Received: 7 January 2022 | Revised: 27 June 2022 | Accepted: 15 July 2022

DOI: 10.1111/cas.15515

ORIGINAL ARTICLE

Cancer Science WILEY

Apelin-mediated deamidation of HMGA1 promotes tumorigenesis by enhancing SREBP1 activity and lipid synthesis

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 81772478, 81974363 and 821733251; the Clinical Research Incubation Project of West China Hospital of Sichuan University, Grant/Award Number: 2018HXFH102; the Hospital Enterprise Cooperative Clinical Research Innovation Project, Grant/Award Number: 2019HXCX04; the Sichuan International/Hong Kong, Macao and Taiwan Science and technology innovation cooperation project, Grant/Award Number: 2018hh0161

Abstract

Enhanced fatty acid synthesis provides proliferation and survival advantages for tumor cells. Apelin is an adipokine, which serves as a ligand of G protein–coupled receptors that promote tumor growth in malignant cancers. Here, we confirmed that apelin increased sterol regulatory element–binding protein 1 (SREBP1) activity and induced the expression of glutamine amidotransferase for deamidating high-mobility group A 1 (HMGA1) to promote fatty acid synthesis and proliferation of lung cancer cells. This post-translational modification stabilized the HMGA1 expression and enhanced the formation of the apelin-HMGA1-SREBP1 complex to facilitate SREBP1 activity for lipid metabolism and lung cancer cell growth. We uncovered the pivotal role of apelin-mediated deamidation of HMGA1 in lipid metabolism and tumorigenesis of lung cancer cells.

KEYWORDS
apelin, glutamine deamidation, HMGA1, lipid metabolism, lung tumorigenesis

1 | INTRODUCTION

As an adipokine, apelin is secreted by adipocytes and participates in multiple processes, including energy metabolism, fluid homeostasis, and angiogenesis.[1–3] The apelin gene (*APLN*) is highly expressed in many malignant cancers, such as lung cancer, and is associated with poor prognosis.[4,5] Plasma apelin and tumor apelin levels are significantly higher in lung cancer patients.[6] Apelin overexpression in NSCLC cells leads to tumor growth in mice, while neither apelin transfection nor exogenous apelin stimulates NSCLC cell proliferation.[7] However, Yang et al. demonstrated that apelin peptide promotes A549 cell proliferation.[6] Therefore, the biological function of apelin in NSCLC cells is controversial and needs to be further studied.

The HMGA1 protein is a transcriptional enhancer that elevates oncogene expression by interacting with DNA and protein in cancer cells.[8,9] High levels of HMGA1 protein drive metabolic alterations that contribute to tumorigenesis through aerobic glycolysis and fatty acid (FA) synthesis.[10,11] Similar to histones, the biological activities of the HMGA1 are highly regulated by their post-translational modifications, including acetylation, methylation, and phosphorylation.[12] Glutamine deamidation is the conversion of glutamine to glutamate.[13–15] Here, we found apelin induced the expression of glutamine amidotransferases (GATs), especially trifunctional enzyme carbamoyl-phosphate synthetase, aspartyl-transcarbamoylase, and dihydroorotase (CAD), for deamidating HMGA1 and enhancing FA synthesis.

Activated de novo lipogenesis accelerates tumor cell growth and is closely associated with poor prognosis in many tumor types.[16,17] Recent studies have demonstrated adipokines might have a key role in cancer development.[18] The level of apelin is also elevated in those cancer types connected with adiposity.[19,20] In this study, we revealed that apelin induced the deamidation of HMGA1 by CAD and enhanced abnormal lipid metabolism in NSCLC cells.

2 | MATERIALS AND METHODS

2.1 | Reagents and antibodies

All the antibody information is listed in Table S1. Hoechst was obtained from Sigma-Aldrich. Nile red and L-6-diazo-5-oxonorleucine (DON) were purchased from MCE.

2.2 | Lentivirus-mediated stable cell line construction

The apelin lentiviral activation particle was used for the construction of stable apelin overexpression cell lines (Santa Cruz). The shRNA lentiviral particles of apelin and HMGA1 were respectively used for the construction of apelin-knockdown and HMGA1-knockdown cell lines (Santa Cruz). Controls were also transfected with control lentiviral particles (Santa Cruz).

2.3 | Plasmid construction and RNAi transfection

All plasmids were constructed by Genecopoeia. siRNAs targeting SREBP1 (sc-36557) and HMGA1 (sc-37115) were purchased from Santa Cruz. The siRNA against CAD and APJ were designed and synthesized by RiboBio.

2.4 | Glycerol gradient analysis

293T cells were transfected with HA-apelin, Flag-HMGA1 WT, or Flag-HMGA1 deamidation mutants expressing plasmids for 48 hours. Cell pellets were collected and resuspended in lysis buffer. The lysed cells were loaded onto a 10%-40% glycerol gradient prepared in lysis buffer. Then, tubes were ultracentrifuged at 250,000 *g* for 16 hours at 4 °C. A total of 700 μl fractions were collected, and proteins were precipitated by the methanol-chloroform precipitation method. Proteins were dissolved in SDS-PAGE sample buffer for immunoblotting.

2.5 | RNA isolation and quantitative PCR analysis

Cells were used for RNA extraction, cDNA synthesis, and quantitative PCR with gene-specific primers. Some metabolism-related gene primers were obtained from the literature.[21] All primers are listed in Table S2.

2.6 | Immunohistochemical (IHC) staining and immunofluorescence

The Department of Pathology, West China Hospital of Sichuan University, provided specimens of primary lung tumor tissues and normal pulmonary tissues surgically resected from patients treated without radiotherapy or chemotherapy. Paraffin embedding, tissue sectioning (5 μm), and IHC staining for tissues were performed.

2.7 | Xenograft mouse model

A549-NC and A549-OE cells (5×10^6 cells) were inoculated into nude mice subcutaneously ($n = 10$ per group), and the tumors were isolated and weighed after 35 days. To evaluate tumor metastasis, A549-NC and A549-OE cells (5×10^6 cells) were injected into the tail vein of the nude mice. The lung tissues were sectioned, and hematoxylin-eosin (H&E) staining was performed after 40 days.

To induce lung adenocarcinoma (LUAD) in situ, LSL-$Kras^{G12D}$ mice were infected with the adeno-associated virus AAV-CAG-EGFP-T2A-Cre (8.19×10^{12} pfu) by tracheal intubation. LSL-$Kras^{G12D}$ mice developed LUAD approximately 8 weeks after infection. Then, lung tissues were removed, and IHC was performed.

2.8 | Bioinformatics analysis

We analyzed the overall survival of lung cancer patients with the apelin and HMGA1 expression using online analysis tools (http://www.kmplot.com). We investigated HMGA1 expression in lung tumor tissues from the Human Protein Atlas database (http://www.proteinatlas.org) and GEPIA database (http://www. gepia.cancer-pku.cn).

