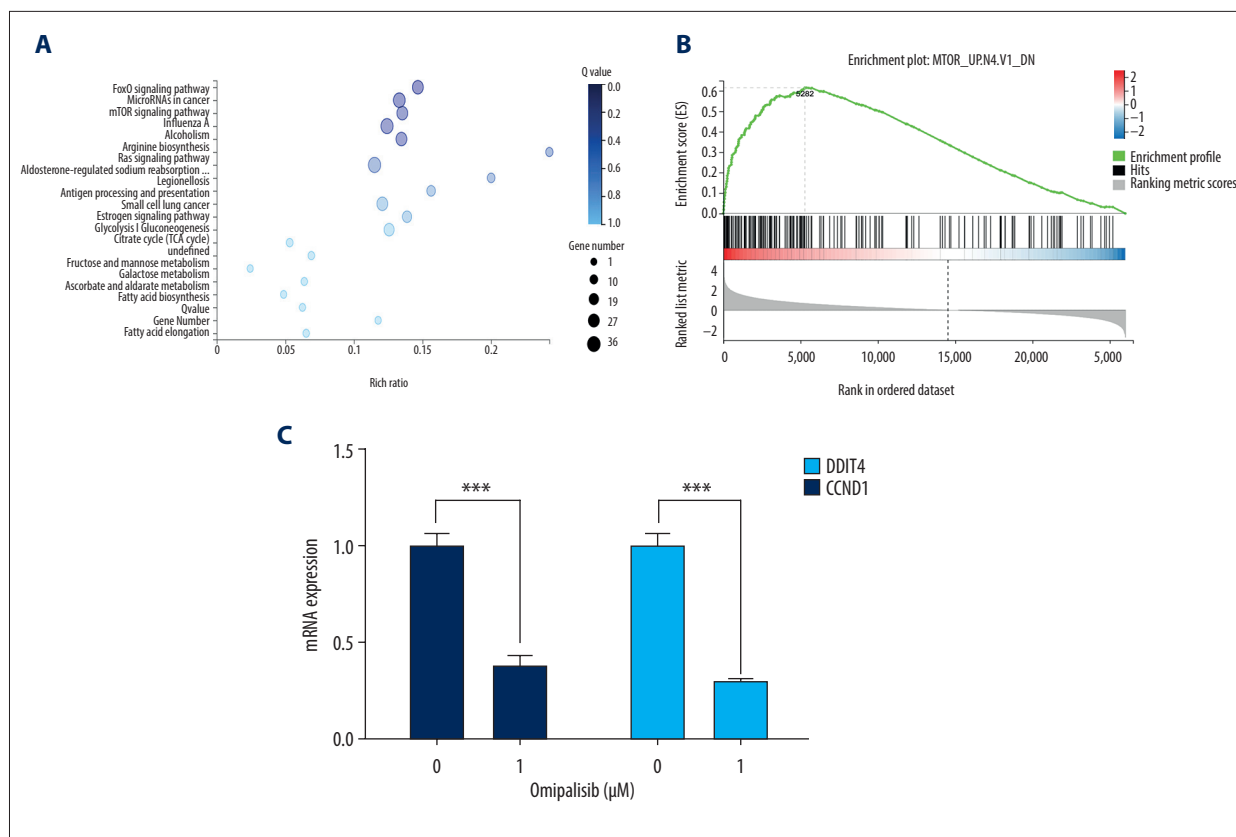
Figure 6. Identification of potential signaling pathways of omipalisib with RNA-seq assays. (**A, B**) DEGs indicated that the mTOR signaling pathway is the major pathway affected by omipalisib treatment according to KEGG pathway enrichment analysis (**A**) and GSEA (**B**). (**C**) qRT-PCR assays showed obvious alterations in DDIT4 and CCND1 gene expression, and the tendency was consistent with the RNA-seq results. (*** *P*<0.001).

Statistical analysis

Data are represented as mean±standard deviation (SD) unless otherwise stated. Statistical analysis was performed using SPSS21.0 software. Two-tailed unpaired *t* test was applied to determine statistical significance. *P*<0.05 was considered statistically significant. Statistical significance was defined as * *P*<0.05, ** *P*<0.01, and *** *P*<0.001.

