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UHMK1 promotes lung adenocarcinoma oncogenesis by regulating the PI3K/AKT/mTOR signaling pathway

Yongmeng Li | Shuai Wang | Kai Jin | Wenxing Jin | Libo Si | Huiying Zhang | Hui Tian

Department of Thoracic Surgery, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China

Correspondence
Hui Tian, Department of Thoracic Surgery, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, 250012, China.
Email: tianhuiql@126.com

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Abstract

Background: Effective targeted therapy for lung adenocarcinoma (LUAD), the number one cancer killer worldwide, continues to be a difficult problem because of the limitation of number of applicable patients and acquired resistance. Identifying more promising drug targets for LUAD treatment holds immense clinical significance. Recent studies have revealed that the U2 auxiliary factor (U2AF) homology motif kinase 1 (UHMK1) is a robust pro-oncogenic factor in many cancers. However, its biological functions and the underlying molecular mechanisms in LUAD have not been investigated.

Methods: The UHMK1 expression in LUAD cells and tissues was evaluated by bioinformatics analysis, immunohistochemistry (IHC), western blotting (WB), and real time quantitative polymerase chain reaction (RT-qPCR) assays. A series of gain- and loss-of-function experiments for UHMK1 were carried out to investigate its biological functions in LUAD in vitro and in vivo. The mechanisms underlying UHMK1's effects in LUAD were analyzed by transcriptome sequencing and WB assays.

Results: UHMK1 expression was aberrantly elevated in LUAD tumors and cell lines and positively correlated with tumor size and unfavorable patient prognosis. Functionally, UHMK1 displayed robust pro-oncogenic capacity in LUAD and mechanistically exerted its biological effects via the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway.

Conclusion: UHMK1 is a potent oncogene in LUAD. Targeting UHMK1 may significantly improve the effect of LUAD treatment via inhibiting multiple biological ways of LUAD progression.

KEYWORDS
lung adenocarcinoma (LUAD), metastasis, PI3K/AKT/mTOR signaling pathway, proliferation, U2AF homology motif kinase 1 (UHMK1)

INTRODUCTION

Lung cancer is a common malignancy, which remains the most cancer-related deaths in China and globally.[1,2] Lung adenocarcinoma (LUAD) is the most prevalent histological subtype accounting for ~40% of lung cancer and causes most deaths from this type of cancer.[3,4] The prognosis of LUAD patients is still unsatisfactory at ~15%.[5] This scenario warrants identifying novel therapeutic and diagnostic biomarkers for improving the clinical outcomes of LUAD patients.

Because of their unique role in signal transduction, protein kinases are the primary drug targets of many anticancer treatments.[6] Several kinase-related inhibitors have been clinically approved in LUAD treatment, such as inhibitors of epidermal growth factor receptor (EGFR) tyrosine kinase, receptor tyrosine kinase (ROS1) and anaplastic lymphoma kinase (ALK).[7–9] Unfortunately, they have limited

clinical effects because they are only suitable for specific patients, with patients' tendency to develop resistance and acquire some side effects such as nausea, headache, diarrhea, fatigue, rash, hypertension, and diabetes. Hence, identifying novel key kinase targets that critically regulate LUAD initiation and progression might potentially lead to developing safer and more effective therapeutics with significant clinical implications.

U2 auxiliary factor homology motif kinase 1 (UHMK1) gene, localized to chromosome 1q23, encodes the protein UHMK1 (also called KIS), is the only serine/threonine kinase with the U2AF homology motif (UHM).[10] The characteristic structure of UHMK1 enables it to participate in multiple biological processes by interacting with other molecules. Specifically, UHMK1 was first reported to regulate Stathmin, the vital protein functions in microtubule dynamics.[11] Moreover, it has been identified to interact with various proteins, such as splicing factors SF1 and SF3b155,[12,13] cyclin-dependent kinase inhibitor (CDKI) $p27^{Kip}$,[14] RNA-binding proteins CPEB1-3,[15] the marker of proliferation PIMREG,[16] and so on. Research on UHMK1 was initially focused on neurological diseases such as schizophrenia[17,18] because of its abundant expression in the nervous system.[19] In the last few years, many researches have revealed the function of UHMK1 in promoting the initiation and progression of different types of cancer, including ovarian cancer,[20,21] melanoma,[22,23] cervical cancer,[24] liver cancer,[25,26] colorectal carcinoma,[27,28] pancreatic cancer,[29–31] and gastric cancer.[32] However, no studies have investigated the function and regulatory mechanism of UHMK1 in LUAD.

Our study aimed to evaluate the expression of UHMK1 and explore its underlying molecular mechanism governing its biological functions in LUAD. This study may provide a novel insight for improving the therapeutic effect of LUAD, which may be beneficial to the further clinical application of LUAD treatment.

METHODS

Clinical tissue specimen collection

Ten paired fresh LUAD and adjacent normal tissues were recruited from patients undergoing surgical resection in our department and stored in liquid nitrogen till used. Ethical approval for this study was granted by the Medical Ethics Committee of Qilu Hospital of Shandong University (KYLL-2021[KS]-1053). Informed consent was obtained from each patient or their legal representatives.

Cell culture, transduction, and selection

Three LUAD cell lines (PC9, A549, H1299) and the human bronchial epithelial (HBE) cell line were purchased from the Shanghai Academy of Science and cultured in Dulbecco's Modified Eagle Medium (Gibco) with 10% fetal bovine serum (FBS) (BI) at 37°C in an environment of 5% CO_2. The lentiviral vectors encoding sh-NC (negative control, TTCTCCGAACGTGTCACGT), sh-UHMK1 (sh-UHMK1-1: CTGCTGAATGTGCTGGATGAT; sh-UHMK1-2: GCCTATCACCTAAGAGACCTT), oe-UHMK1, and its corresponding negative control were purchased from Jikai Company and delivered into cells by lentiviral transduction. Approximately, 48 h later, the stably transduced cells were selected using puromycin (4 μg/mL) for 3 days.