2.9 | Statistical methods

All analyses were performed using GraphPad Prism 7. The data was represented as mean ± SEM. The two groups were compared with Student *t* test. The asterisks in the figures indicate statistical significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3 | RESULTS

3.1 | Apelin increased the proliferation and metastasis of lung cancer cells

Firstly, we confirmed the high expression of apelin in lung squamous cell carcinoma (LUSC) and LUAD tissues compared with normal lung tissues (Figure 1A). Lung cancer patients with high expression of apelin exhibited a decreased trend in overall survival from the KMplot website (Figure S1A). It indicated apelin gene is involved in the development of NSCLC. APJ, an apelin receptor, is highly coexpressed with apelin in many tissues.[2] To determine whether apelin and APJ are coexpressed in a cell type–specific manner to regulate lung cancer proliferation, we detected the expression of apelin and APJ in four human NSCLC cell lines (A549, H1299, H1975, and H460 cell). Apelin was highly expressed in H1299 and H460 cells (Figure 1B,C), while APJ was highly expressed only in A549 cells (Figure S1B). Next, we established apelin-overexpressing cell lines (A549-OE and H1975-OE cells) to investigate the function of apelin in NSCLC cells (Figure 1D). Apelin increased cell viability (Figure 1E) and colony formation (Figure 1F) in A549-OE and H1975-OE cells. To explore whether apelin works through its receptor APJ, we performed siRNA knockdown of APJ in A549-NC and A549-OE cells. siRNA-mediated knockdown of APJ (si-APJ) did not completely inhibit apelin-mediated cell proliferation (Figure S1C,D), which suggested that apelin might induce cancer growth of NSCLC cells in a manner independent of the apelin-APJ system. Then, we injected A549-NC and A549-OE cells into nude mice by subcutaneous implantation and tail vein injection, respectively. Compared with A549-NC cells, A549-OE cells accelerated the formation of subcutaneous tumors and significantly increased the tumor volume and tumor weight after implantation (Figure 1G,H, and Figure S1E). Tail vein injection of A549-OE cells resulted in more metastatic nodules than tail vein injection of A549-NC cells (Figure 1I). All these results indicated that apelin promoted the growth and metastasis of lung cancer cells.

3.2 | Apelin induced metabolic reprogramming in lung cancer cells by enhancing lipid synthesis

Next, we assayed variations in gene expression and signaling pathways in A549-NC and A549-OE cells by RNA-Seq. We discovered multiple genes involved in metabolic metabolism and FA metabolism that were specifically upregulated in A549-OE cells (Figure 2A). Key lipogenic proteins were highly expressed in both A549-OE and H1975-OE cells, including SREBP1 and ACC (Figure 2B). Subsequently, we analyzed total cellular lipid extracts by gas chromatography and found that apelin elevated the levels of cellular lipids such as glycerophospholipids (GP), glycerolipids (GL), and sphingolipids (SL) in A549-OE and H1975-OE cells in vitro (Figure 2C). We then performed Nile red staining and confirmed more lipid accumulation in A549-OE and H1975-OE cells (Figure 2D). In addition, we found that apelin promoted glucose uptake in A549-OE cells (Figure 2E). Glucose is a major resource for de novo lipid synthesis in cancer cells, and elevated glucose consumption is often accompanied by increased lipogenesis (Figure 2F). Cultured with [U-^{13}C6] glucose for 48 hours, apelin promoted the synthesis of both saturated FAs (palmitate [PA], stearate, and myristate; Figure 2G) and unsaturated FAs (palmitoleate, oleate, and arachidonate; Figure S2A) in A549-OE cells. The intermediaries of the TCA cycle were mostly decreased in A549-OE cells (Figure 2H), which indicated that apelin induced metabolic reprogramming by promoting lipid synthesis.

To further investigate the role of apelin in lipogenesis and cell proliferation, we established H460-apelin knockdown (KD) cells and found lipogenic proteins were also dramatically inhibited (Figure 2I). Knockdown of apelin dramatically inhibited glucose uptake and decreased lipid content (Figure 2J and Figure S2B). At the same time, downregulation of apelin significantly inhibited cell proliferation (Figure 2K) and colony formation (Figure S2C). We added PA to a culture medium containing delipidated FBS and found that cellular proliferation was dramatically rescued in H460-apelin KD cells (Figure 2K). Taken together, these findings demonstrated that apelin promoted the proliferation of lung cancer cells by inducing FA synthesis.

3.3 | Activation of SREBP1 was critical in apelin-mediated lipid metabolism

An active N-terminal cleavage of SREBP1 is a key transcription factor that activates many lipogenic genes required for FA synthesis, such as *ACC* and *FASN*.[22,23] In our study, we observed that apelin promoted the expression of active N-terminal cleavage of SREBP1 (SREBP1-N) expression in A549-OE and H1975-OE cells (Figure 3A). Recent evidence has shown that the N-terminal cleavage of SREBP1 in the nucleus is highly upregulated and promotes FA synthesis in a variety of malignancies.[24] Therefore, we further investigated whether active SREBP1 was involved in apelin-induced abnormal lipid metabolism and cellular growth. Using confocal fluorescence imaging, we found that apelin promoted the nuclear translocation of

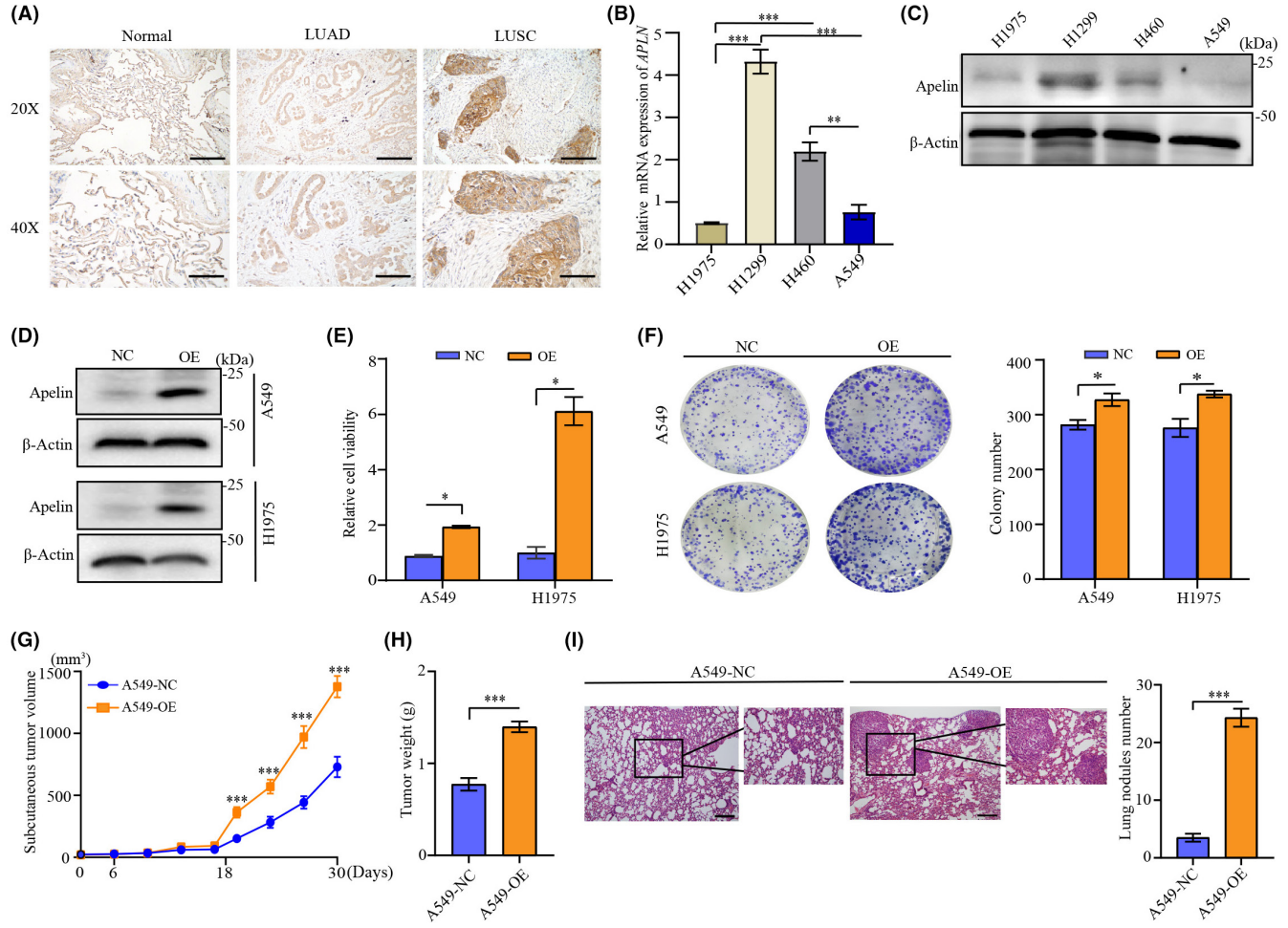

FIGURE 1 Apelin increased the proliferation of lung cancer cells. A, Typical images of apelin immunohistochemical (IHC) staining in lung cancer tissues and normal lung tissues. Scale bars: 20X = 100 μm, 40X = 50 μm. B, C, mRNA and protein expression of apelin in lung cancer cell lines (A549, H1299, H1975, and H460). D, Apelin protein expression in apelin-overexpressing cells (A549-OE and H1975-OE cells) and negative control cells (A549-NC and H1975-NC cells). E, F, Cell viability and colony formation in apelin-overexpressing cells and negative controls. G, H, Tumor volume and weight in nude mice injected with A549-NC and A549-OE cells. I, Metastasis nodules in nude mice with tail vein injection of A549-NC and A549-OE cells. Scale bars: 100 μm. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

