

PIK3R1 Promotes Lung Cancer Proliferation Through Activating PI3K/AKT/mTOR Signaling Pathways

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Research

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Abstract

Background: Lung cancer is a common malignant neoplasm worldwide. Phosphoinositide-3-kinase, regulatory subunit 1 (PIK3R1) plays as a therapeutic target in many cancers. The role PIK3R1 plays in lung cancer still remains unclear. Our study aims to explore the role of PIK3R1 in lung cancer.

Methods: We used quantitative real-time PCR (qPCR) to detect the PIK3R1 mRNA expression level in our 20 paired lung cancer patients. We then used A549^{sh-PIK3R1}, H1299^{sh-PIK3R1}, H1650^{LV-PIK3R1}, H292^{LV-PIK3R1}, A549^{sh-PIK3R1+LV-PIK3R1} and H1299^{sh-PIK3R1+LV-PIK3R1} cells for in vitro analysis. Cell viability assay was used to detect the ability of PIK3R1 in regulating proliferation of lung cancer. Western blot analysis was used to detect the PIK3R1 protein expression level and proteins in PI3K/AKT/mTOR signaling pathway.

Results: PIK3R1 mRNA expression level was higher in tumour tissues than the corresponding adjacent noncancerous among the 20 lung cancer patients. Increased PIK3R1 promoted lung cancer proliferation and downregulated PIK3R1 inhibited lung cancer proliferation. Furthermore, PIK3R1 downregulation suppressed p-PI3K, p-Akt, and p-mTOR in A549^{sh-PIK3R1} and H1299^{sh-PIK3R1} cells. Overexpressed of PIK3R1 upregulated p-PI3K, p-Akt, and p-mTOR in H1650^{LV-PIK3R1} and H292^{LV-PIK3R1} cells. Cell viability was increased in both A549 and H1299 cells following sh-PIK3R1+LV-PIK3R1 co-transfection, thus reversed the effect of sh-PIK3R1 on cell proliferation.

Conclusions: We identified that overexpressed of PIK3R1 could promote lung cancer proliferation via PI3K/AKT/mTOR signaling pathway, thus may provide PIK3R1 as a therapeutic target for effective strategy in lung cancer.

Introduction

Lung cancer is a commonly diagnosed malignant neoplasm in men and women [1]. Lung cancer has two histological types for better treatment. The most common type is non-small cell lung cancer (NSCLC, 85%) and the rest is small cell lung cancer (SCLC, 15%) [2]. In China, an highest incidence (3.57/100,000) as well as mortality (45.57/100,000) of lung cancer were expected in 2009 [3]. Most lung carcinomas are diagnosed at an advanced stage and even with chemotherapy, its overall 5-year survival rate is still poor [4, 5]. In recent years, survival outcomes have been improved by molecularly targeted therapeutic agents. Therefore, it is imperative to identify new lung cancer biological markers that allow us to develop its early diagnosis approaches.

Phosphoinositide-3-OH kinase (PI3K) belongs to a conserved lipid kinases family, which comprised of a SH2-containing regulatory subunit (p85a, p55a, p50a, p85b, p55g) and a catalytic subunit (p110a, p110b, p110d) [6]. PI3K pathway is one of the most frequently dysregulated pathways in cancer and it represents an important therapeutic target [7]. The regulatory subunit p85a encoded by PIK3R1 binds to the catalytic subunit p110a encoded by PIK3CA and induces stabilization and membrane recruitment of p110a [8].

p85 also acts as an important negative regulator to limit the extent of PI3K signaling, such as the suppression of tumorigenesis driven by the loss of PTEN and EGF receptor/ErbB3 [9, 10].

Studies have showed PIK3R1 exerts both positive and negative effects on signaling. It has been reported that PIK3R1 (p85a) regulatory subunit mutations could interact with and stabilize p110 α and promote cell survival and oncogenesis in colon cancer [11]. Another study identified that high frequency and nonrandom distribution of somatic PIK3R1 (p85a) mutations lead to functional activation of the PI3K pathway in endometrial carcinoma [12]. Philp AJ et al. demonstrated PIK3R1 induced constitutive activation of PI3k and had been shown as an oncogene in ovarian and colon tumors [13]. Other studies showed PIK3R1 had a tumor-suppressive role in hepatocellular carcinoma [14] and downregulation of PIK3R1 promoted propagation, migration, EMT in renal cancer [15]. However, the possible biological mechanisms of PIK3R1 in lung cancer remains unclear. In the present study, we detected the PIK3R1 expression in lung cancer patients and lung cancer cells. We further explored the potential mechanism of PIK3R1 in lung cancer progression.

Methods

Subject characteristics

The study population was composed of 20 lung cancer patients (14 men, 6 women) with a median age of 49.9 years (range 32–65 years) at the time of diagnosis in our hospital between May 2016 and June 2018 (Table 1). All patients were diagnosed with NSCLC confirmed by pathological tissue examination. 8 (40%) patients were classified as TNM stage grade I or II, while 12 (60%) were stage III or IV. All patients underwent surgical resection and we obtained tissues from the surgical specimens. Both cancerous and noncancerous tissues were histologically confirmed. Written informed consent was signed by all the participants. This study was approved by the ethics committee of Huai'an Second People's Hospital, The Affiliated Huai'an Hospital of Xuzhou Medical University.