RNA isolation, cDNA preparation, and real-time quantitative polymerase chain reaction

The RNA extraction kit (RNAfast200; Fastagen Biotech) was used to extract the total cellular RNA and the reverse transcription kit (AG11706; Accurate Biology) to reverse transcription. Real-time quantitative polymerase chain reaction (RT-qPCR) reactions were run on a Bio-Rad IQ 5 RT-PCR detection system using SYBR Green Premix Pro Taq HS qPCR Kit (AG11701; Accurate Biology). The primers were ordered from Biosune company, having the following sequences: UHMK1 (F: 5'-AGCACGCTGTCTGTTGCTTG-3', R: 5'-AACATCTCGGGCACAATGCT-3′) and GAPDH (F: 5'-GCACCGTCAAGGCTGAGAAC-3′, R: 5'-TGGTGAAGACGCCAGTGGA-3′). The expression of target genes was evaluated using the $2^{-\Delta\Delta CT}$ method. This experiment was repeated three times.

Western blotting assay

Protein from cells or tissues was lysed using radioimmunoprecipitation assay buffer lysis buffer with 1% protease and phosphatase inhibitor cocktails (Beyotime Biotechnology). The western blotting (WB) assay was performed as previously described.[33] Antibodies used in this experiment were as follows: UHMK1 (11624-1-AP; Proteintech), LC3 (A19665; ABclonal), Beclin1 (A21191; ABclonal), p62 (A19700; ABclonal), Bcl-2 (A19 693; ABclonal), phosphoinositide 3-kinase (PI3K) (A16950; ABclonal), protein kinase B (AKT) (A18675; ABclonal), phosphorylated AKT (p-AKT) (AP1208; ABclonal), mammalian target of rapamycin (mTOR) (A11345; ABclonal), phosphorylated m-TOR (p-mTOR) (AP0115; ABclonal), E-cadherin (A20798; ABclonal), N-cadherin (A19083; ABclonal), vimentin (A19607; ABclonal), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (AC033; ABclonal). This experiment was repeated three times.

Tissue microarray, immunohistochemistry, and hematoxylin/eosin staining

The tissue microarray (HLugA180Su07), which contained 157 available tissues, including 70 paired LUAD and

adjacent tissues, was purchased from Shanghai Outdo Biotech. Immunohistochemistry (IHC) and hematoxylin/eosin (HE) staining were performed as previously described.[33] The IHC results were scored separately by two pathology experts. The scoring criteria were as follows: Hscore (histochemistry score) = staining intensity × percentage of positive cells, staining intensity (0, none; 1, weak; 2, moderate; and 3, strong); percent positivity of cells (0%–100%). Finally, images were acquired with a light microscope (Olympus).

Cell Counting Kit 8 assay

Cells were incubated in 96-well microplates (2×10^3/well). Next, 10% Cell Counting Kit 8 (CCK8) solution was added into each well and incubated for 2 h in darkness when the cells were cultured for 0, 24, 48, 72, and 96 h. Finally, a microplate reader was used to record the absorbance of each well at 450 nm. This experiment was replicated thrice.

Colony formation assay

Cells were incubated in 6-well culture plates (1×10^3/well) until cell colonies were visible. Next, the cells were stained with 0.5% crystal violet for 30 min after being fixed in 4% paraformaldehyde for 20 min. Finally, the plates were washed with phosphate-buffered saline (PBS) and photographed with a digital camera. This experiment was replicated thrice.

5-Ethynyl-2'-deoxyuridine staining

The transduced cells were incubated at 2×10^4 cells/well into 96-well plates, and the 5-ethynyl-2'-deoxyuridine (EdU) staining was carried out with an EdU staining kit (RiboBio) following the manual instruction. EdU imaging was finally done using a fluorescent microscope (Olympus).

Flow cytometry analysis and electron microscopy

A Cell Cycle Assay Kit (Vazyme) and an Annexin V-FITC/PI Apoptosis Detection Kit were used for cell-cycle and cell apoptosis assays according to the manufacturer's instructions. EM was performed as previously described.[34]

Wound-healing assay

Cells were cultured in 6-well plates with complete media till they were fully attached. Next, scratches were made using 200 μL pipette tips, and the media was replaced with serum-free media following three PBS washes. Finally, the wound regions were photographed and measured using the Image J software. This assay was replicated thrice.

Transwell assay

A total of 24-well transwell chambers (8 μm; Corning) were used in the transwell assay. The invasion (with Matrigel) and migration (without Matrigel) assays were carried out as previously described.[33] Finally, an inverted microscope (Olympus) was used to obtain the images. All the assays were independently replicated thrice.

In vivo experiments

BALB/c-nude mice (4 weeks old, female) were obtained from GemPharmatech and housed in individual cages (5 per cage). For the tumor xenograft experiment, the transduced A549 and H1299 cells were injected (5×10^6 cells per mouse) into the right flank of mice. The size of the tumors was measured once a week, and tumor volumes were calculated using the equation: volume = length × width^2/2. The mice were euthanized at the end of this experiment and the tumors were collected for HE and IHC staining. The ethics committee of Qilu Hospital of Shandong University approved this study (DWLL-2022-074).

Data processing and transcriptome sequencing

The data of UHMK1 expression, along with the prognostic information of the LUAD patients, were obtained from The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/) and the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) databases. Transcriptome sequencing (RNA-seq) was performed by Lianchuan Biotechnology using the Illumina HiSeq4000 platform.

Statistical analysis

GraphPad Prism (GraphPad8) and R (version 4.0.3) software were used to analyses the data. Kruskal–Wallis test, Wilcoxon test, Kaplan–Meier survival analysis and Student's *t*-test (two-sided) were performed as indicated. The experimental results were expressed as mean ± standard deviation and a *p*-value <0.05 ($p < 0.05$) revealed significant differences between groups.