SREBP1 in A549-OE and H1975-OE cells (Figure 3B). Translocation of endogenous SREBP1 into the nucleus in A549-OE and H1975-OE cells was also demonstrated by Western blotting (Figure 3C). In contrast, apelin KD in H460 cells inhibited SREBP1 expression and restrained SREBP1 translocation into the nucleus (Figure S3A,B), which suggested the important role of apelin in SREBP1 activation.

To explore whether activation of SREBP1 participates in lipid metabolism and promotes cell growth, A549-OE and H1975-OE cells were transfected with SREBP1-targeting siRNA (si-SREBP1), and the results indicated that si-SREBP1 significantly inhibited the mRNA and protein expression of SREBP1 and ACC (Figure 3D,E). In addition, siRNA-mediated inhibition of SREBP1 in apelin-overexpressing cells reduced cell viability, but cell viability was rescued after PA addition (Figure 3F). These results strongly suggested the importance of SREBP1 activity in abnormal lipogenesis and cell viability in apelin-overexpressing cells.

3.4 | The apelin-HMGA1 complex facilitated lipid metabolism and lung cancer cell proliferation

To identify apelin regulatory protein in enhancing SREBP1 activity, we performed immunoprecipitation (IP) to seek potential apelin-binding proteins in the lung cancer cells, followed by mass spectrometry analysis. Apelin increased 21 binding proteins in A549-OE cells (Figure S4A), such as high-mobility group A 1 (HMGA1), insulin-like growth factor 2 mRNA-binding protein 2 (IF2B2), and plexin-C1 (PLXC1), which are mainly involved in cellular function including transcription, translation, and receptor activity (Figure 4A). HMGA1 is overexpressed in diverse solid tumors for enabling malignant transformation and metastasis.[25] As an architectural transcription factor, HMGA1 participates in regulating abnormal energy metabolism in tumors.[26] This indicated that HMGA1 might be involved in apelin-mediated lipid metabolism and cell proliferation. We confirmed the

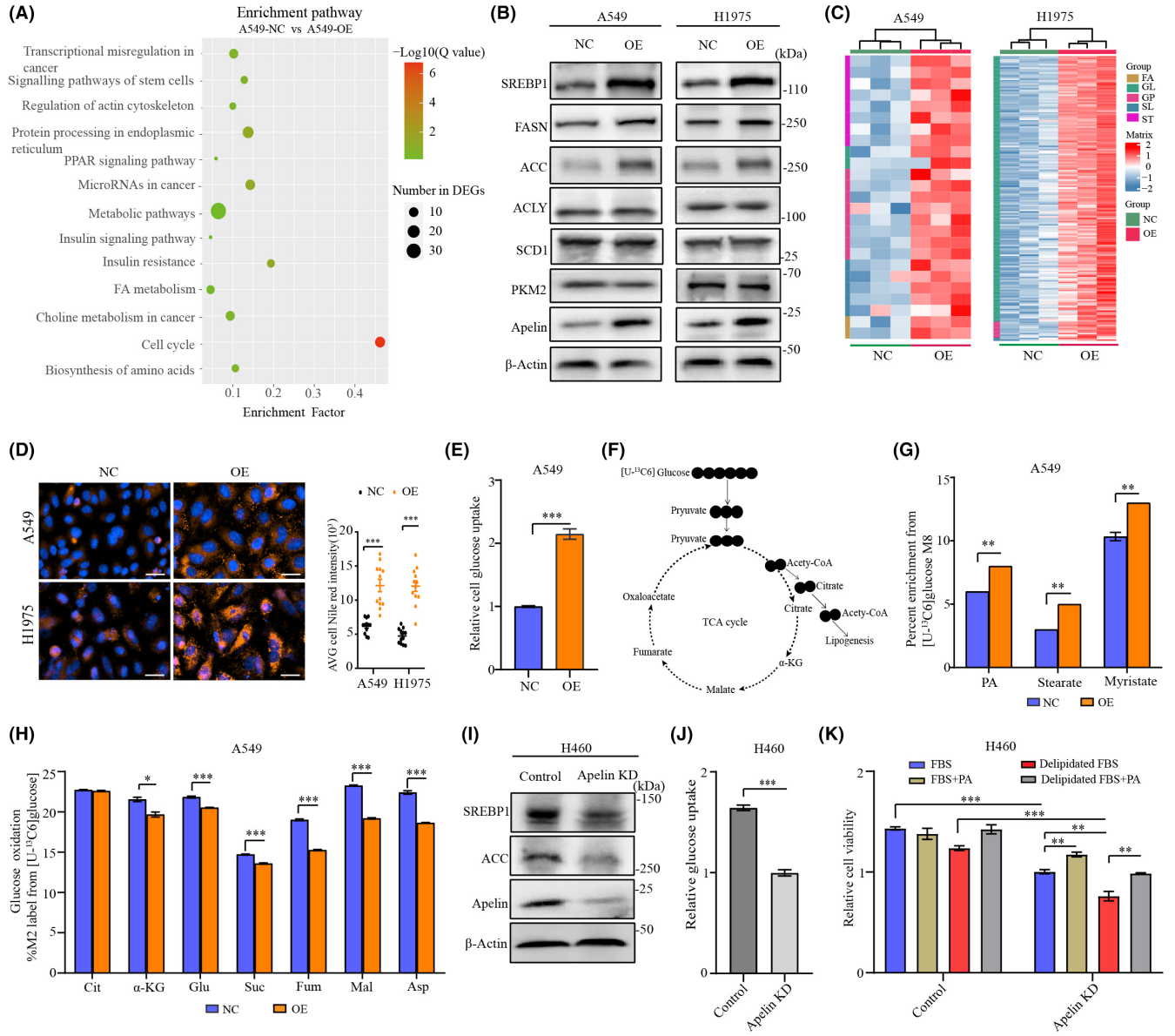

FIGURE 2 Apelin promoted lung cancer cell growth by enhancing de novo fatty acid (FA) synthesis. A, Different enrichment signaling pathways in A549-OE cells compared with A549-NC cells by RNA-Seq analysis. B, Lipid synthesis–related proteins in A549-OE and H1975-OE cells. C, Lipidomics results for lung cancer cells obtained by gas chromatography. D, Lipid levels in A549-OE and H1975-OE cells as determined by Nile red staining. Scale bar: 100 μm. E, Glucose uptake in A549-OE and H1975-OE cells. F, The carbon atom (circle) transitions and tracers are used to detect de novo fatty acid (FA) synthesis. G, H, Enrichment of saturated FAs and glucose oxidation with [U-^{13}C6] glucose in A549-NC and A549-OE cells. Cit, citrate;α-KG, α-ketoglutarate; Glu, glutamate; Suc, succinate; Fum, fumarate; Mal, malate; Asp, aspartate. I, Lipid synthesis–related proteins in H460-control and apelin knockdown (KD) cells. J, Glucose uptake in H460-control and H460-apelin KD cells. K, Cell viability in H460-control and H460-apelin KD cells for 72 h cultured in medium containing FBS or delipidated FBS supplemented with 2 μM exogenous palmitate (PA). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

interaction between apelin and HMGA1 in A549-OE and H1975-OE cells (Figure 4B) and found that the mRNA levels of HMGA1 were increased in A549-OE and H1975-OE cells (Figure S4B). Knockdown of apelin in H460 cells decreased the mRNA and protein expression of HMGA1 (Figure S4C,D). From the Human Protein Atlas database, HMGA1 is highly expressed in NSCLC tumor cells (Figure 4C). The expression of HMGA1 is substantially increased in the tissues of patients with LUAD and LUSC from the GEPIA database (Figure 4D). High HMGA1 levels were associated with poor prognosis in patients with LUAD (Figure 4E). In addition, we found lung cancer tissues exhibited strong coexpression of apelin with HMGA1 and SREBP1 (Figure 4F). These results suggested that HMGA1 might be involved in apelin-mediated lipid metabolism and cell proliferation of lung cancer cells.

