SPAG1 Inhibits Cell Proliferation and Tumor Growth of Lung Adenocarcinoma via the AKT/mTORC1 Signaling Axis

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Research

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Abstract

Background

Sperm-associated antigen 1 (SAPG1) expression is increased in multiple cancer tissues, but the role and mechanisms of SPAG1 in lung cancer remain unknown. The present study was aimed to investigate SPAG1's function and mechanisms in lung adenocarcinoma (LUAD).

Methods

SPAG1 expression in LUAD tissues was evaluated by analyzing three LUAD datasets, and its association with prognosis of patients with LUAD was accessed by using Kaplan-Meier Plotter. The role of SPAG1 and its mechanisms were investigated in LUAD cells *in vitro.* The effect of SPAG1 on tumor growth was evaluated in LUAD tumor xenografts. In addition, the molecular domain involved in the regulation of cell proliferation was mapped.

Results

The bioinformatical results showed that SPAG1 expression was increased significantly in LUAD tissues compared with normal lung tissues, and its high expression was associated with favorable prognosis, including overall survival (OS), first progression (FP) and post-progression survival (PPS). The results of *in vitro* experiments showed that SPAG1 suppressed cell proliferation, but enhanced autophagy via inhibiting AKT/mechanistic target of rapamycin (mTOR) complex 1 signaling axis, and that the region of 130~170 amino acid residues in SPAG1 is involved in the regulation of AKT and cell proliferation. The results of tumor xenografts demonstrated that SPAG1 knockdown promotes LUAD tumor growth and enhances AKT/mTORC1 signaling.

Conclusion

SPAG1 is upregulated in LUAD tissues and associated with favorable prognosis of patients with LUAD, and plays an inhibitory role in cell proliferation and tumor growth of LUAD through the AKT/mTORC1 signaling axis.

Background

Lung cancer is one of the leading causes of cancer death worldwide (1). The latest statistics show that in 2020 the global estimated incidence cases of lung cancer is over 1.4 million, and mortality cases of lung cancer is over 1.1 million. According to histological characteristics, lung cancer is classified into small cell lung carcinoma and non-small cell lung carcinoma (NSCLC), of which lung adenocarcinoma (LUAD) and lung squamous cell carcinoma are the most common subtypes. NSCLC accounts for about 85% of lung cancer (2). Current treatments for lung cancer mainly include surgery, radiotherapy, chemotherapy, targeting therapy, and immunotherapy (3). Despite the progress in the treatments of cancer during last few decades, the survival time of more than half of the patients is still less than one year due to the

limitation of the available drugs and the drug resistance, and the 5-year survival rate is only 17.8% (4). Therefore, it is necessary to discover new therapeutic targets and drugs for lung cancer.

AKT is a serine/threonine protein kinase, consisting of three subtypes, namely AKT1, AKT2 and AKT3 (5), which is essential for cell proliferation, growth, survival, glucose metabolism, cytoskeleton rearrangement, invasion and metastasis and other important cell functions (5). AKT is regulated by a variety of factors, including insulin and growth factors (5). Studies have found that AKT signaling pathway is one of the most common over-activated signaling pathways in human cancer cells, which leads to the drug resistance (6). AKT is an upstream positive regulator of mechanistic target of rapamycin (mTOR), which is a serine/threonine protein kinase that is present in the cytoplasm and belongs to the phosphoinositine-associated protein kinase family (7). mTOR can form two signal complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (7). mTORC1 can phosphorylate ribosomal protein S6 kinase beta-1 (S6K1), which promotes protein translation, nucleotide and lipid synthesis, lysosomal biogenesis (7). mTORC1 can also phosphorylate Unc-51 like autophagy activating kinase 1/2, resulting in autophagy inhibition (8). mTOR is hyperactivated in more than 70% cancers, suggesting that mTOR is involved in the occurrence and development of tumors (9).

Sperm-associated antigen 1 (SAPG1) gene encodes two protein isoforms, one of which consists of 926 amino acids and contains 9 tetratricopeptide repeat (TPR) motif involved in protein interaction; and another isoform misses 417 ~ 926 amino acids and thus only has 3 TPR motif (10). The analysis results of transcriptomics and proteomics demonstrated that SPAG1 RNA exists in all organs examined, but SPAG1 protein only exists in few organ, such as testis and lung (11). A number of studies investigated the function of SPAG1 in different cells. Lin et al proposed that SPAG1 has GTPase activity (12), which was questioned by a recent study in which the authors observed that SAPG1 does not bind and hydrolyzed GTP efficiently (13). Liu et al reported that a SPAG1 truncation (1 ~ 245 amino acids) can interact with a G protein subunit β1 at the presence of GDP when they were co-expressed in HEK-293 cells, and this truncation activated extracellular signal-regulated kinases 1/2 (ERK1/2) in COS-7 cells (14). Horani et al found that long SAPG1 is involved in regulating the ciliogenesis in normal human tracheobronchial epithelial cells by forming preassembly protein complex with other key proteins (15). Huang et al showed that SPAG1 knockdown compromised meiosis of mouse oocytes and disrupted actin filament assembly (16). Hu et al domonstrated that SPAG1 was required for spindle morphogenesis in Sertoli cells (17). Biochemical studies revealed that SPAG1 binds to Hsp70 and Hsp90 (13). Few studies have indicated that SPAG1 is associated with cancer. Neesse et al reported that SPAG1 expression is increased in pancreatic cancer tissues and knockdown of SPAG1 impairs the migration of Panc1 pancreatic cancer cells (18). Silina et al observed that SPAG1 mRNA is increased in a variety of cancer tissues, including breast, stomach, rectal, lung, and melanoma (19). Lin et al proposed that knockdown of SPAG1 inhibited cell proliferation and colony formation in breast cancer cells (20). However, there is no report regarding the role and mechanisms of SPAG1 in lung cancer yet.

In the present study, we analyzed SPAG1 expression in LUAD tissues and the association of SPAG1 with the prognosis of patients with LUAD, and investigated the mechanism of SPAG1 in lung adenocarcinoma.