RESULTS

UHMK1 expression was aberrantly high in LUAD

We first used high-throughput sequencing data to compare UHMK1 expression between normal lung tissues and LUAD tissues. In both TCGA and several GEO datasets (GSE 19188, GSE 32665, GSE 32863), compared with normal lung tissues, the expression of UHMK1 was

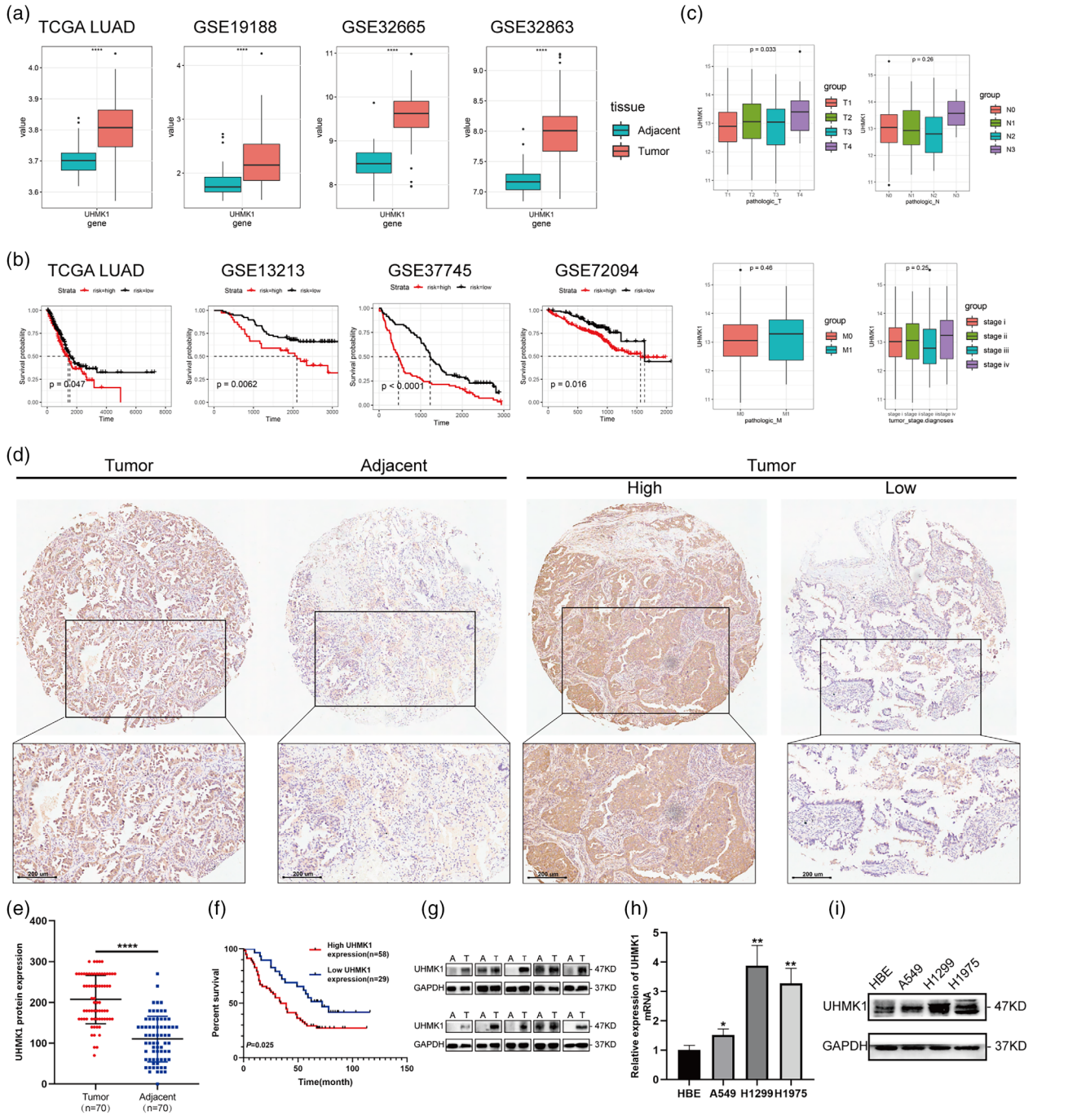

FIGURE 1 U2 auxiliary factor homology motif kinase 1 (UHMK1) was highly expressed in lung adenocarcinoma (LUAD) tissues and cells and predicted poor prognosis. (a) The expression of UHMK1 was much higher in LUAD compared with that in normal lung tissues according to The Cancer Genome Atlas (TCGA) and several Gene Expression Omnibus (GEO) databases (GSE 19188, GSE 32665, GSE 32863). (b) Kaplan–Meier survival analysis of the data from TCGA database and some GEO datasets (GSE 13213, GSE 37745, GSE 72094) showed high UHMK1 expression was associated with poor prognosis. (c) The relationship analysis between UHMK1 expression and clinical features of LUAD patients in the TCGA database revealed the expression of UHMK1 was positively correlated with tumor size. Kruskal-Wallis test was used for pathologic T stage, N stage, and tumor stage analysis; the Wilcoxon test was used for pathologic M stage analysis. (d) Representative tissue microarray immunohistochemistry (IHC) images depicting UHMK1 expression. (e) The expression of UHMK1 protein was significantly elevated in LUAD compared with that in adjacent normal lung tissues according to H-score analysis for IHC of 70 paired LUAD tissues and adjacent normal lung tissues. (f) Kaplan–Meier survival analysis of 87 LUAD patients from the tissue microarray showed high UHMK1 expression was associated with poor prognosis. (g) UHMK1 protein expression in 10 paired LUAD (T) and adjacent (a) normal lung tissues were measured by western blotting. (h), (i) The expression of UHMK1 mRNA and protein was abnormally elevated in LUAD cell lines. The data was represented by the mean ± standard deviation (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$).