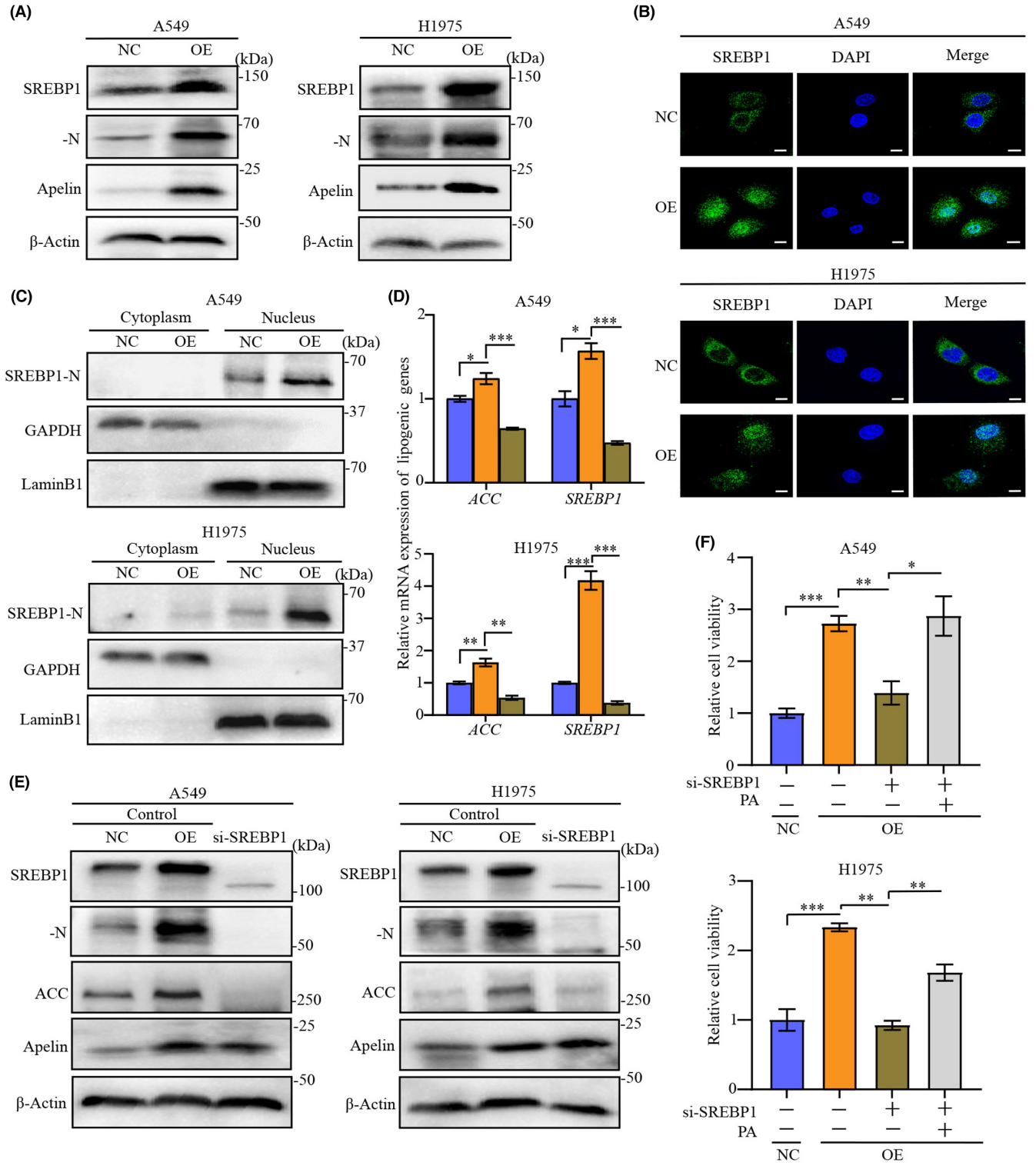

FIGURE 3 Activation of SREBP1 is critical in apelin-mediated lipid metabolism. A, The N terminal of SREBP1 in A549-OE and H1975-OE cells. B, Confocal microscopy images of SREBP1 trafficking to the nucleus in A549-OE and H1975-OE cells. Scale bar: 10 μm. C, SREBP1-N detection in the nuclear extracts of A549-OE and H1975-OE cells. D, E, The mRNA and protein expression of SREBP1 and ACC in A549-OE and H1975-OE cells treated with small-interference RNA (si-SREBP1). F, Cell viability in A549-OE and H1975-OE cells was inhibited after treatment with si-SREBP1 but rescued after palmitate (PA) (2 μM) treatment. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