Materials And Methods

Cell culture and transfection

The cell lines A549 was from the Kunming cell bank of the Chinese Academy of Sciences (Kunming, Yunnan, China). HCC827, NCI-H1975, and NCI-H1993 were from the Meisen CTCC (Meisen Cell Biotechnology Co., Ltd, Hangzhou, Zhejiang, China). Beas-2B, HCC515, and HeLa were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in an incubator with 5% CO_2. The cells (1.2×10^5) were seeded into 12-well plates and cultured overnight, and transfection was performed with the Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. 1μg plasmids per well was used for overexpression experiments. SPAG1 small interfering RNA (si-SPAG1 hereafter, 100 pmol) and control small interfering RNA (si-control hereafter, 100 pmol) per well were used for knocking down experiments. The si-SPAG1 and si-control were synthesized by GenePharma (Shanghai, China). si-SPAG1 sequence: 1#, 5’-CCACUGUAGUUGCCUAUAATT-3’; 2#, 5’-CCAAGGGAUUACGCGGAAUTT-3’; 3#, 5’-GCUGCAUUCAAGAUUGUAATT-3’

SPAG1 cDNA was synthesized and cloned into vector by GenePharma. Bafilomycin (working concentration 100 nM) was obtained from Cell Signaling Technology, Inc. (CST, cat. no. 54645S).

Western blot analysis and antibodies

48 hours post-transfection, the cells were collected and lysed with radioimmunoprecipitation assay lysis buffer (cat. no. R0020; Beijing, Solarbio Science and Technology Co., Ltd.). The protein concentration of the cell lysates was measured with a BCA kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's instructions. Immunoblot analysis were performed as previously described(21). The primary antibodies used were as follows: rabbit anti-SPAG1 polyclonal antibody (cat. no. PA5-54879; dilution 1:800; Thermo Fisher Scientific, Waltham, MA, USA); rabbit anti-AKT polyclonal antibody (cat. no. 9272; dilution 1:1,000; CST); rabbit anti-phosphorylated-AKT (cat. no. 4060; dilution 1:1,000; CST), rabbit anti-S6 kinase (cat. no. 2708; dilution 1:1,000; CST), mouse anti-phosphorylated-S6 kinase (cat. no. 9206; dilution 1:1,000; CST), rabbit anti-LC3B polyclonal antibody (cat. no. NB100-2220; dilution 1:1,000; Novus Biologicals, LLC), rabbit anti-p62 polyclonal antibody (cat. no. 39749; dilution 1:1,000; CST) and mouse anti-β-actin monoclonal antibody (cat. no. TA811000; dilution 1:1,000; OriGene Technologies, Inc.). Anti-GAPDH and anti-actin were from ZSGB-BIO (Beijing, China). The secondary antibodies used were as follows: Horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (IgG; cat. no. 7074; dilution 1:5,000; CST) and HRP-conjugated anti-mouse IgG (cat. no. 7076; dilution 1:5,000; CST).

Colony formation assay

Transfected cells were seeded into 6-well plates in 2 ml growth medium at a density of 600 cells per well and cultured in RPMI-1640 with 10% FBS at 37˚C in a humidified incubator with 5% CO_2 for 10 days. The cell culture medium was replaced with fresh media every 2 days. At the end of experiments, the cells were washed three times with PBS and fixed with 4% neutral paraformaldehyde solution at room temperature for 30 min, followed by another three washes with PBS. Then, 2 mL 1% crystal violet solution was added to each well and incubated at 4˚C overnight. The cells were washed three times with PBS. The plates were dried and scanned with Epson Perfection V370 Photo scanner (Seiko Epson Corporation). Cell colonies visible by the naked eye were manually counted.

Autophagy assays

Autophagy was examined as described previously (22). Cells were transfected for 48 h, and were harvested to detect autophagy markers microtubule-associated proteins 1A/1B light chain 3 (LC3) and p62/sequestosome 1 (SQSTM1) using western blot (23). Autophagy inhibitor bafilomycin A1 (cat. no. 54645S, CST) was added into culture media (final concentration 100 nM) and incubated at 37˚C for 1 h prior to harvesting cells in autophagy inhibition experiment. GFP-LC3 stable transformant HCC827 cells were transiently transfected with si-SPAG1 or si-control for 48 h. The cells were fixed with 2% paraformaldehyde at room temperature for 10 min, followed by washing with PBS three times. Cells were observed under a fluorescent microscope (magnification, x400, Zeiss Axio Imager Z2, Carl Zeiss Microscopy, LLC), and the GFP-LC3 fluorescent puncta (autophagosome) were counted manually.

Gene set enrichment analysis (GSEA)

Gene set enrichment analysis was conducted as previously described (24). Tumour samples in The Cancer Genome Atlas (TCGA) LUAD dataset were classified into high- and low- SPAG1 groups using median value of SPAG1 expression as cut-off. Then gene set enrichment was analyzed using GSEA 4.0.3 software (downloaded from http://www.broad.mit.edu/gsea/) with the pre-defined curated gene sets. Permutation number was set as 1000. A gene set is considered significantly enriched when the false discovery rate (FDR) score < 0.25.

Mice xenograft models

Stable transformant HCC827 cell lines were constructed by transfected with SPAG1 shRNA or control shRNA respectively, and screened with G418. NOD/SCID mice (male, 8 weeks) were provided by the Hunan Saike Jingda Experimental Animal Co. Ltd. 1.5×10^7 HCC827/shSPAG1 and HCC827/shControl cells were injected subcutaneously to the mice flanks. Xenograft was measured every four days from day 10 until the end. The volume was calculated by using the equation V (mm^3) = (length×$width^2$)/2. All mice were sacrificed at day 30, and the tumor tissues were collected for subsequent studies.

LUAD datasets collection, SPAG1 expression in LUAD and prognosis analysis

LUAD gene expression datasets GSE75037 and GSE31210 were obtained from the Gene Expression Omnibus (GEO) public database. SPAG1 expression data in LUAD and normal tissues were downloaded

from GEO and analyzed using GraphPad Prism 5 (GraphPad Software Inc, San Diego, CA, USA). The SPAG1 expression in TCGA LUAD and normal tissues was analyzed using online tool UALCAN (http://ualcan.path.uab.edu/) (25). The prognostic values of SPAG1 in LUAD were analyzed using online software Kaplan-Meier Plotter (http://kmplot.com)(26). Log rank test was used for survival probability difference.

Statistical analysis

Statistical analysis was performed with SPSS 19.0 software (SPSS, Chicago, USA). Data were presented as the mean ± SD. Student's t test was used for comparison between two groups, and ANOVA was used to comparison among multiple groups. $P < 0.05$ was considered as indicating statistical significance.