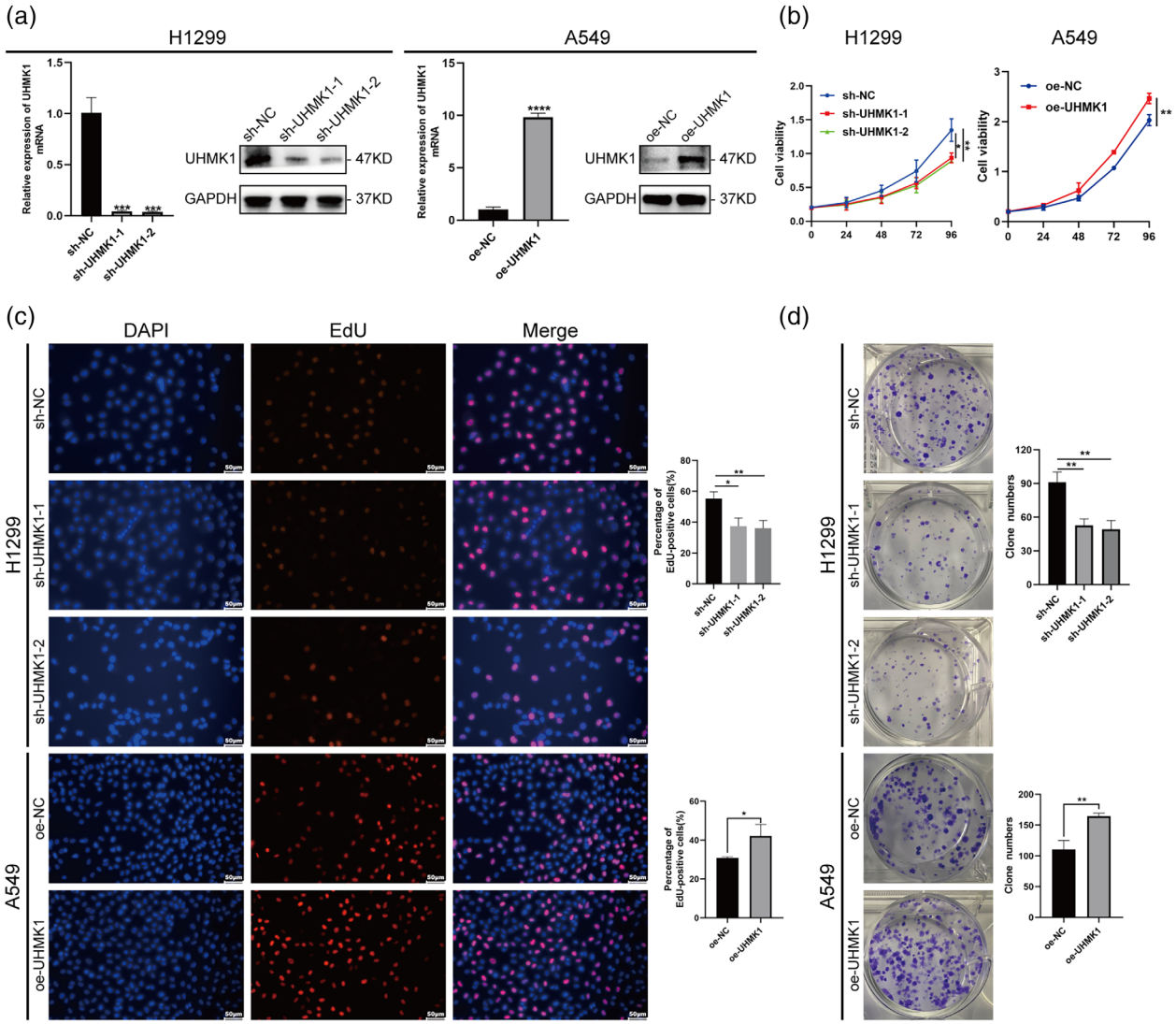

FIGURE 2 U2 auxiliary factor homology motif kinase 1 (UHMK1) enhanced lung adenocarcinoma (LUAD) cell proliferation in vitro. (a) The expression of UHMK1 was measured by real-time quantitative polymerase chain reaction and western blotting assays after lentiviral transduction. (b)–(d) Cell Counting Kit 8, 5-ethynyl-2'-deoxyuridine (EdU), and clone formation assays of the transduced H1299 and A549 cells showed UHMK1 could increase the cell proliferation ability in LUAD cells. The data was represented by the mean ± standard deviation (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

significantly upregulated in LUAD (Figure 1(a)) ($p < 0.0001$). Next, valuation of the association between UHMK1 expression and the prognosis of LUAD patients in TCGA LUAD dataset and some GEO datasets (GSE 13213, GSE 37745, GSE 72094) revealed elevated UHMK1 expression indicated a poor prognosis (Figure 1(b)). Additionally, analyzing the correlation between clinical features and UHMK1 expression of LUAD patients in TCGA database showed a positive correlation with tumor volume (Figure 1(c)) ($p = 0.033$). We, then, performed tissue microarray IHC to measure the expression difference of UHMK1 protein in LUAD tissues and their corresponding adjacent normal lung tissues. Coherently with the high-throughput sequencing data, we observed that UHMK1 protein was markedly upregulated in LUAD tissues (Figure 1(d),(e)), and patients with high UHMK1 expression had a significantly worse prognosis (Figure 1(f)) ($p = 0.025$). We also examined UHMK1 protein expression in 10 paired LUAD and adjacent normal lung tissues by WB assay. Likewise, UHMK1 protein expression was highly upregulated in LUAD tissues (Figure 1(g)). Finally, we determined the expression of UHMK1 mRNA and protein in different cell lines by RT-qPCR and WB assays, respectively. LUAD cell lines exhibited significantly higher expression of UHMK1 compared to the HBE cell line (Figure 1(h),(i)). Taken the above results together, UHMK1 was highly expressed in LUAD cells and tissues and predicted a poor prognosis of patients with LUAD.