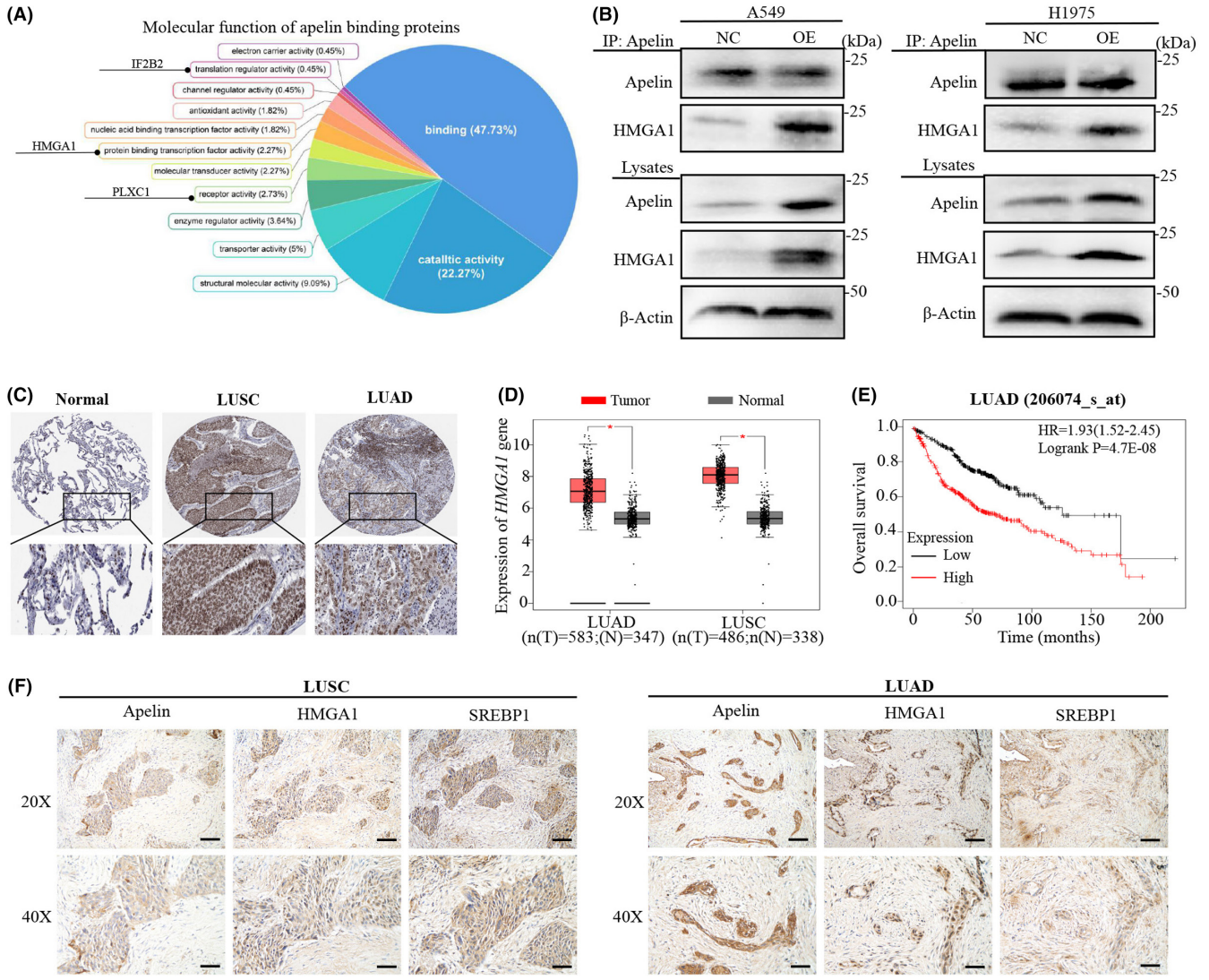

FIGURE 4 The apelin-HMGA1 complex facilitated lipid metabolism in lung cancer cells. A, Mass spectrometry analysis of apelin-binding proteins in A549-NC and A549-OE cells. B, Immunoprecipitation (IP) detected apelin-HMGA1 interaction in A549-OE cells and H1975-OE cells. C, Immunohistochemical (IHC) images from the Human Protein Atlas database on the expression of HMGA1 in patient tissues with lung squamous carcinoma (LUSC), lung adenocarcinoma (LUAD), and normal controls. D, The HMGA1 expression in human LUAD and LUSC tissues; the data is from the GEPIA database. E, The effect of HMGA1 expression on the overall survival time of LUAD patients; the data is from the Kaplan-Meier Plotter website. F, IHC staining for apelin, HMGA1 and SREBP1 expression in lung cancer tumor tissues. Scale bars: 20X = 100 μm, 40X = 50 μm

3.5 | HMGA1 enhanced SREBP1 activity via the formation of a multiprotein complex

Next, HMGA1-targeted siRNA (si-HMGA1) was used to investigate the role of HMGA1 in apelin-mediated SREBP1 activity and cell growth. Knockdown of HMGA1 expression inhibited the mRNA and protein expression of SREBP1 and downstream kinase ACC in apelin-overexpression cells (Figure 5A,B). Glucose uptake capacity and lipid contents were both restrained in A549-OE and H1975-OE cells upon si-HMGA1 treatment (Figure 5C,D). The cell viability of A549-OE and H1975-OE cells was suppressed after the knockdown of HMGA1 expression but was rescued by the addition of PA (Figure 5E,F). These results suggested that HMGA1 may regulate apelin-mediated lipid metabolism and cell growth by activating SREBP1. Next. we extensively explored the mechanisms by which HMGA1 regulated the activity of SREBP1. Co-IP assays revealed that apelin formed a multiprotein complex with HMGA1 and SREBP1, but the formation of this complex was interrupted in A549-OE cells after silencing HMGA1 (Figure 5G). This indicated that HMGA1 might be recognized as a regulator protein, which is capable of regulating SREBP1 activity and lipid synthesis gene expression by forming a multiprotein complex. In addition, knockdown of HMGA1 in H460 cells (H460-HMGA1 KD cells) with endogenous overexpression of apelin also reduced the expression of SREBP1 and ACC and restrained active N-terminal SREBP1 into the nucleus, leading to a decrease in the proliferation of H460-HMGA1 KD cells (Figure S5A–C). These results further confirmed that HMGA1 was involved in apelin-mediated lipid metabolism and SREBP1 activity.

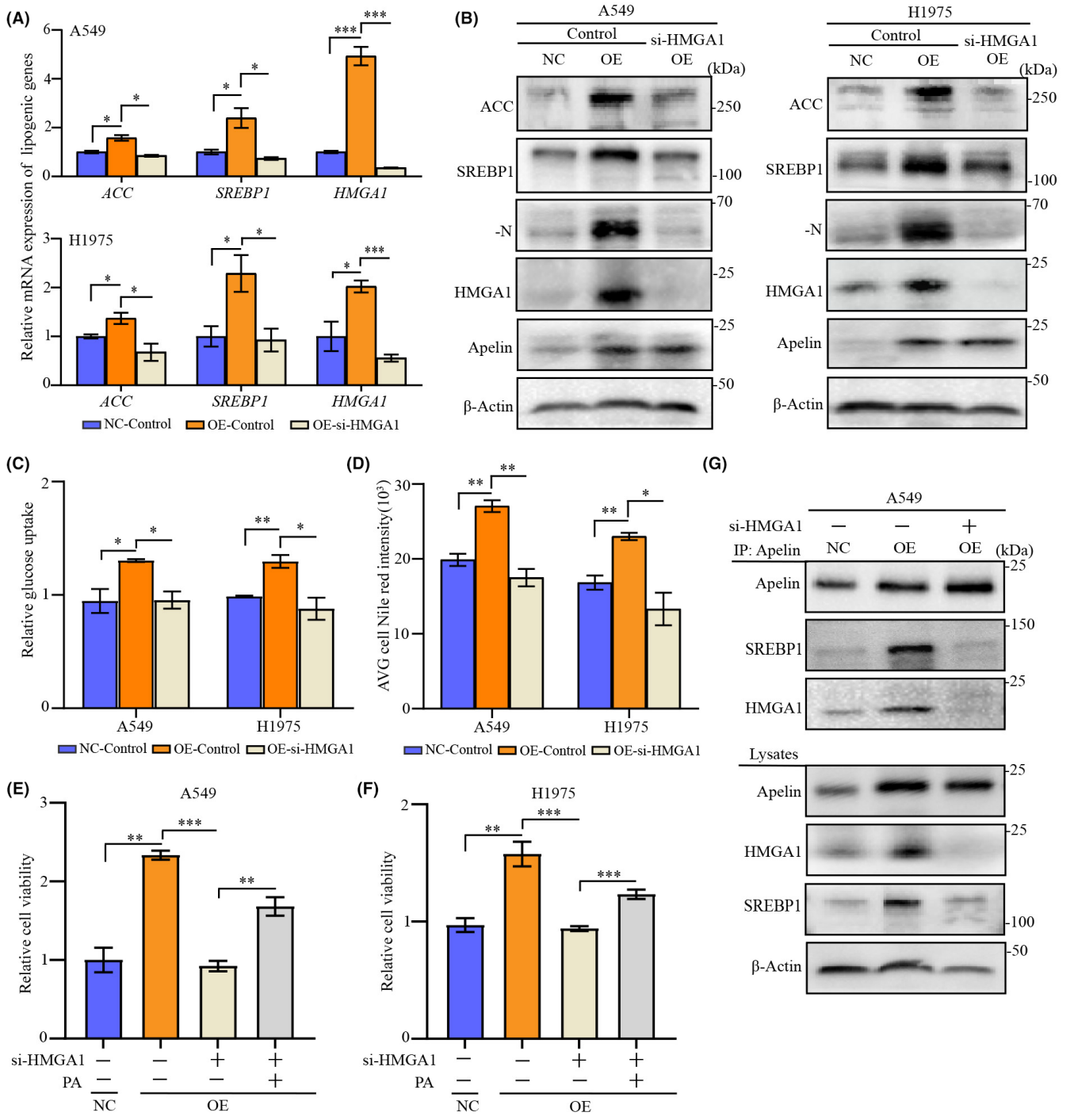

FIGURE 5 HMGA1 activated SREBP1 via the formation of a multiprotein complex. A, B, The mRNA and protein expression of ACC and SREBP1 in A549-OE and H1975-OE cells treated with si-HMGA1. C, Glucose uptake in A549-OE and H1975-OE cells treated with si-HMGA1. D, E, and F, Lipid contents and cell viability in A549-OE and H1975-OE cells treated with si-HMGA1. G, Immunoprecipitation (IP) detected the formation of a multiprotein complex of apelin, HMGA1, and SREBP1 with si-HMGA1 treatment in A549-OE cells. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