Results

SPAG1 expression is up-regulated in LUAD tissues and its high expression is associated with favorable prognosis of patients with LUAD

We analyzed SPAG1 expression in TCGA LUAD dataset using the online tool UALCAN (http://ualcan.path.uab.edu/) (25). The results indicated that SPAG1 expression was significantly higher in LUAD tissues (n = 515) compared with normal lung tissues (n = 59) ($P < 0.001$; Fig. 1A). To further confirm the results, we analyzed SPAG1 expression in LUAD of anther two datasets (GSE75037 and GSE31210) from the Gene Expression Omnibus (GEO) database (27, 28). The results also showed that SPAG1 expression in LUAD tissues was significantly up-regulated ($P < 0.001$ and $P < 0.05$ respectively, Fig. 1A). To reveal the potential role of SPAG1 in LUAD progression, we analyzed the association between SPAG1 expression levels in LUAD tumors and the prognosis of patients with LUAD, including the overall survival (OS), first progression (FP) and post-progression survival (PPS), by using the online tool Kaplan-Meier Plotter (kmplot.com) (29). The results indicated that the patients in SPAG1 high expression group had better OS, FP and PPS than those in SPAG1 low expression group (OS: logrank P = 0.0034, HR = 0.69(0.54–0.89); FP: logrank P = 0.0079, HR = 0.56 (0.48–0.9); PPS: logrank P = 0.00043, HR = 0.44 (0.27–0.7)) (Fig. 1B), implying that SPAG1 might play a role to negatively affect tumor progression in LUAD patients.

SPAG1 suppresses the proliferation of LUAD cells

To study the function of SPAG1 *in vitro*, we first examined the SPAG1 protein expression in multiple LUAD cell lines (HCC827, HCC515, H1975 and H1993), HeLa cell line and normal lung cell line BEAS-2B by western blot analysis. The results showed that SPAG1 was highly expressed in HCC827 and HeLa cells, but weakly expressed in A549 cells (Fig. 2A). Therefore, LUAD A549 and HCC827 were selected for subsequent SPAG1 overexpression and knockdown experiments, respectively, and HeLa was used to confirm the findings from LUAD cells. To deplete SPAG1 expression, three siRNAs against SPAG1 were designed and their efficacy on SPAG1 expression was examined by western blot analysis. HCC827 cells were individually transfected with SPAG1 siRNA-1 to -3 or control siRNA, then the SPAG1 protein was

detected 48 h after transfection. The results showed that SPAG1siRNA-2 had the most potent inhibitory effect (Fig. 2B). Thus, SPAG1 siRNA-2 was used in subsequent experiments.

The cell counting and colony formation assays were conducted to determine the effects of SPAG1 on cell proliferation. The results suggested that, compared with the control, SPAG1 knockdown significantly increased cell proliferation (Fig. 2C), colony formation (Fig. 2D) of HCC827 cells, whereas overexpression of SPAG1 in A549 cells had the opposite effects (Fig. 2C&D). The results in HeLa cells confirmed that SPAG1 suppressed the cells proliferation (Fig. 2C). These results suggested that SPAG1 suppresses the proliferation of LUAD cells.

SPAG1 inhibits AKT/mTORC1 signaling axis

To elucidate the mechanisms through which SPAG1 negatively affects proliferation, we analyzed TCGA LUAD gene expression data using GSEA software. The results suggested that the gene set induced by EGF was inhibited by SPAG1 (Fig. 3A), indicating that SPAG1 might inhibit EGF signaling. To verify the bioinformatical finding, SPAG1 was overexpressed in A549 cells for 48 hours, and followed by EGF treatment for 30 min. The results of western blot analysis showed that SPAG1 inhibited basal level and EGF-induced AKT phosphorylation (Ser473) compared with the control groups (Fig. 3B), suggesting SPAG1 inhibits AKT activity. To further confirm this finding, SPAG1 was depleted with si-SPAG1 in HCC827 and HeLa for 48 hours and overexpressed in A549. The western blot results of AKT phosphorylation (Ser473) showed that SPAG1 depletion led to the increase of AKT phosphorylation (Ser473), meanwhile SPAG1 overexpression resulted in the decrease (Fig. 3C). As mTORC1 is key signaling node downstream AKT, we also examined mTORC1 activity upon SPAG1 depletion and overexpression by detecting the phosphorylation (Thr421/Ser424) of ribosomal protein S6 kinase beta-1 (S6K1), a well-known substrate of mTORC1. The results demonstrated that SPAG1 knockdown led to enhanced phosphorylation of S6K1 (Thr421/Ser424), whereas overexpression of SPAG1 exerted the opposite effects (Fig. 3C). Taken together, these findings suggest that SPAG1 may suppress cell proliferation via inhibiting AKT-mTORC1 signaling axis.

SPAG1 enhances autophagy

As mTORC1 is an inhibitor of autophagy, we then examined whether SPAG1 can affect autophagy. The autophagic markers microtubule-associated protein 1 light chain 3B (LC3B) and p62/sequestosome 1 (p62/SQSTM1) were determined by western blot analysis upon SPAG1 knockdown or overexpression. The results indicated that in HCC827 SPAG1 knockdown resulted in the decrease of LC3-II and the ratio of LC3-II to LC3-I, as well as the increase of p62/SQSTM1 protein compared with the control siRNA group. Whereas overexpression of SPAG1 had the opposite effects on LC3B and p62/SQSTM1 in A549 cells (Fig. 4A). We measured autophagy flux by treating cells for 1 h with a lysosomal inhibitor, bafilomycin A1 48 h post-transfection of SPAG1 plasmids in A549 and HeLa cells. The results demonstrated that autophagy inhibition caused apparent accumulation of LC3B-II (Fig. 4B), indicating that the decrease of LC3-II here was due to enhanced degradation, but not decreased conversion of LC3-I to LC3-II. To confirm the role of SPAG1 in autophagy regulation, we depleted SPAG1 expression with si-SPAG1 in

HCC827/GFP-LC3 cells that expresses GFP-LC3 fusion protein and thus the autophagosomes within the cells may be monitored under fluorescence microscope. The result showed that down-regulation of SPAG1 decreased autophagy puncta in HCC827/GFP-LC3 cells (Fig. 4C). Taken together, these findings suggest that SPAG1 enhances autophagy.