Results

Chemical screening

A pilot screening of 1404 compounds from an FDA-approved compound library was conducted by MTT assay with 3 biological replicates using a panel of 4 ESCC cell lines: KYSE150, KYSE70, KYSE520, and KYSE180 (Figure 1A). A total of 69 compounds demonstrated obvious antineoplastic effect, with an inhibitory rate above 50% for at least 1 ESCC cell line. A literature review identified 13 compounds that were reported to have pharmaceutical potential against cancers, and most of

these compounds were histone deacetylase inhibitors, proteasome inhibitors, or kinase inhibitors. Of the 13 compounds, 5 had an inhibitory rate above 50% in all 4 ESCC cell lines (Figure 1B, C). Of the 5 compounds tested, 2 were proteasome inhibitors: digoxin is a Na^+, K^+-ATPase inhibitor; and omipalisib is a dual inhibitor for PI3K and mTOR (Table 1). Further, we compared the PIK3CA and mTOR mRNA expression levels between esophageal cancer tissues and paired normal samples in GEPIA (*http://gepia.cancer-pku.cn*) (Figure 2A, 2B). PIK3CA and mTOR mRNA expression levels were significantly higher in esophageal cancer tissues than in those of normal esophageal tissues (Figure 2C, 2D), indicating the PI3K/AKT/mTOR signaling pathway could be a target for esophageal cancer treatment. Therefore, we focused on omipalisib in the next experiment.

Omipalisib exerted a significant antineoplastic effect on ESCC cells

To evaluate the inhibitory effect of omipalisib, ESCC cells were treated with a series of concentrations of omipalisib for 72 hours. Omipalisib inhibited growth in all 4 ESCC cell lines in a dose-dependent manner (Figure 3A). The clone formation assay

confirmed that omipalisib significantly suppressed the growth and proliferation of the 4 cell lines (Figure 3B, 3C). Moreover, omipalisib demonstrated better bioactivity against the 4 ESCC cell lines than did cisplatin, a common chemotherapy drug for patients with ESCC (Figure 3D).
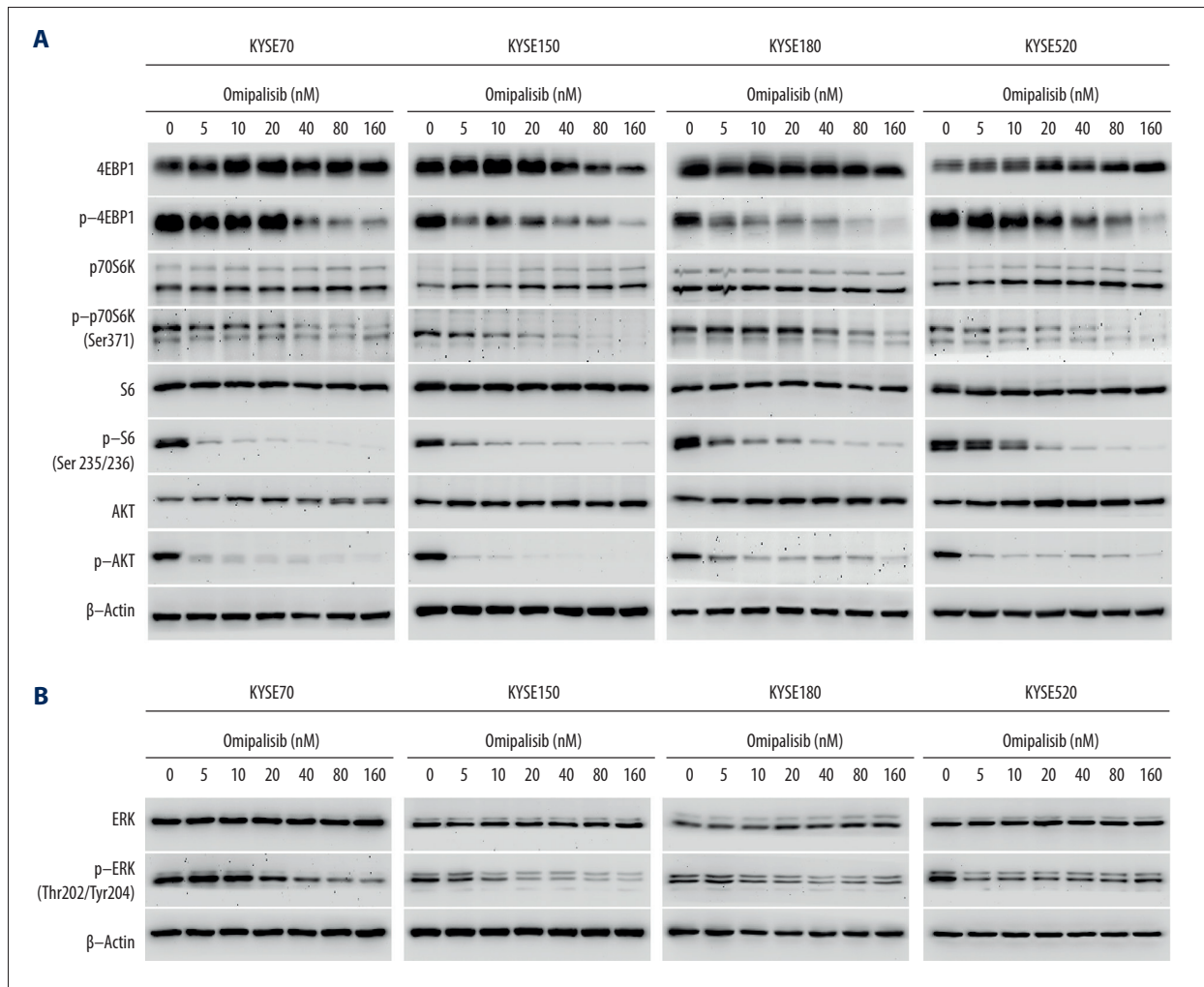
Omipalisib induced cell cycle arrest and apoptosis in ESCC cells