3.6 | Deamidation of HMGA1 enhanced the binding affinity of multiprotein complex and SREBP1 activity

HMGA1 carries an acidic carboxyl-terminus and three AT-hooks, which are involved in binding to the minor groove of AT-rich DNA.[27,28] The binding affinity of HMGA1 to both DNA and other proteins is highly regulated by their post-translational modifications.[29,30] Here, we found that two fragments of HMGA1 were directly bound to apelin, in which the HMGA1 Gln32 site had undergone deamidation of glutamine into glutamate (Figure 6A). It indicated that deamidated HMGA1 might be involved in lipid

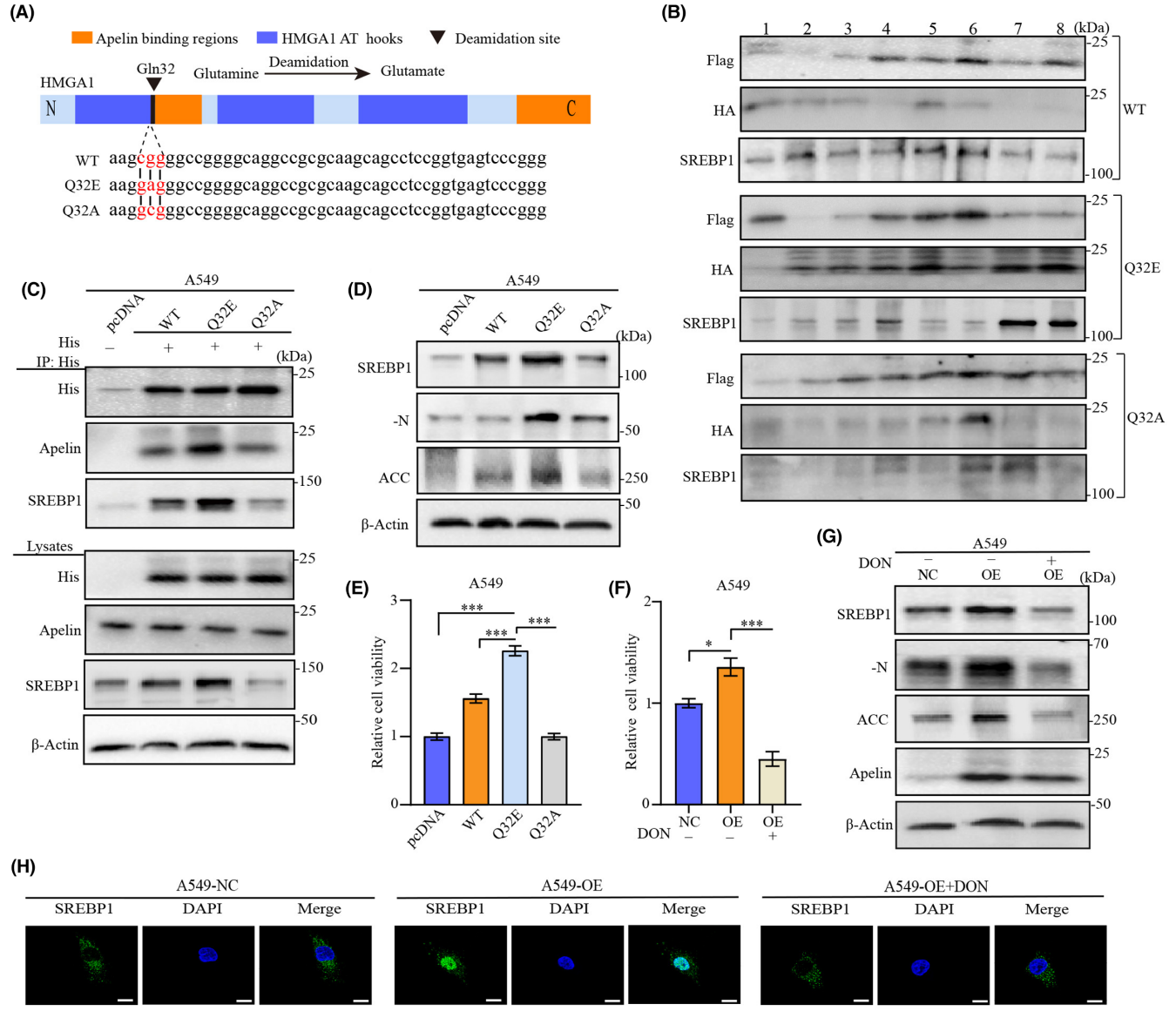

FIGURE 6 Deamidation of HMGA1 improved the SREBP1 activity by enhancing the binding affinity of the multiprotein complex. A, The genomic location of the deamidation sequence at HMGA1 Gln 32 and the two HMGA1 mutations, Q32E (glutamic acid, Glu) and Q32A (alanine, Ala), are shown in red. Orange boxes, apelin-binding regions; blue boxes, HMGA1 AT-hooks; triangle, HMGA1 deamidation site. B, Glycerol gradient analysis of cell lysates from 293T cells transfected with plasmids coexpressing HA-apelin, Flag-WT-HMGA1, or Flag-HMGA1 mutants. C, Immunoprecipitation (IP) results for A549 cells expressing His-WT-HMGA1 and His-HMGA1 deamidation mutants. D, Protein expression of SREBP1 and ACC in A549 cells transfected with His-WT HMGA1 and His-HMGA1 deamidation mutants. E, Cell viability of A549 cells transfected with His-WT HMGA1 and His-HMGA1 deamidation mutants. F, Cell viability in A549-OE cells after addition of DON (500 nM) for 24 h. G, The protein expression of SREBP1 and ACC in A549-OE cells after addition of DON (500 nM) for 24 h. H, Confocal microscopy images of SREBP1 trafficking related to the nucleus in the presence of DON (500 nM) for 24 h. DAPI, nuclear staining. Scale bar: 10 μm

metabolism in apelin-overexpression lung cancer cells. HMGA1 mutation plasmids containing Q32E (glutamic acid, Glu) and Q32A (alanine, Ala) mutations were generated and transfected into 293T and A549 cells (Figure 6A). HMGA1-Q32E showed be more potently deamidation activity than wild-type HMGA1, while HMGA1-Q32A showed be no deamidation activity. The effect of HMGA1 amidation on the binding of the apelin-HMGA1-SREBP1 complex was analyzed by glycerol gradient analysis (Figure 6B). In cell lysates with coexpressing HA-apelin and Flag-WT-HMGA1, the presence of apelin, SREBP1, and HMGA1 in the same fractions (fractions 1, 3, 5, 6) proved their existence in the form of a complex in 293T cells. And we detected a high level of the apelin-HMGA1-SREBP1 complex in the fractions from the HMGA1-Q32E cells (fractions 7, 8) but not in fractions from WT-HMGA1 or HMGA1-Q32A cells, which showed that HMGA1 deamidation enhanced the binding affinity of a multiprotein complex. We further confirmed that HMGA1-Q32E