The fragment of 130 ~ 170 amino acid residues in SPAG1 can inhibit AKT phosphorylation and cell proliferation

To map the molecular domain of SPAG1 involved in the inhibition of AKT and proliferation, we firstly constructed three truncations of SPAG1 that encodes cDNA of 1 ~ 400 amino acids (aa), 300 ~ 700 aa and 600 ~ 925 aa, respectively. The truncations were transfected into A549 cell, and their effects on AKT phosphorylation and proliferation were examined. The results showed only SPAG1 (1 ~ 400 aa) had the inhibitory effect on AKT phosphorylation and proliferation (Fig. 5A & B), implying the region involved in this function should be within 1 ~ 300 aa. Then we constructed three truncations that encodes cDNA of 1 ~ 150 aa, 150 ~ 300 aa and 100 ~ 200 aa, and tested their effects in A549 cells. The results showed that SPAG1 (100 ~ 200) had the inhibitory effect, but the other two truncations had no effect at all (Fig. 5A & B). Based on the results, we speculated that the functional region might be around 150 aa. Thus, we constructed two truncations that encodes cDNA of 130 ~ 170 aa and 140 ~ 160 aa, and tested their effects in A549 cells. The results showed SPAG1(130 ~ 170) had the inhibitory effect on AKT phosphorylation and proliferation (Fig. 5A & B). These results suggest that the region of 130 ~ 170 amino acids in SPAG1 is the key domain in the regulation of AKT and cell proliferation.

SPAG1 inhibits tumor growth

To further explore the effect of SPAG1 on tumor growth, we established HCC827/SPAG1 shRNA cell line within which SPAG1 was down-regulated by stably expressing SPAG1 shRNA and the control cell line, and then xenograft models were created. The result indicated that the tumors of HCC827/SPAG1 shRNA cells grew faster than the control group, which led to bigger final tumor mass and volume (Fig. 6A, B & C). Western blot analysis of xenografts revealed that SPAG1 knockdown resulted in a remarkable increase of p-AKT (Ser473) and p-S6K1 (Thr421/Ser424) (Fig. 6D), which was consistent to the above *in vitro* results. Moreover, the immunohistochemistry results demonstrated that the percentage of Ki-67 positive cells was increased significantly in HCC827/SPAG1 shRNA tumors compared with that in the control tumors (Fig. 6E), indicating increased cell proliferation in HCC827/SPAG1 shRNA tumors. Taken together, the results from xenografts suggested that SPAG1 suppresses LUAD tumor growth.

Discussion

The present study demonstrated that SPAG1 is upregulated in LUAD tissues and associated with favorable prognosis of patients with LUAD. Silina et al reported that SPAG1 RNA was increased in 5 out of 7 lung cancer samples while was not detected in normal lung samples (19), which might result in bias in conclusion as the sample size was small. To overcome the drawback of their study, we analyzed three

LUAD gene expression datasets (TCGA LUAD, GSE75037 and GSE31210) in which the gene expression data of a relatively large numbers of LUAD samples were included. The results from three datasets coincidently exhibited that SPAG1 is significantly upregulated in LUAD tissues, which verified the conclusion of the study by Silina et al (19). Furthermore, Kaplan-Meier Plotter analysis in our study demonstrated that SPAG1 high expression is associated with favorable prognosis, including OS, FP and PPS, implying that SPAG1 might has an inhibitory role in LUAD progression, and it might be a potential prognostic biomarker.

The present study revealed a novel function of SPAG1 that it inhibits cell proliferation and tumor growth of LUAD. Silina et al found SPAG1 increase in lung cancer (19), but they did not investigate the role and mechanisms of SPAG1 in lung cancer. As sustaining proliferative signaling is a hallmark of cancer cells (30), which results in uncontrolled proliferation of cancer cells, we analyzed the effects of SPAG1 on cell proliferation and colony formation by up- and down-regulating its expression. We observed that SPAG1 inhibited the proliferation LUAD and HeLa cells *in vitro*, and tumor growth *in vivo*. Moreover, we observed that SPAG1 knockdown resulted in faster growth and bigger mass of xenograft tumors of LUAD. These results provided cellular evidences to support our prognosis results.

The present study proposed a novel mechanism that SPAG1 suppresses AKT/mTORC1 signaling axis, which provides support to SPAG1's role on prognosis, cell proliferation and tumor growth in LUAD. Moreover, we identified the domain of SPAG1 involved in the inhibition of AKT activity and cell proliferation. As AKT signaling pathway is often excessively activated in lung cancer due to the oncogenic mutations of the upstream molecules, such as EGFR and KRAS, it is worth to investigate in the future whether SPAG1 and/or its inhibitory domain have the potential to become therapeutic agents for LUAD. However, we have to point out that it is regretful that we could figure out how SPAG1 and AKT are linked due to poor performance of commercial SPAG1 antibody in co-immunoprecipitation experiment. Since Hsp90 binding to AKT is important to AKT activity (31), and SPAG1 can bind to Hsp90 (13), we hypothesized that SPAG1 probably competes with AKT to bind Hsp90, and thus is involved in regulation of AKT activity and cell proliferation. Future studies that test this hypothesis are warranted.

We noticed that our finding are different from a previous study, which showed in breast cancer that SPAG1 expression was not associated with OS, distant metastasis-free survival as well as post-progression survival of patients with breast cancer, but was associated with poor relapse-free survival (20), and that SPAG1 promotes cell proliferation, colony formation and cell cycle progression in breast cancer cells (18, 20). The possible explanation for these differences between breast cancer and LUAD might be due to that SPAG1 functions through distinct mechanisms in different cancer cell context.

Conclusions

SPAG1 is upregulated in LUAD tissues and associated with favorable prognosis of patients with LUAD, and plays an inhibitory role in cell proliferation and tumor growth of LUAD through the AKT/mTORC1 signaling axis.

Abbreviations

SAPG1
Sperm-associated antigen 1
LUAD
Lung adenocarcinoma
NSCLC
Non-small cell lung carcinoma
OS
Overall survival
FP
First progression
PPS
Post-progression survival
mTOR
Mechanistic target of rapamycin
mTORC1
mTOR complex 1
S6K1
Ribosomal protein S6 kinase beta-1
TPR
Tetratricopeptide repeat
ERK1/2
Extracellular signal-regulated kinases ½
Hsp70
70-kDa heat-shock protein
Hsp90
90-kDa heat-shock protein
GSEA
Gene set enrichment analysis
FDR
False discovery rate
GEO
Gene Expression Omnibus
TCGA
The Cancer Genome Atlas
LC3
Microtubule-associated proteins 1A/1B light chain 3
aa
Amino acids

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Author contributions

AL, JG, YW, QH and RG performed the experiments. KH performed the bioinformatical analysis. GH conceived of the study, and designed experiments. AL, YG and GH analyzed the data, and drafted the manuscript. All authors read and approved the final manuscript.