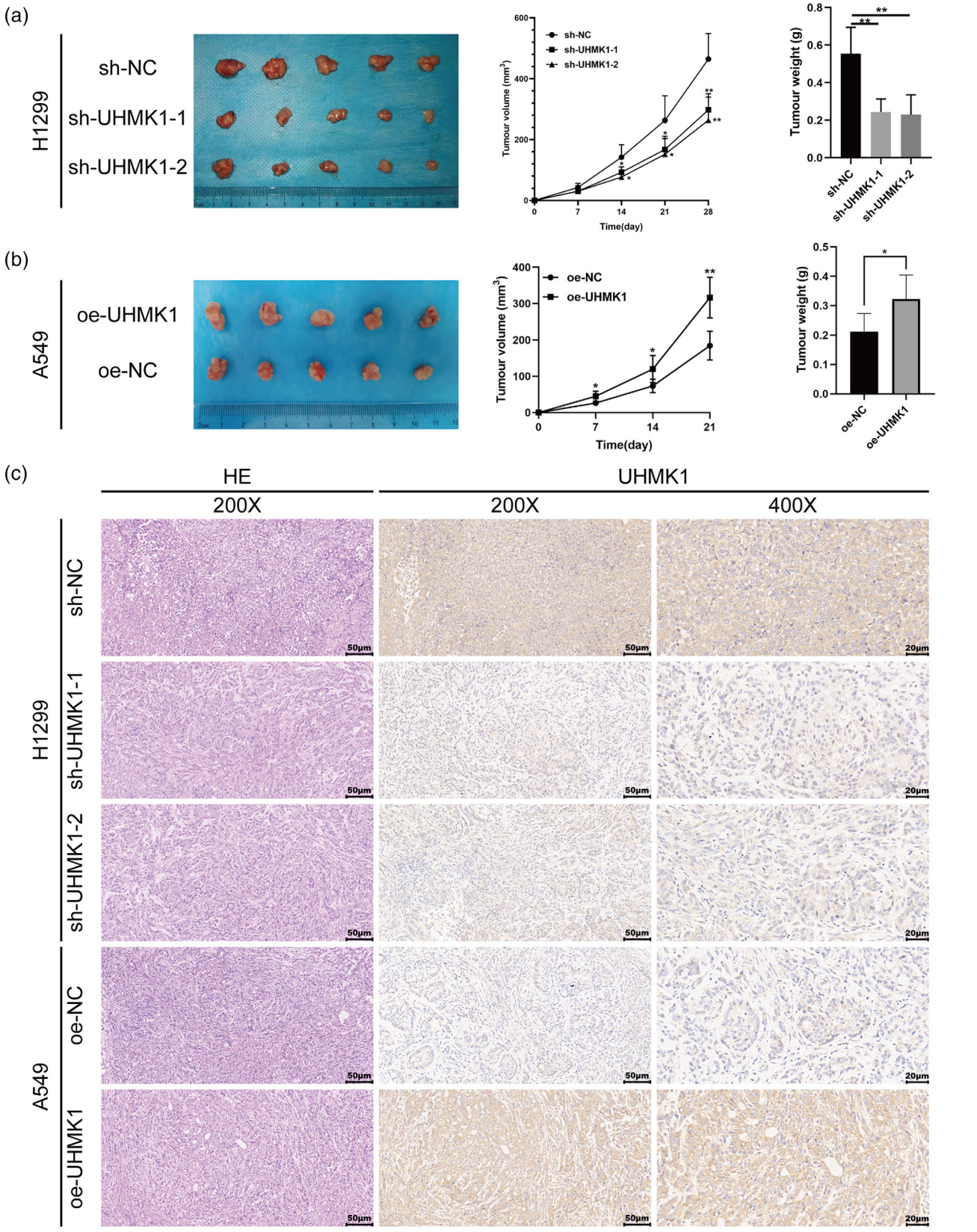

FIGURE 3 U2 auxiliary factor homology motif kinase 1 (UHMK1) enhanced lung adenocarcinoma cell proliferation in vivo. (a), (b) The pictures, the growth curve, and the weight of tumors in different groups. (c) Representative photographs of hematoxylin/eosin immunohistochemical staining of the tumor tissues in different groups. Data were articulated as the mean ± standard deviation (*$p < 0.05$, **$p < 0.01$).