To demonstrate the underlying mechanisms of omipalisib, flow cytometry was used to clarify its effect on cell cycle distribution and cell apoptosis. Treatment of ESCC cells with omipalisib (0, 20, 40, 80, and 160 nM) for 24 hours led to the significant accumulation of cells in the G_0/G_1 phase and a reduction of cells in the S phase among all ESCC cell lines (Figure 4A). Furthermore, we identified the expression levels of cell cycle-associated proteins CCND1 and CDKN1B. Expression of CCND1 was significantly decreased with 24 hours of omipalisib treatment at doses above 20 nM in KYSE70 and KYSE150 cells, whereas CCND1 expression was significantly decreased with

160 nM omipalisib in KYSE180 and KYSE520 cells. Omipalisib decreased CDKN1B expression at the doses above 20 nM in all 4 cell lines (Figure 4B, 4C). With extended treatment time, omipalisib induced apoptosis in ESCC cells. Omipalisib treatment for 72 hours significantly increased apoptosis in ESCC cells compared to that of the control (Figure 5A, 5B).

RNA-seq identified the mTOR signaling pathway as the prominent target of omipalisib

We next examined the effects of omipalisib on global gene expression in KYSE150 cells using RNA-seq. The results of RNA-seq were deposited in the GEO database (accession no. GSE143462). Omipalisib treatment downregulated 500 genes and upregulated 1535 genes; specifically, genes in the mTOR signaling pathway were markedly altered by omipalisib treatment according to the KEGG pathway enrichment analyses ($P=0.0003$, q=0.1356) (Figure 6A). GSEA confirmed that the mTOR signaling pathway was involved in omipalisib treatment (Figure 6B). We verified the RNA-seq data with a qRT-PCR assay. Similarly,
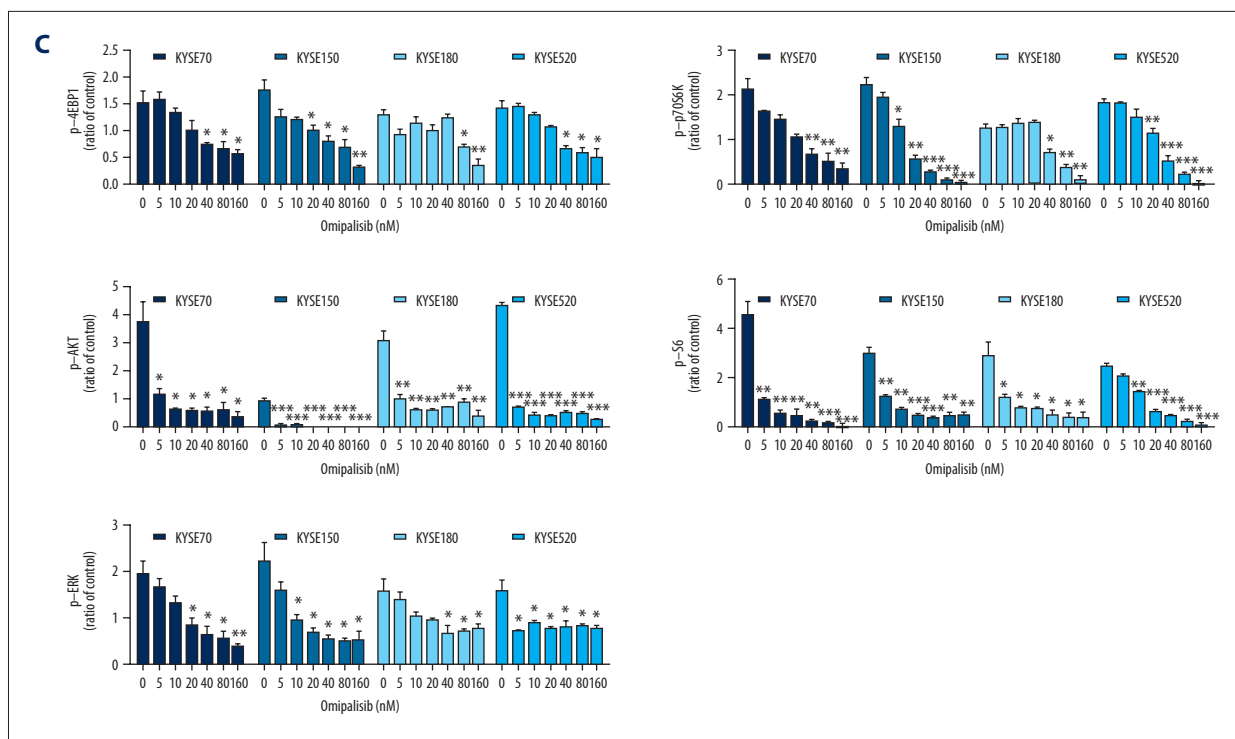

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Figure 7. Omipalisib decreased phosphorylation of proteins in mTOR and ERK signaling pathway. (**A**) Esophageal squamous cell carcinoma (ESCC) cells were treated with the various concentrations of omipalisib for 24 hours. The protein expression levels of 4EBP1, p-4EBP1, p70S6K, p-p70S6k, S6, p-S6, AKT, and p-AKT were identified by Western blot analysis. (**B**) ERK and p-ERK protein levels in the 4 ESCC cell lines treated with omipalisib at the indicated concentrations for 24 hours. (**C**) Density analysis of protein bands performed with ImageJ software. Plot is the average of 3 independent experiments. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

the expression levels of DDIT4 and CCND1 mRNA were decreased by omipalisib treatment (Figure 6C).

Omipalisib decreased protein expression in PI3K/AKT/ mTOR and ERK signaling

To confirm the RNA-seq data, the protein expression of the PI3K/AKT/mTOR signaling pathway was detected by Western blot analysis, which revealed that omipalisib treatment caused similar protein expression changes among the 4 ESCC cell lines. Omipalisib significantly decreased the expression of p-AKT at concentrations above 5 nM. The phosphorylation levels of P70S6K, 4EBP1, and S6, downstream proteins of the mTOR signaling pathway, were decreased by omipalisib in a dose-dependent manner (Figure 7A, 7C). Additionally, p-ERK levels were significantly decreased by the 24-hour treatment with omipalisib (Figure 7B, 7C).

Omipalisib exerted antineoplastic effects *in vivo*

Based on the inhibitory activity of omipalisib on ESCC *in vitro*, we established an ESCC xenograft model and administered omipalisib (control, 1, 2, and 3 mg/kg) for 4 weeks. At the end of

treatment, the average tumor volumes were: 1036.01±183.44 (control), 485.95±160.34 (1 mg/kg), 191.04±37.56 (2 mg/kg), and 155.79±36.52 mm^3 (3 mg/kg). Compared to the control, the 3 doses of omipalisib treatment significantly delayed tumor growth (Figure 8A, 8B). The body weight of the mice did not significantly change during the treatment with omipalisib, indicating it was well tolerated. (Figure 8C). We further determined the expression of p-AKT, CCND1, and p-S6 in tumor tissues by Western blotting, which showed that omipalisib treatment led to decreased p-AKT, CCND1, and p-S6 expression levels (Figure 8D, 8E). These results further illustrated that omipalisib exerted antineoplastic effects by disrupting the PI3K/AKT/mTOR signaling pathway.