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Figures

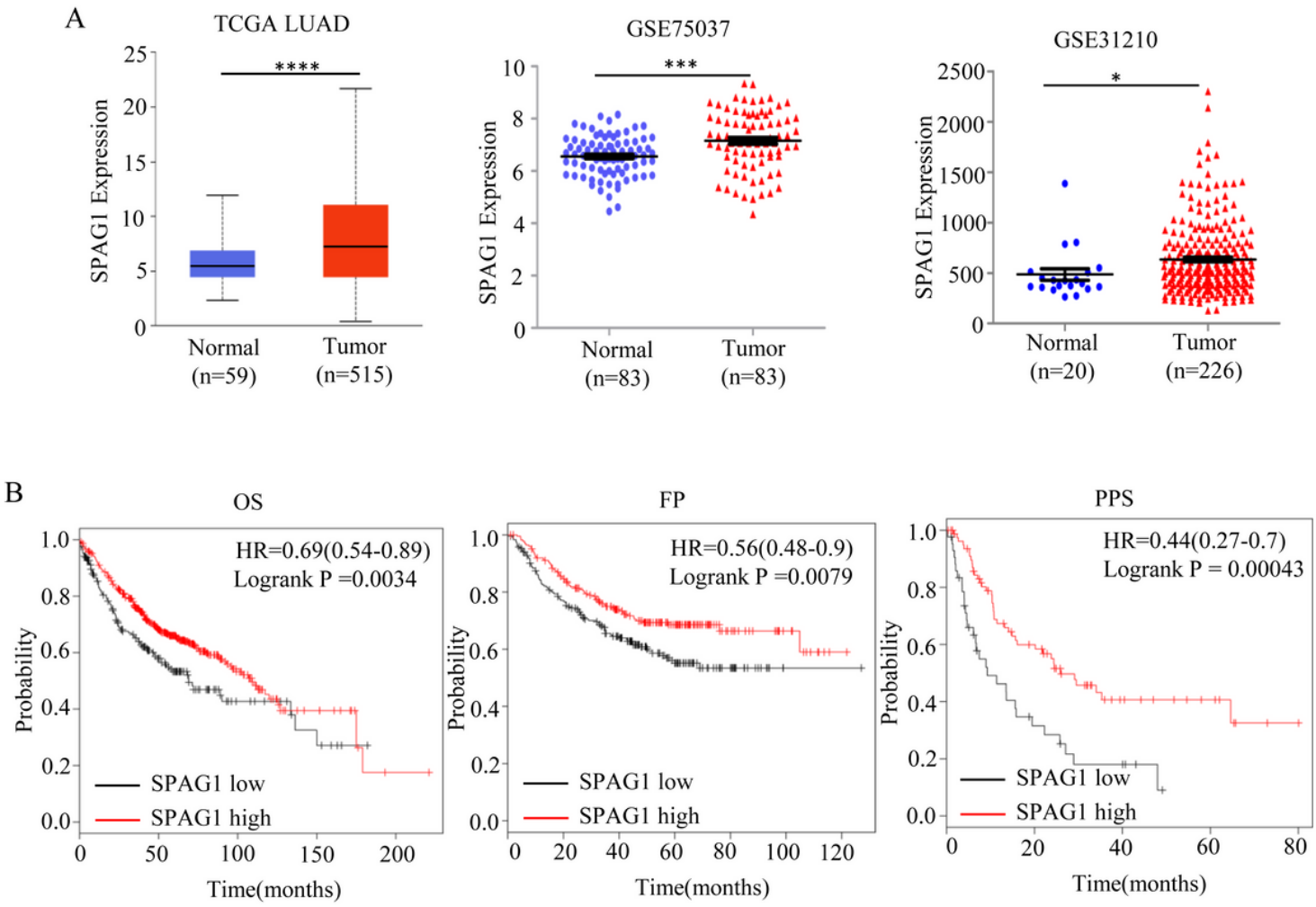

Figure 1

SPAG1 expression is significantly upregulated in LUAD tissues, and associated with favorable prognosis of patients with LUAD. (A)SPAG1 expression was up-regulated significantly in LUAD tissues of TCGA, GSE75037 and GSE31210 datasets. (B) High expression of SPAG1 was associated with favorable overall survival (OS), first progression (FP) and post-progression survival (PPS) of patients with LUAD. *P < 0.05, ***P < 0.001, ****P < 0.0001.