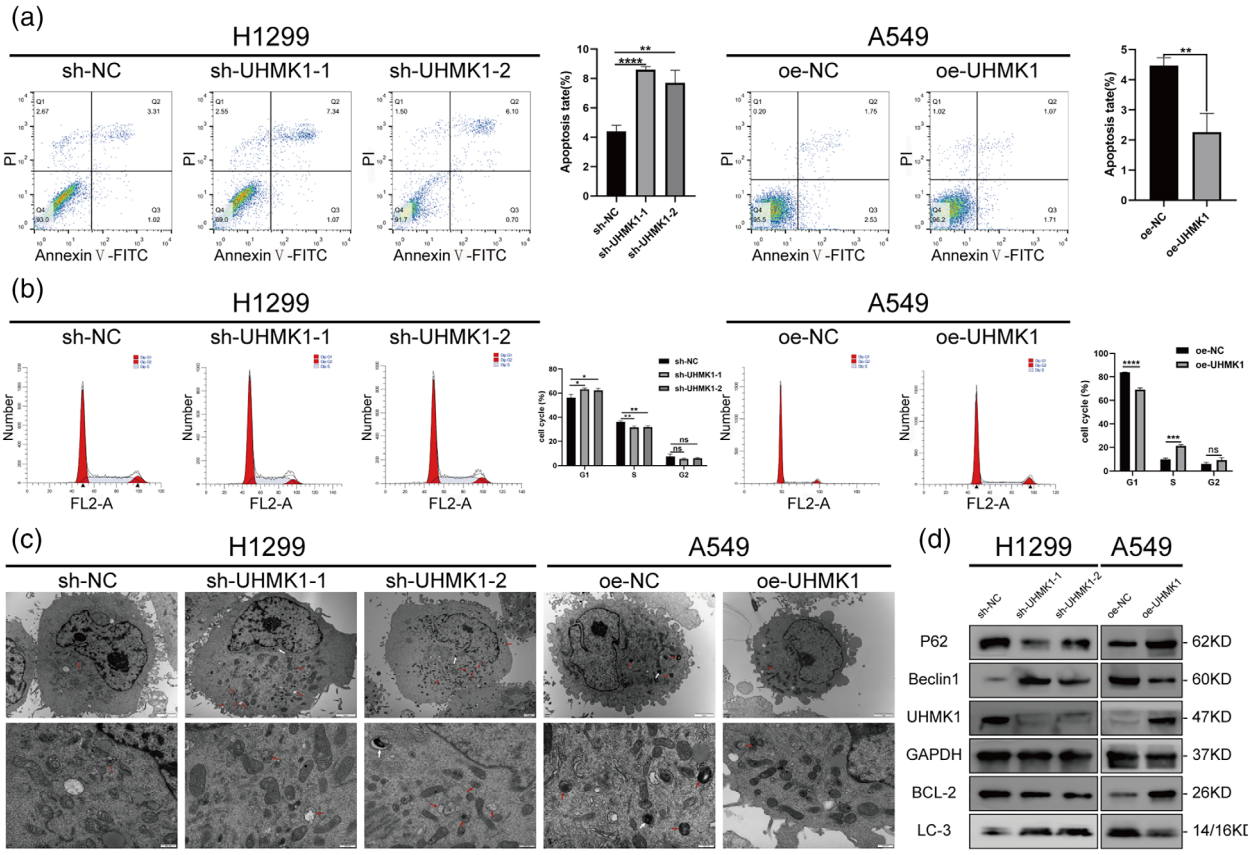

FIGURE 4 U2 auxiliary factor homology motif kinase 1 (UHMK1) promoted cell-cycle progression while inhibited apoptosis and autophagy in lung adenocarcinoma cells. (a) Flow cytometry analyses revealed downregulation of UHMK1 increased the proportion of apoptotic H1299 cells and upregulation of UHMK1 decreased the proportion of apoptotic A549 cells. (b) Flow cytometry analysis of cell cycle showed that UHMK1 knockdown induced significant G1/S phase arrest. In contrast, overexpression of UHMK1 markedly accelerated the G1/S phase transition in the transduced cells. (c) Electron microscopy representative images of autophagosomes (white arrows) and autolysosomes (red arrows). (d) The expression of key biomarkers associated with cell autophagy and apoptosis were measured by western blotting assay. Data were articulated as the mean ± standard deviation (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

UHMK1 enhanced LUAD cell proliferation in vitro and in vivo

As UHMK1 expression was relatively high and low in H1299 and A549 cell lines, respectively (Figure 1(h),(i)). We, therefore, silenced and overexpressed UHMK1 in H1299 and A549 cells, respectively. The efficiencies of lentiviral transduction were tested by RT-qPCR and WB assays. The sh-UHMK1 H1299 and oe-UHMK1 (overpressed UHMK1) A549 cells displayed marked down- and upregulation of UHMK1 expression, respectively, compared with the cells in the NC groups (Figure 2(a)). Next, CCK-8, EdU, and colony formation assays examined the proliferative capacity of the stably transduced cells. Our results showed that the proliferative capacity of the cells significantly decreased on UHMK1 silencing in H1299 cells, whereas it increased on UHMK1 overexpression in A549 cells (Figure 2(b)–(d)). To further investigate the functional role of UHMK1, we used a subcutaneous xenograft tumor model to explore whether UHMK1 affected the proliferation of LUAD cells in vivo. The growth rate and weight of the tumors were significantly lower in the UHMK1 knockdown groups while significantly higher in the UHMK1 upregulated group over the NC groups (Figure 3(a),(b)). The tumors were then subjected to HE and IHC staining, which revealed decreased UHMK1 expression in the sh-UHMK1 groups while significantly increasing in the oe-UHMK1 group than in the NC groups (Figure 3(c)). The above results indicated that UHMK1 promoted LUAD cell proliferation both in vitro and in vivo.

UHMK1 promoted cell-cycle progression while inhibited apoptosis and autophagy in LUAD cells

To further identify the specific reason underlying UHMK1-mediated promotion of proliferation, we conducted flow cytometry and electron microscopy assays. We found a significant increase in the proportion of apoptotic cells on downregulation of UHMK1 in H1299 cells. At the same time, it decreased in A549 cells on UHMK1 upregulation (Figure 4(a)). We also observed that UHMK1