Discussion

Although chemotherapy is an indispensable and important part of treatment for advanced ESCC, serious adverse effects limit its clinical application [21]. Thus, the discovery and development of novel drugs are urgently needed for the treatment of ESCC. The screening of existing drug libraries provides a convenient method to promote drug discovery and development.
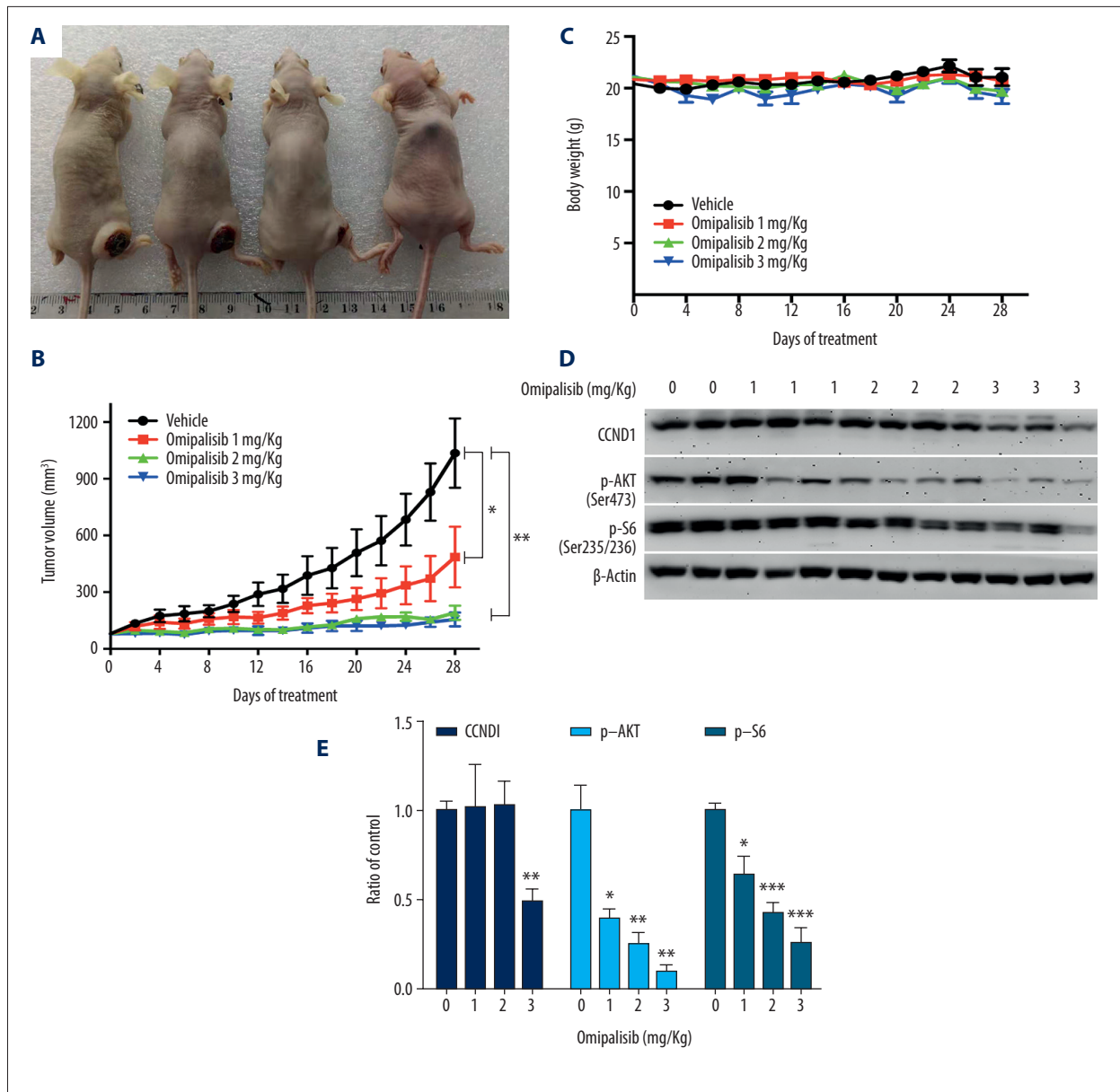
Figure 8. Omipalisib inhibited the growth of Esophageal squamous cell carcinoma (ESCC) *in vivo*. (**A**) Representative image of mice administered different doses of omipalisib at the end of the experiment. (**B**) Tumor volume was calculated by the following equation: volume=long diameter×(short diameter)^2/2. (**C**) Body weight was recorded every 2 days during the course of omipalisib administration. (**D**) Protein expression in tumor tissue from KYSE150-bearing mice by Western blot. (**E**) Density analysis of protein bands were performed with ImageJ software. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