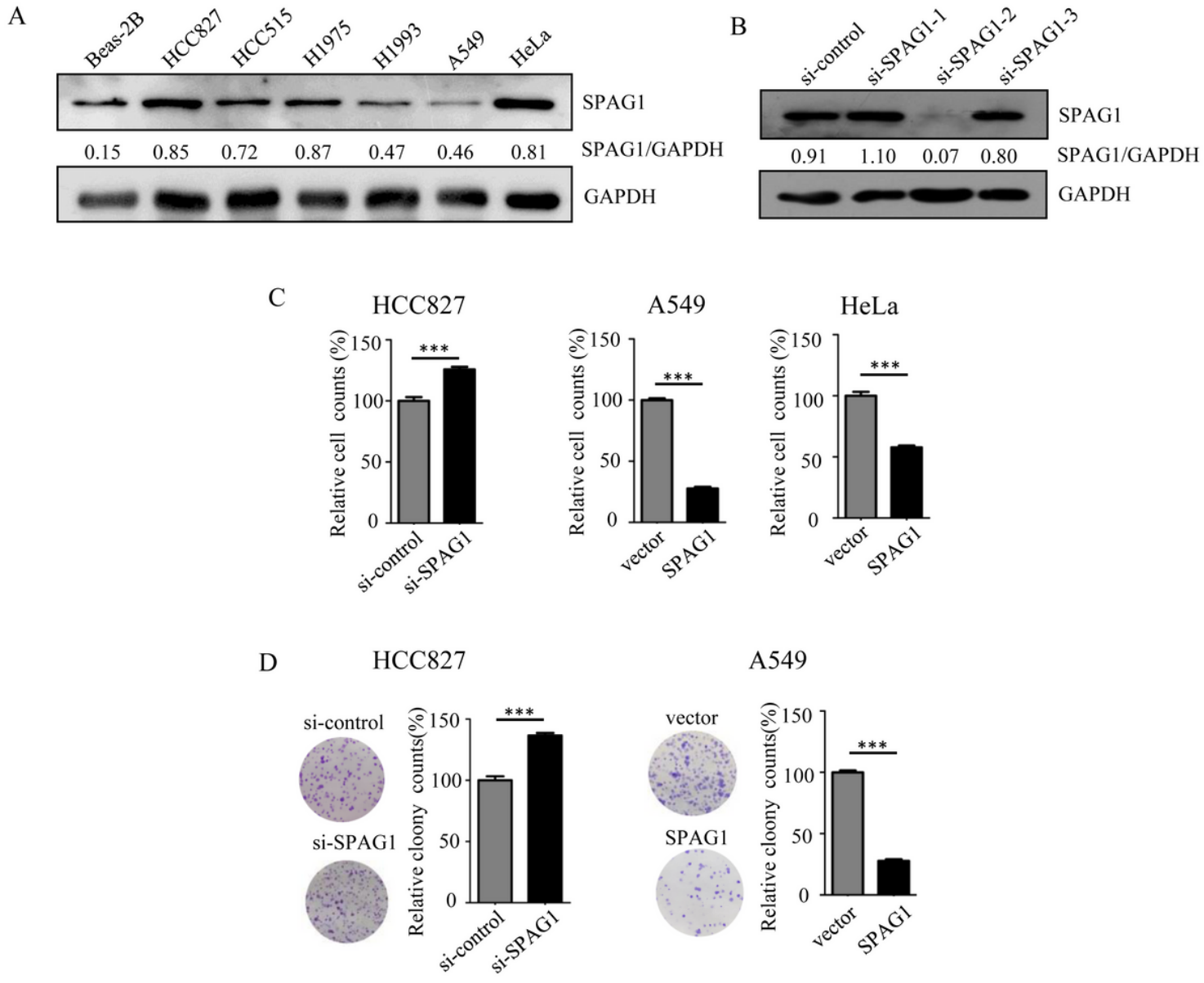

Figure 2

SPAG1 inhibits the proliferation and colony formation of lung adenocarcinoma cells (A) SPAG1 protein levels in LUAD cells, Beas-2B and HeLa cells. (B) si-SPAG1-2 had the highest efficacy in HCC827 cells. (C) SPAG1 knockdown promoted cell proliferation in HCC827, whereas SPAG1 overexpression had the opposite effects on cell proliferation in A549 and HeLa cells. (D) SPAG1 knockdown promoted colony formation in HCC827 cells, whereas SPAG1 overexpression had the opposite effects in A549 cells. ***P<0.001.

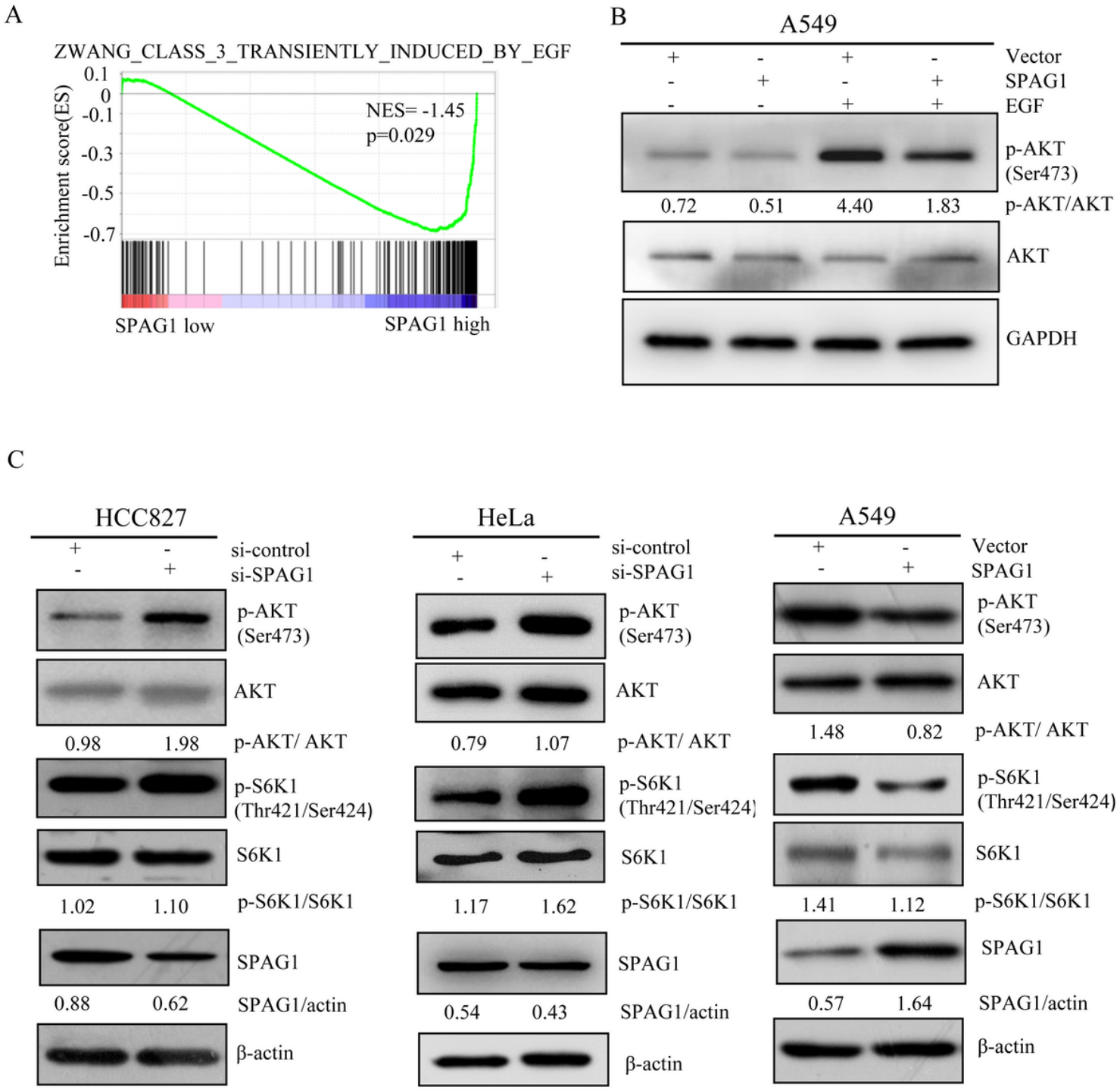

Figure 3

SPAG1 inhibits AKT/mTORC1 signaling axis (A) The gene set induced by EGF was inhibited by SPAG1 high expression. FDR, false discovery rate; NES, normalized enrichment score. (B) SPAG1 overexpression inhibited the basal and EGF-induced phosphorylation of p-AKT (Ser473) in A549 cells. (C) SPAG1 knockdown resulted in increased p-AKT (Ser473) and p-S6K1(Thr421/Ser424) in HCC827 and HeLa cells, whereas SPAG1 overexpression had the opposite effects on p-AKT (Ser473) and p-S6K1(Thr421/Ser424) in A549 cells.