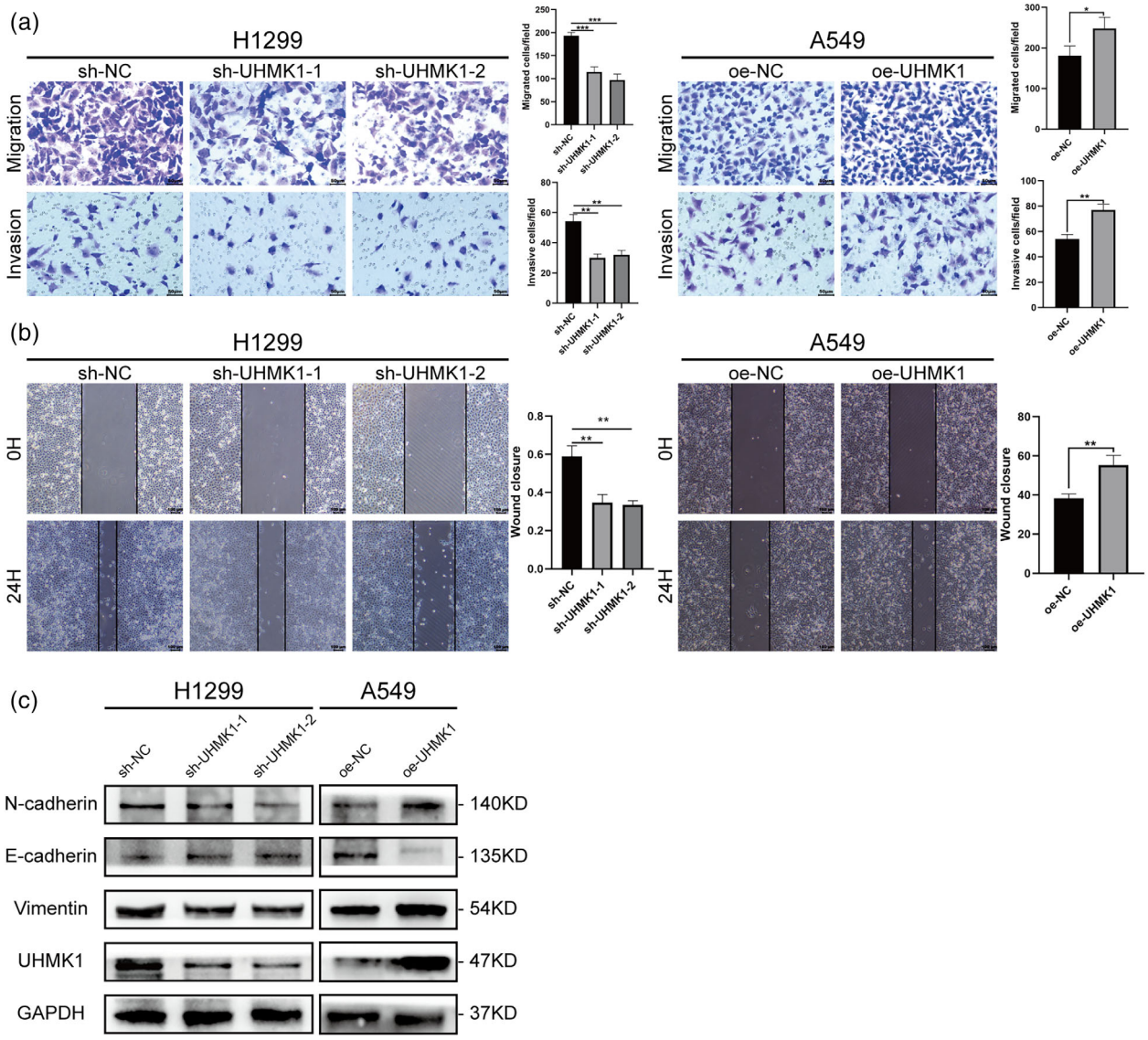

FIGURE 5 U2 auxiliary factor homology motif kinase 1 (UHMK1) promoted lung adenocarcinoma cell invasion and migration. (a), (b) Transwell and wound-healing assays showed UHMK1 knockdown reduced the migratory and invasive capacities of H1299 cells, whereas overexpression of UHMK1 markedly enhanced these capacities. (c) The expression of epithelia-mesenchymal-transition-related proteins was examined by western blotting assay. Data were articulated as the mean ± standard deviation (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

knockdown induced significant G1/S phase arrest. In contrast, overexpression of UHMK1 markedly accelerated the G1/S phase transition (Figure 4(b)). Additionally, EM revealed that UHMK1-knockdown led to significantly more autophagosomes (white arrows) and autolysosomes (red arrows). In contrast, UHMK1-upregulation markedly decreased these numbers compared with the cells in NC groups (Figure 4(c)). We, then, examined how the expression of some critical biomarkers associated with cell apoptosis and autophagy varied on manipulating the expression of UHMK1. The result showed that UHMK1 knockdown increased Beclin1 and LC3, whereas decreased the expression of p62 and Bcl-2. Moreover, A549 tended to behave oppositely when UHMK1 expression was upregulated (Figure 4(d)). Our results collectively showed UHMK1 promoted LUAD cell proliferation by promoting cell-cycle progression while inhibiting apoptosis and autophagy.

UHMK1 increased LUAD cell invasion and migration

We, then, carried out the transwell and wound healing assays to further detect the effects of UHMK1 on migration and invasion in LUAD cells. We found UHMK1 knockdown significantly reduced the number of invaded and migrated H1299 cells. In contrast, overexpression of UHMK1 significantly increased the number of invaded and migrated A549 cells (Figure 5(a)). Moreover, wound healing assay showed that UHMK1 knockdown notably delayed while UHMK1

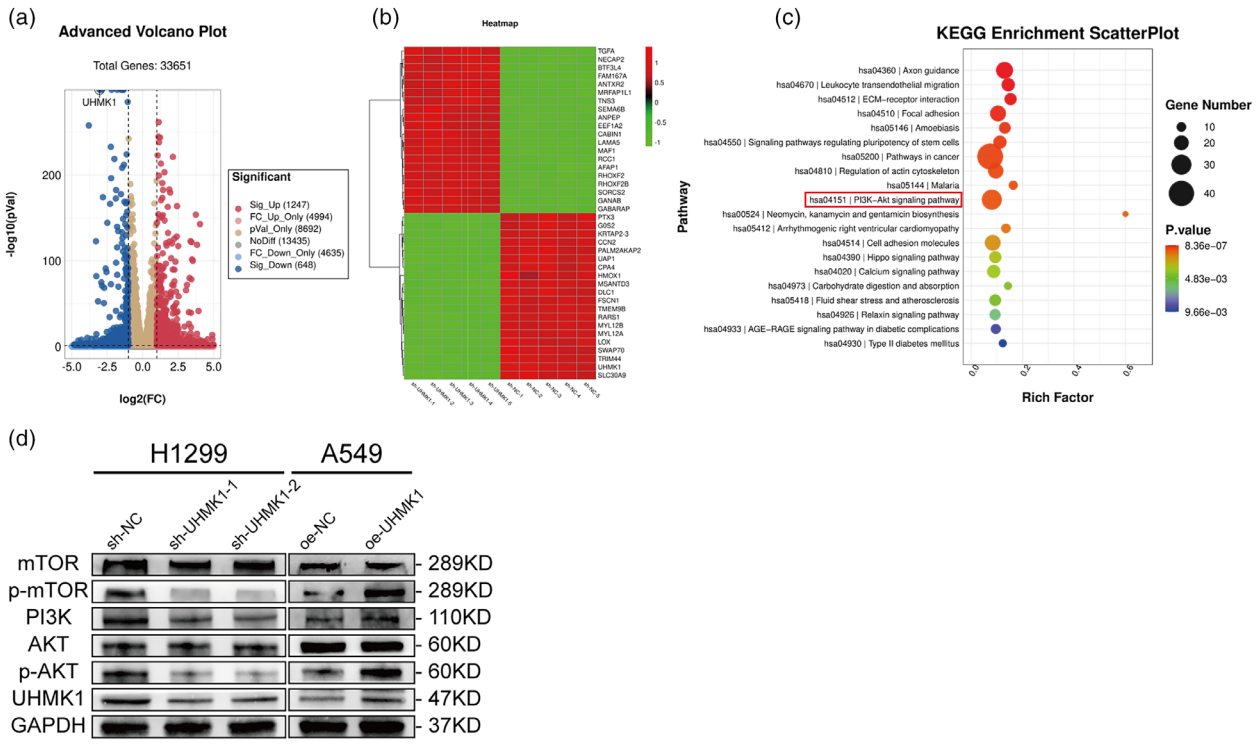

FIGURE 6 U2 auxiliary factor homology motif kinase 1 (UHMK1) activated the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway in lung adenocarcinoma cells. (a) Volcano plot analysis of our transcriptome data. (b) The heat map of the top 20 significantly upregulated and downregulated genes. (c) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the transcriptome sequencing data. (d) Evaluation of the levels of proteins related to the PI3K/AKT/mTOR signaling pathway by western blotting assay.

overexpression significantly accelerated the wound closure areas compared with that in the control groups (Figure 5(b)). Finally, we tested the expression of some critical epithelia-mesenchymal-transition (EMT) regulatory proteins (E-cadherin, N-cadherin, and vimentin) by WB assay. UHMK1 knockdown downregulated N-cadherin and vimentin while upregulated E-cadherin. In contrast, UHMK1 overexpression increased E-cadherin expression while decreased the expression of N-cadherin and vimentin (Figure 5(c)). Collectively, these data suggested that UHMK1 promoted LUAD cell invasion and migration.