Using this method, we identified omipalisib as a promising therapeutic agent for ESCC.

The PI3K/AKT/mTOR pathway has been identified as a potential therapeutic target for multiple types of cancer [22–24]. Further, the inactivation of the PI3K/AKT/mTOR signaling pathway is considered an effective therapeutic strategy for ESCC [25,26]. Everolimus and temsirolimus, 2 mTOR inhibitors, have exhibited potent antineoplastic effects against ESCC [27,28].

Compared to these 2 mTOR inhibitors, omipalisib targets both PI3K and mTOR, while showing greater efficacy and fewer toxic effects [13]. Moreover, omipalisib could overcome the drug resistance of cancer cells in combinational therapy [29,30]. To the best of our knowledge, the present study is the first to investigate the antineoplastic effect of omipalisib on ESCC. RNA-seq analysis confirmed that the PI3K/AKT/mTOR pathway is the prominent target of omipalisib. We demonstrated that omipalisib suppressed the activation of AKT and the downstream

targets of mTOR and significantly inhibited the proliferation of ESCC cells *in vitro* and *in vivo*.

Hou et al. revealed that inhibiting the phosphorylation of P70S6K and 4EBP1 induced G_0/G_1 arrest and apoptosis in ESCC cells [26]. Liu et al. found that omipalisib suppressed phosphorylation of the AKT, S6, and 4EBP1 proteins, thereby inducing apoptosis and cell cycle arrest [14]. Similarly, the present study showed that omipalisib inhibited the proliferation of ESCC cells by inducing G_0/G_1 arrest and apoptosis. The decreased CCND1 expression and increased CDKN1B expression with omipalisib treatment further confirmed that cell cycle arrest is a partial cause of omipalisib-induced decreased cell viability. Basu et al. showed that the cell death induced by omipalisib in NRAS mutant neurocutaneous melanocytosis cells occurs mainly via autophagy [31]. However, we did not find any evidence of autophagy in the ESCC cells.

There were negative feedback loops between the mTORC1 and MAPK signaling pathways, and LY294002, a PI3K inhibitor, could block feedback by reducing rapamycin-mediated MAPK/ERK activation [32]. Dual PI3K-mTOR inhibitors possibly overcome the feedback of MAPK signaling by targeting PI3K and mTOR simultaneously [33]. Consistent with our results, it was recently reported that BEZ235, another dual PI3K-mTOR inhibitor, has an inhibitory effect on ESCC, but its effect on feedback inhibition was not mentioned [34]. Our results demonstrated that omipalisib suppressed the activation of both PI3K/AKT/mTOR and ERK signaling in all ESCC cell lines, indicating that omipalisib can suppress the feedback activation of MAPK. Because the PI3K/AKT/mTOR and MAPK signaling pathways play crucial roles in cell proliferation and survival, omipalisib on its own can act effectively in ESCC cells.

Conclusions

In summary, omipalisib inhibited the proliferation of ESCC cells by inducing cell cycle arrest and apoptosis by inactivating PI3K/AKT/mTOR and ERK signaling. The present study supports the rationale for using omipalisib as a therapy for ESCC and could promote the rapid clinical evaluation of this therapeutic approach.

Acknowledgements

Ethics statement

All work was done at the Bethune Institute of Epigenetic Medicine, the First Hospital of Jilin University. All experimental personnel conducted experiments under the supervision and guidance of the Laboratory Animal Ethics Committee of the First Hospital of Jilin University, whose license number is SYXK (Ji) 20180006. The approval no. is 20190457. All procedures were done in compliance with the statute on the Administration of Laboratory Animal approved by China's Council (November 1988).

Conflict of interest.

None.

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