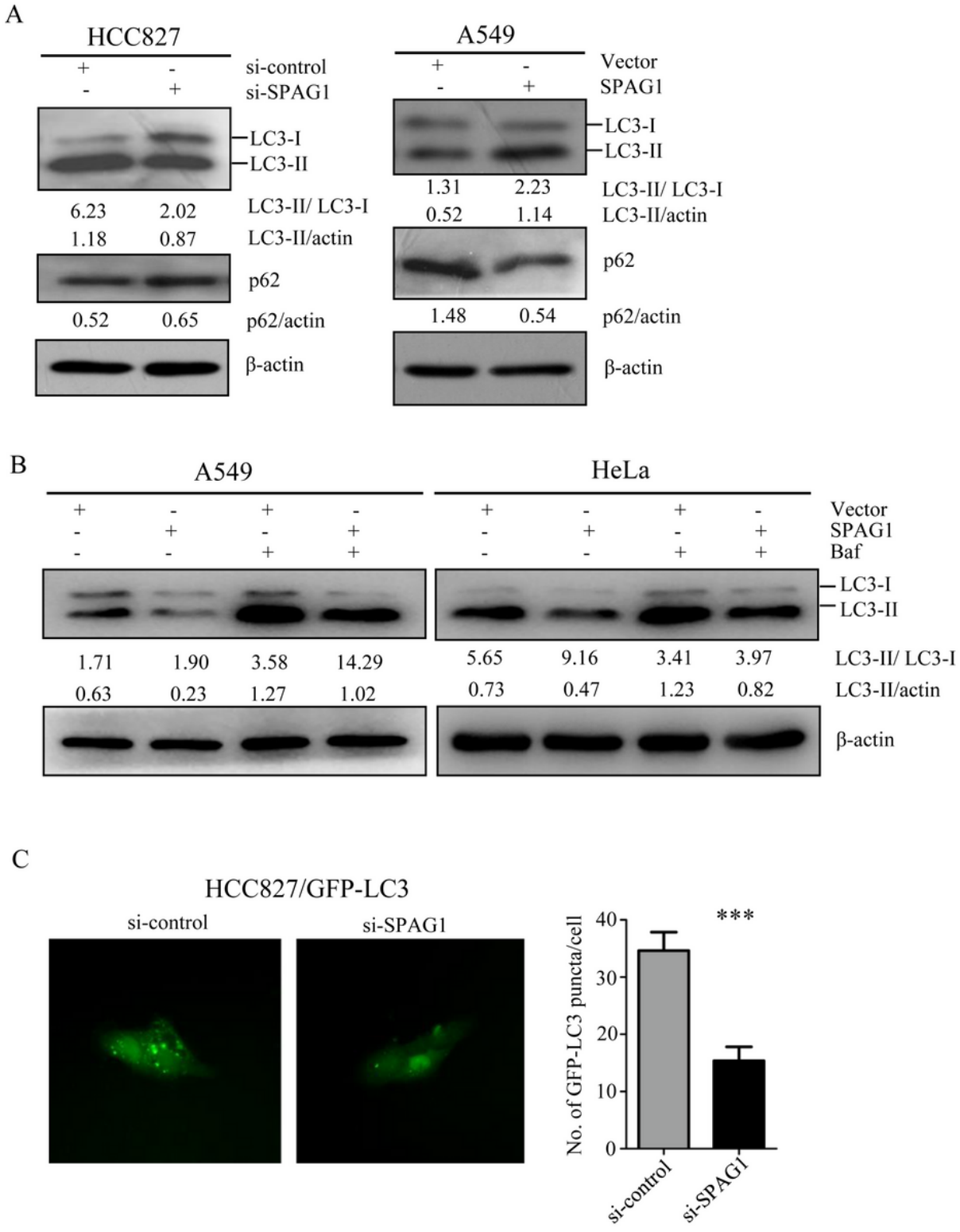

Figure 4

SPAG1 enhances autophagy (A) Autophagy was suppressed by SPAG1 knockdown, whereas enhanced by SPAG1 overexpression in LUAD cells. The autophagic markers p62/SQSTM1 and LC3 were detected by Western blot assay. The bands were quantified using ImageJ and normalized to the loading control. (B) Bafilomycin A1 (Baf) treatment led to LC3-II accumulation in A549 and HeLa cells, which confirmed that autophagy was enhanced by SPAG1 overexpression. (C) The autophagosomes (green puncta) were

decreased significantly upon SPAG1 knockdown in HCC827-GFP-LC3 cells, indicating reduced autophagy. ***P<0.001.