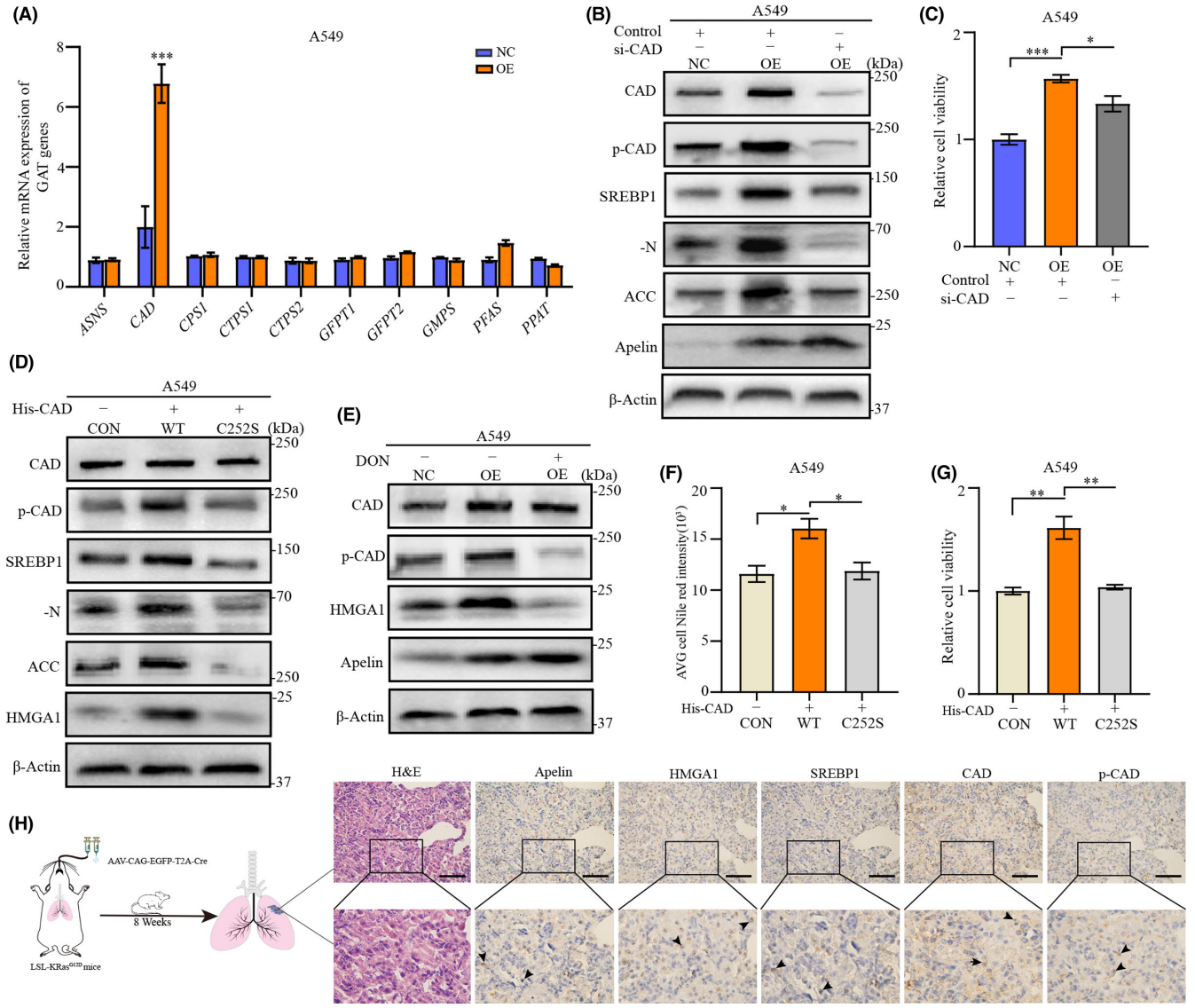

FIGURE 7 CAD deamidated and stabilized the expression of HMGA1 for lipid metabolism and cell growth. A, The mRNA expression of glutamine amidotransferase genes (GATs) in A549-OE cells. B, C, The protein levels of lipid kinases and cell viability in A549-OE cells treated with small-interference RNA (si-CAD). D, Immunoblots of cell lysates of A549 cells transfected with plasmids containing the His-WT CAD and enzyme-deficient mutant of CAD-C252S. E, The protein level of HMGA1 in A549-OE cells treated with DON (500 nM) for 24 h. F, G, Lipid contents and cell viability in A549 cells transfected with His-WT CAD and His-CAD C252S. H, Hematoxylin and eosin (H&E)-stained section and immunohistochemical (IHC) sections of $Kras^{G12D}$ mice lung adenocarcinoma. Scale bar: 50 μm. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

exhibited a more potent ability to form an apelin-HMGA1-SREBP1 complex in A549 cells by IP (Figure 6C). Deamidated HMGA1 Q32E also induced the protein expression for ACC, and enhanced SREBP1 activity to promote A549 cell proliferation (Figure 6D,E). In contrast, the addition of the transglutaminase inhibitor DON (500 nM) to A549-OE and H1975-OE cells inhibited cell viability and decreased protein levels of lipid kinases (Figure 6F,G and Figure S6A,B). Active N-terminal fragment of SREBP1 could not enter the nucleus after DON treatment on A549-OE cells (Figure 6H). These results demonstrated that apelin might induce HMGA1 amidation and enhance the binding affinity of the apelin-HMGA1-SREBP1 complex to regulate lipid metabolism and cell growth.

3.7 | CAD deamidated and stabilized the HMGA1 for lipid metabolism and cell growth

Glutamine amidotransferases constitute a family of metabolic enzymes that transform glutamine to glutamate for extracting nitrogen to synthesize nucleotides, amino acids, and glycoproteins for cell growth.[31] As a member of the GATs family proteins, CAD could deamidate the RelA subunit of NF-kB to promote aerobic glycolysis and cell proliferation in cancer cells.[32] CAD also catalyzes the first three steps in de novo pyrimidine synthesis, and can be phosphorylated and activated to stimulate de novo synthesis of pyrimidines.[33] We found that CAD was highly expressed and phosphorylated in

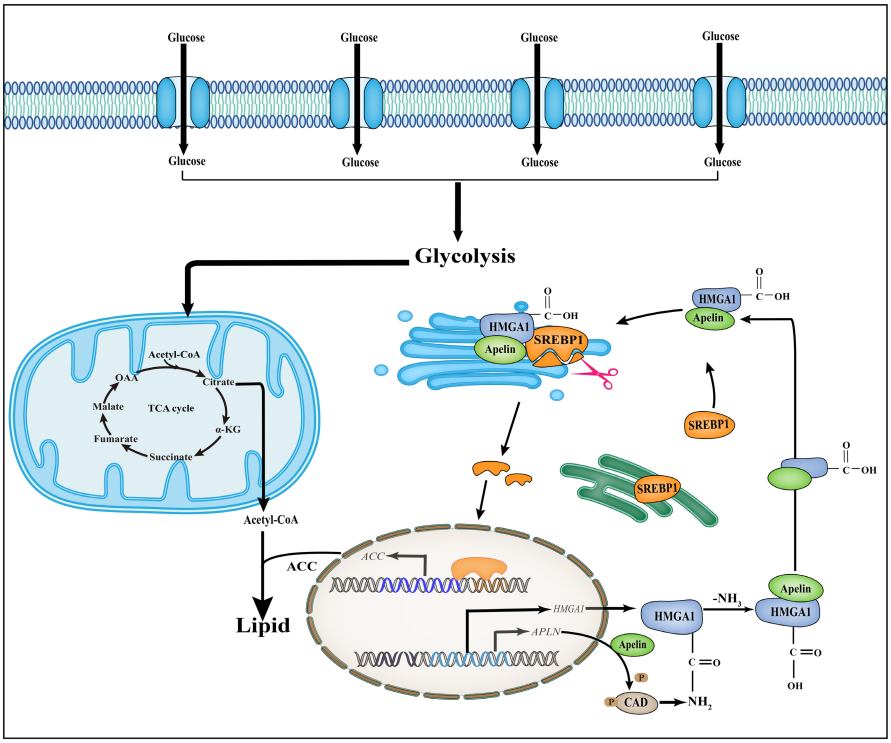

FIGURE 8 The mechanism diagram of this study. Apelin enhances the expression and phosphorylation of CAD for the deamidation of HMGA1. HMGA1 deamidation enhances the formation of the apelin-HMGA1-SREBP1 complex and promotes the activity of SREBP1, thereby inducing the expression of lipid metabolism gene and lipid synthesis