UHMK1 activated the PI3K/AKT/mTOR signaling pathway in LUAD cells

To explore the molecular mechanism underlying UHMK1-mediated LUAD progression, we performed RNA-seq in sh-UHMK1 H1299 cells and sh-NC H1299 cells ($n = 5$). We found 1895 genes to be significantly differentially regulated (FoldChange >2; $p < 0.05$) in expression, including 1247 and 648 genes up- and downregulated, respectively (Figure 6(a)). The top 20 most significantly up- and downregulated genes were shown in Figure 6(b). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the differentially expressed genes indicated that the alteration in UHMK1 expression affected numerous signaling pathways, including the PI3K-AKT signaling pathway (Figure 6(c)). We focused mainly on this pathway and examined the expression of some major genes in it: PI3K (110α), AKT, and p-AKT. As mTOR is a crucial downstream target of AKT and critically regulates cell apoptosis,[35] autophagy,[36] and metabolism,[37] we also tested the expression of mTOR and p-mTOR. The expression of PI3K, p-AKT, and p-mTOR significantly reduced on UHMK1 knockdown in H1299 cells and an evident elevation following the UHMK1 upregulation in A549 cells (Figure 6(d)). Collectively, these results demonstrate UHMK1 activated the PI3K/AKT/mTOR signaling pathway in LUAD cells.

DISCUSSION

The U2AF heterodimer, which defines ~88% of functional 3′ splice sites, plays a predominant role in RNA splicing.[38,39] Being the only identified kinase with the U2AF homology motif, UHMK1 is equipped with both the functions of phosphorylation and mRNA splicing. UHMK1 has been implicated as an attractive therapeutic target by exerting its potent oncogenic functions in different ways in many types of cancer.[20–23,25–32,40] This motivated us to explore the role and possible mechanism of action of UHMK1 in LUAD. In our study, we identified UHMK1 as

significantly overexpressed in LUAD patients, leading to a poor prognosis. Moreover, we demonstrated for the first time that UHMK1 promoted the progression of LUAD. Considering the significant biological and clinical implications of UHMK1, this study indicated UHMK1 to be a promising target for LUAD therapy.

In the present study, we showed for the first time that UHMK1 inhibited LUAD cell autophagy. Although autophagy promotes or suppresses the development of cancer has been controversial, its role in regulating tumor cell death and survival and drug resistance should not be overlooked.[41–43] Under certain conditions, autophagy contributes to cell survival and improves the tolerance to drug therapy.[44,45] Interestingly, some studies have indicated that UHMK1 can mediate oxaliplatin,[28] 5-fluorouracil[46] resistance in colorectal cancer, and vemurafenib resistance in melanoma.[22] Our findings might provide insights into UHMK1-mediated drug resistance in tumors. Apoptosis and autophagy have a profound crosstalk during cancer progression; they may antagonize or assist each other.[47,48] Our findings corroborated the inhibitory influence of UHMK1 on the apoptosis of LUAD cells. This phenomenon that UHMK1 inhibited both apoptosis and autophagy could be accounted for by our finding that UHMK1 affected the PI3K/AKT/mTOR signaling pathway.

Previous studies have indicated that UHMK1 promotes cell-cycle progression by phosphorylation of $p27^{Kip}$.[14,49] In this study, we also found that UHMK1 overexpression accelerated while UHMK1 knockdown delayed the cell cycle in LUAD cells, respectively. However, studies also have reported that UHMK1 cannot regulate the cell-cycle progression of U937 leukemia cells[10] and only affects the cell-cycle progression of breast cells in combination with erlotinib.[49] These studies collectively suggest that the function of UHMK1 in cell-cycle regulation may not be generalizable. The underlying mechanisms need more intense investigations.

In addition to accelerating the proliferation ability, UHMK1 also promoted the invasion and migration of LUAD cells by contributing to EMT. Multiple studies have pointed out how UHMK1 activates Stathmin,[15,25] an essential regulator of EMT in lung cancer[50,51] and other cancers.[52–54] Moreover, Zhang et al.[32] recently demonstrated UHMK1 significantly improves the invasive abilities of gastric cancer cells by regulating purine metabolism. These studies sufficiently provide strong evidence for the oncogenic role of UHMK1 in promoting tumor invasion and metastasis by regulating various biological processes.

The aberrant UHMK1 expression is always accompanied by changes in cancer-related pathways, like the mitogen-activated protein kinases (MAPK)/extracellular-signal-regulated-kinase (ERK) and Hippo/yes-associated protein 1 (YAP) signaling pathways in liver cancers,[25,26] and the Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) signaling pathway in colorectal cancers.[28] KEGG pathway analysis of our transcriptome data revealed that altered UHMK1 expression significantly affected the PI3K/AKT signaling pathway. As a serine/threonine kinase, we reasonably speculated that UHMK1 activates the PI3K/AKT/mTOR signaling pathway mediated by serine/threonine kinase cascades. Therefore, we primarily focused on PI3K/AKT/mTOR signaling pathway and demonstrated the critical role of UHMK1 in significantly affecting this pathway. This finding helped to explain the results of our phenotypic assays that UHMK1 inhibited both apoptosis and autophagy, with mTOR being a well-known apoptosis and autophagy inhibitor.[55,56] However, the specific direct molecular mechanism of UHMK1 underlying PI3K/AKT/mTOR signaling modulation warrants future investigations.

In conclusion, we uncover that the serine/threonine kinase UHMK1 is a strong promoter of LUAD. Therefore, targeting UHMK1 might bring a new therapeutic approach for treating LUAD patients. However, larger sample size and more in-depth studies are needed in the future to investigate this possibility.

AUTHOR CONTRIBUTIONS

Hui Tian and Yongmeng Li designed this study. Yongmeng Li performed the experiments and Kai Jin analyzed the data. Libo Si, Yongmeng Li, and Huiying Zhang wrote and revised the manuscript. Shuai Wang and Wenxing Jin collected the samples and information of patients. All authors approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data supporting this research are presented in the article, further inquiries can be directed to the corresponding author.

ORCID

Hui Tian https://orcid.org/0000-0003-4516-2313

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