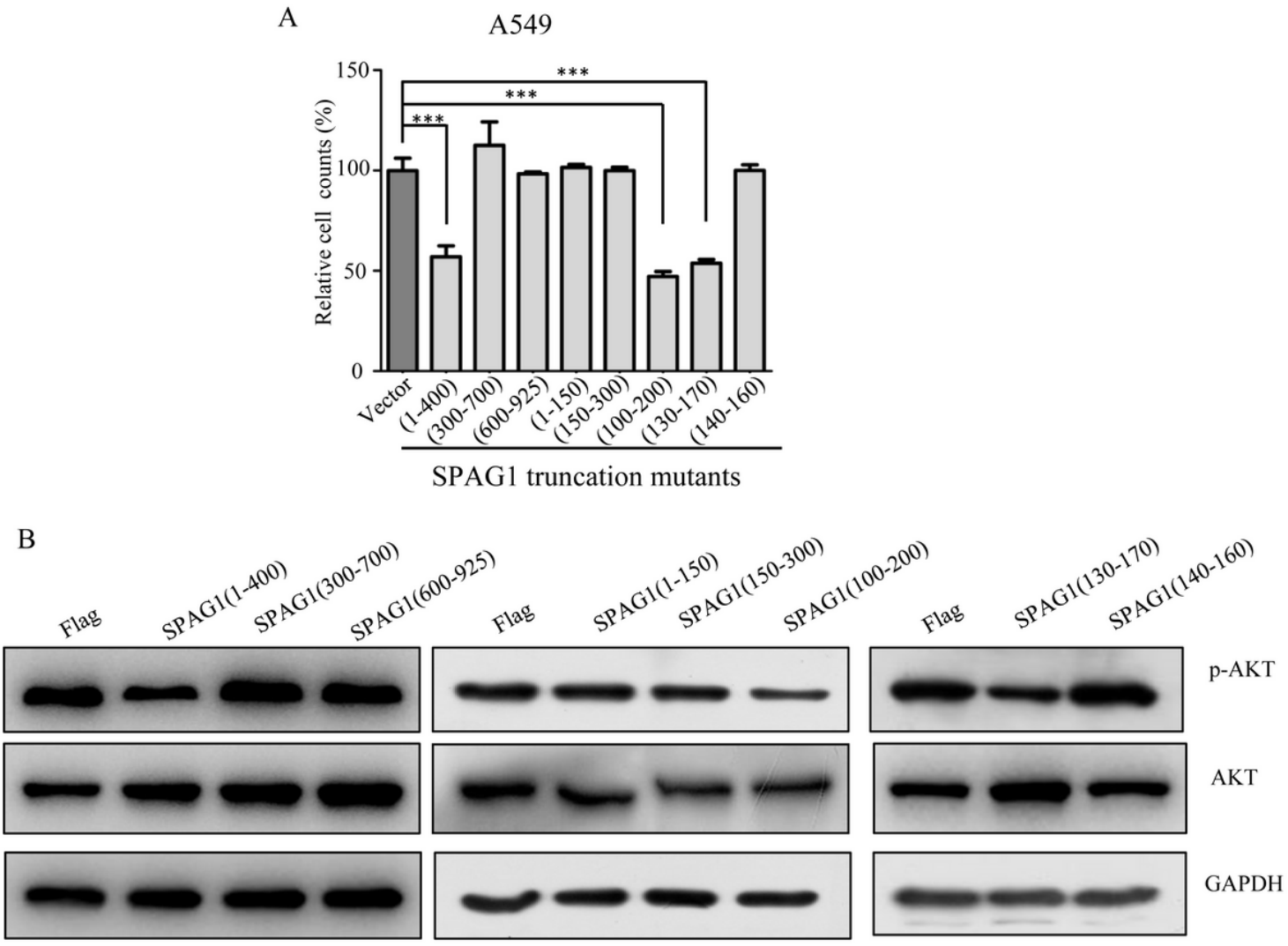

Figure 5

The molecular domain of SPAG1 involved in cell proliferation and AKT phosphorylation (A) The effects of SPAG1 truncation mutants on cell proliferation of A549 cells. (B) The effects of SPAG1 truncation mutants on AKT phosphorylation in A549 cells.

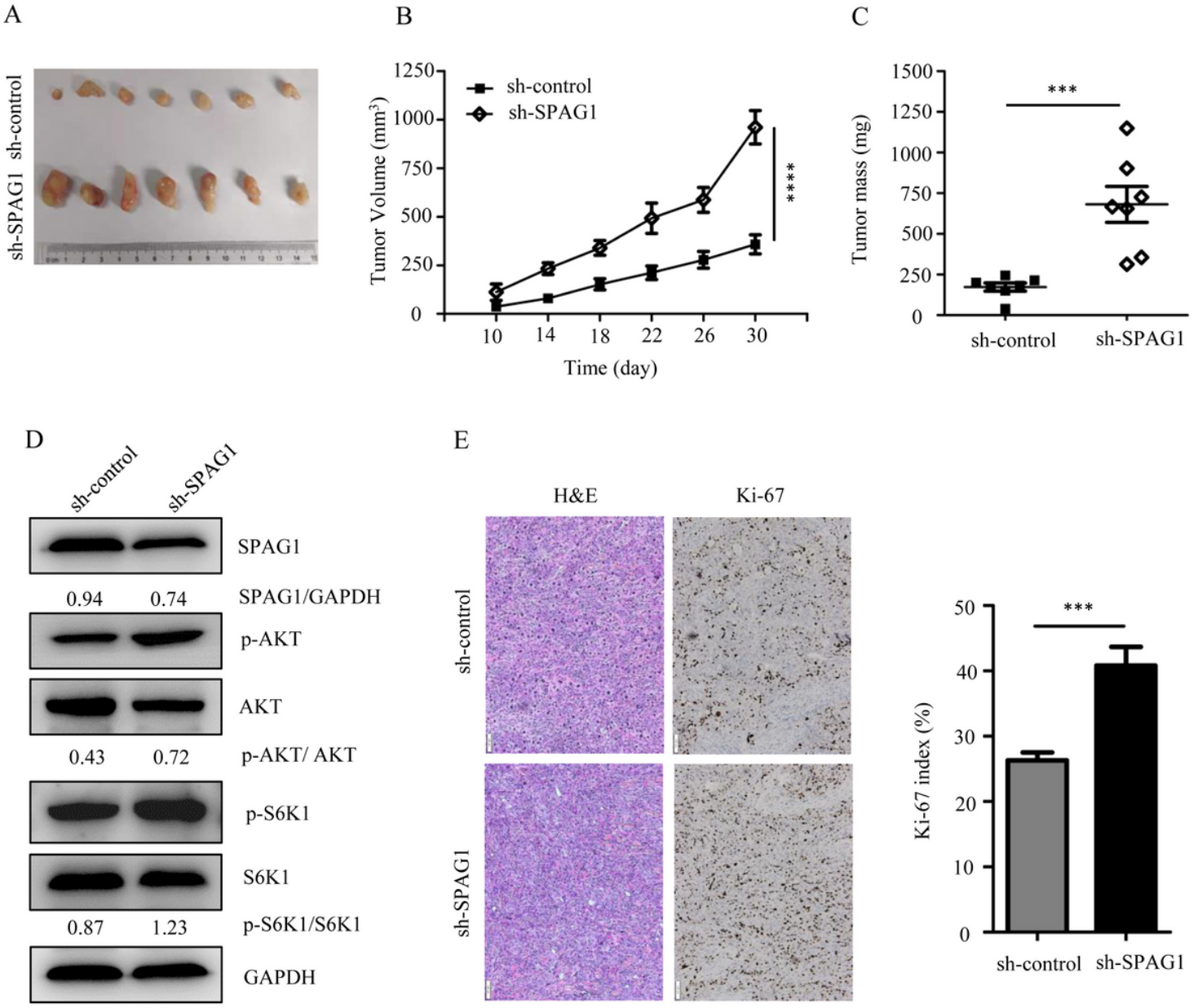

Figure 6

SPAG1 inhibits tumor growth and AKT/mTORC1 signaling in xenografts (A) The images of xenograft tumors of HCC827/sh-SPAG1 and the control. (B) The growth curves of xenograft tumors of HCC827/sh-SPAG1 and the control. (C) The mass of xenograft tumors of HCC827/sh-SPAG1 and the control. (D) Western blot results of SPAG1, AKT, p-AKT, S6K and p-S6K in xenograft tumors of HCC827/sh-SPAG1 and the control. (E) The representative images of H&E staining and Ki-67 IHC staining of xenograft tumors of HCC827/sh-SPAG1 and the control. Column graph shows Ki-67 index in xenograft tumors of HCC827/sh-SPAG1 and the control. ***p < 0.001, ****p < 0.0001.