A549-OE and H1975-OE cells (Figure 7A,B and Figure S7A,B). To verify whether CAD contributed to apelin-mediated lipid metabolism and cell growth, CAD siRNA was transfected in A549-OE and H1975-OE cells. Silencing of CAD decreased the protein expression of lipid kinases and blocked cell proliferation in A549-OE and H1975-OE cells (Figure 7B,C and Figure S7B,C). We then purified HMGA1 with exogenously expressed CAD and confirmed the CAD-mediated HMGA1 deamination at Gln32 by tandem mass spectrometry (Figure S7D). Next, to investigate whether CAD contributed to apelin-mediated lipid metabolism by deamidating HMGA1, we created an enzyme-deficient mutant of CAD-C252S, in which the active site cysteine 252 was substituted by serine. The mTOR-regulated phosphorylation of CAD promotes the oligomerization and activity of CAD.[33] We also found that CAD-C252S decreased the phosphorylation level of CAD, and inhibited the mTOR signaling pathway of de novo pyrimidine synthesis (Figure 7D and Figure S7E). Notably, compared with CAD-WT, CAD-C252S not only inhibited SREBP1 activity but also strongly decreased the protein expression level of HMGA1 (Figure 7D). These results were consistent with the addition of GAT inhibitor DON in apelin-overexpressing lung cancer cells (Figures 6G and 7E and Figures S6A and 7F). In addition, overexpression of CAD-C252S inhibited both lipid accumulation and cell proliferation of lung cancer cells (Figure 7F,G). These results indicated that apelin might activate CAD to deamidate and stabilize HMGA1 protein for lipid metabolism and cell growth.

In tumor tissues derived from LUSC and LUAD patients, we found that CAD was coexpressed with apelin, HMGA1, and SREBP1 (Figure S7G). To verify the contributions of apelin, SREBP1, HMGA1, and CAD to NSCLC tumor growth, LSL-Kras^{G12D} mice expressing oncogenic KRAS were infected with AAV-CAG-EGFP-T2A-Cre to induce LUAD in situ. LSL-Kras^{G12D} mice developed LUAD in situ approximately 8 weeks after AAV-CAG-EGFP-T2A-Cre infection (Figure 7H). The protein expression of apelin, SREBP1, HMGA1, CAD, and p-CAD were all detected in some tumor cells of mice LUAD (Figure 7H). It further confirmed the contributions of apelin, SREBP1, HMGA1, and CAD to NSCLC tumor growth. Finally, we subcutaneously injected A549-NC and A549-OE cells into nude mice to investigate the effect of HMGA1 deamination on apelin-mediated tumor growth. The data showed that DON blocked apelin-induced tumor growth (Figure S7H) and decreased the tumor weight (Figure S7I). Collectively, these data demonstrated that HMGA1 deamination is a critical factor for apelin-induced lipid metabolism and tumorigenesis.

4 | DISCUSSION

The development and progression of cancer are frequently associated with increased de novo lipogenesis in tumor cells.[34,35] In this study, we found that apelin could promote lung cancer proliferation by enhancing lipid synthesis. The theoretical model (Figure 8) illustrates that apelin enhances the expression and phosphorylation of glutamine aminotransferase (CAD) for the deamidation of HMGA1 to promote lipid metabolism and cell growth of lung cancer cells. This post-translational modification stabilized the expression of HMGA1 and increased the formation of the apelin-HMGA1-SREBP1 complex for facilitating the SREBP1 activity, leading to the translocation of SREBP1 into the nucleus, thereby inducing the expression of lipid metabolism gene and the growth of lung cancer cells.

Based on the coexpression of apelin and APJ in the tumor, the autocrine and paracrine apelin-APJ pathways have been considered

to play a crucial role in cancer development and progression.[36,37] However, we found that APJ silencing in apelin-overexpression lung cancer cells did not completely inhibit apelin-mediated cell proliferation. Although APJ is the only known receptor for apelin, it remains possible that apelin might act in lung cancer cells by another signaling pathway.[38,39] Apelin-induced activation of APJ suppresses the expression of active SREBP1 and lipid accumulation in hepatocytes.[40] These research results imply a functional role of the apelin-APJ receptor complex in inhibiting adipogenesis. However, apelin and APJ were not highly coexpressed in lung cancer cells. Apelin might enable independent regulation of abnormal lipogenesis in lung cancer cells by increasing the synthesis of FAs, as well as by upregulating lipid-related proteins, such as ACC and SREBP1.

The HMGA1 protein is a transcriptional enhancer that elevates oncogene expression by interacting with DNA and protein in cancer cells.[41] It was reported that post-translational modification on HMGA1 proteins was correlated with a more aggressive malignancy in human cancer cells.[30,42] As a GAT, CAD not only can catalyze de novo pyrimidine synthesis, but it can also induce protein deamidation for promoting aerobic glycolysis in cancer cells.[33] Our study revealed that apelin upregulated the expression and activity of CAD to deamidate HMGA1 for lipid synthesis in lung cancer cells. HMGA1 deamidation by CAD could stabilize HMGA1 expression and increase the formation of the apelin-HMGA1-SREBP1 complex for lipid metabolism and cell growth. In summary, we identified an important mechanism by which apelin-mediated HMGA1 deamidation promoted the synthesis of FAs and tumor progression.

AUTHOR CONTRIBUTIONS

Li Zhang contributed to financial support; Weimin Li contributed to the experiment design; Yihan Zhu performed the experiments and wrote the manuscript; Yihan Zhu and Hong Bu contributed to design and edit the article; Ying Yang, Hong Huang, Jingjing Ran, Hongyu Chen, Liwen Qin and Mufeng Li contributed to the acquisition and analysis of data; Menglin Yao, Tingting Song, Yongfeng Yang, Yinyun Ni, Tingting Song, Ningning Chao and Zhiqing Liu reviewed the manuscript for critical content. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

ACKNOWLEDGMENTS

We thank Ms Sisi Wu and Ms Hongying Chen from Research Core Facility, West China Hospital, Sichuan University for the technical support. We also thank West China Biobanks at the Department of Clinical Research Management in West China Hospital for their continuous support.

FUNDING INFORMATION

The study was funded by the National Natural Science Foundation of China (Grant No. 81974363, 821733251 and 81772478); the Sichuan International/Hong Kong, Macao, and Taiwan Science and Technology Innovation Cooperation Project (Grant No. 2018hh0161); the Clinical Research Incubation Project of West China Hospital of Sichuan University (Grant No. 2018HXFH102); and the Hospital Enterprise Cooperative Clinical Research Innovation Project (Grant No. 2019HXCX04).

DISCLOSURE

The authors have no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: N/A. Informed Consent: N/A. Registry and the Registration No. of the study/trial: N/A. Animal Studies: All animal procedures were approved by the Institutional Animal Ethical Committee of West China Hospital of Sichuan University (2020415A).

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SUPPORTING INFORMATION

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

How to cite this article: Zhu Y, Yang Y, Bu H, et al. Apelin-mediated deamidation of HMGA1 promotes tumorigenesis by enhancing SREBP1 activity and lipid synthesis. *Cancer Sci*. 2022;113:3722-3734. doi: 10.1111/cas.15515