Table 1
Clinical characteristics of the 20 study participants.

Variables	N	Percent (%)
Age at diagnosis		
≤ 50	16	80.00%
> 50	4	20.00%
Sex		
Male	14	70.00%
Female	6	30.00%
Histology		
Adenocarcinoma	17	85.00%
Squamous cell	3	15.00%
TNM stage		
I-II	8	40.00%
III-IV	12	60.00%
Total	20	100.00%

Cell culture

A549, H1299, H1650, H292 and 293T cells were purchased at American Type Culture Collection (ATCC). A549 H1299, H1650 and H292 were grown in RPMI-1640 (Hyclone). 293T were grown in DMEM. The cell medium were added with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were cultured under 37 °C with 5% CO₂.

Transfection

Lentiviruses plasmid for knockdown and overexpression of PIK3R1 were provided by Genepharma (Shanghai, China). 293T cells were used to produce lentiviruses. The supernatants were collected 48 hours after transfection, filtered by a 0.45-um filter, and obtained at -80°C until use. sh-NC, sh-PIK3R1 were transfected into A549 and H1299 cells and LV-NC, LV-PIK3R1 were transfected into H1650 and H292 cells according to the manufacturer's protocol.

Cell viability assay

CCK-8 assay was used to detect the cell viability. 5000 cells/well cells were cultured in a 96-well plate. After transfection, each well was replaced with 100 ul mixture of 10 ul CCK8 (Dojindo, Japan) and 90 ul medium and kept at 37°C with 5% CO₂. Absorbance at 450 nm as valibility value was determined using an enzyme microplate reader (LabSystems) 2 hours later. A cell growth curve was plotted every 24 h for five days.

Quantitative real-time PCR

We used TRIzol (Invitrogen Corp) to extract total RNA. Reverse Transcriptase cDNA Synthesis Kit (Cat. no. RR037A; Taraka) was utilized to reverse transcribe total RNA. After that, SYBR Premix Ex Taq II (TaKaRaBio Technology) were used for quantitative real-time PCR (qPCR). Fianlly, the qPCR run on an Applied Biosystems 7500 Fast Real-Time PCR System (ABI, Foster, USA). The primers used were as follows: PIK3R1 Forward: 5'-CATCCCAGGACCTCTCT-3', Reverse: 5'-CGGGGGACTGGCGA-3'; GAPDH Forward: . 5'-CAAGGTCATCCATGACAACCTTTG-3', Reverse: 5'-GTCCACCACCCTGTTGCTGTAG-3'.

Western blot

Proteins were run on a 10% SDS-PAGE. We used polyvinylidene fluoride (PVDF) membranes to transfer protein. The primary antibodies for anti-PIK3R1, anti-p-PI3K, anti-PI3K, anti-p-AKT, anti-AKT, anti-p-mTOR, anti-mTOR and anti-GAPDH (Abcam, Cambridge, UK) and secondary antibodies conjugated with horse radish peroxidase were used for analysis. The enhanced chemiluminescence assay was used to visualize the protein bands.

Statistical analysis

Student paired t-tests were used for the comparison between primary tumors and adjacent noncancerous tissues. Student's unpaired t-test were used to measure the differences between two groups. Data were shown as the standard deviation of the mean (mean±standard deviation (SD)). Differences were considered significant with a P value <0.05.

Results

PIK3R1 was overexpressed in lung cancer

The PIK3R1 mRNA expression levels were detected at 20 paired tissues. PIK3R1 mRNA levels were significantly higher in tumour tissues than the corresponding noncancerous tissues (Fig. 1A, P < 0.001). Furthermore, PIK3R1 mRNA expression levels was higher in stage III-IV than in stage I-II (Fig. 1B, P < 0.001). PIK3R1 mRNA expression levels was higher in stage III (n = 5) and stage IV (n = 7) than in stage I (n = 4) and stage II (n = 4) (Fig. 1C, P < 0.01).

Knockdown of PIK3R1 inhibited cell proliferation of lung cancer cells

To access the underlying mechanisms PIK3R1 plays in lung cancer. We chose A549, H1299, H1650, and H292 for further analysis. sh-PIK3R1 were used to knockdown PIK3R1. PIK3R1 expression level was downregulated in A549^{sh - PIK3R1} and H1299^{sh - PIK3R1} cells (Fig. 2A and 2B&C). Cell viability assay showed loss of PIK3R1 inhibited cell proliferation of lung cancer. Figure 4A and 4B showed that the ability of proliferation in A549^{sh - PIK3R1} and H1299^{sh - PIK3R1} cell lines were markedly decreased compared with their respective controls. Furthermore, we downregulated the PIK3R1 expression in A549 and H1299 cells using 2 clones derived from 2 shRNAs and found inhibition of PIK3R1 suppressed cell proliferation (Supplementary Fig. 1).

Upregulated of PIK3R1 promoted cell proliferation of lung cancer cells

LV-PIK3R1 were used to increase PIK3R1 expression level. PIK3R1 expression level was upregulated in H1650^{LV - PIK3R1} and H292^{LV - PIK3R1} cells (Fig. 3A and 3B&C). The ability of proliferation in H1650^{LV - PIK3R1} and H292^{LV - PIK3R1} cell lines were markedly increased compared with their respective controls (Fig. 4C and 4D).

PIK3R1 activated PI3K/Akt/mTOR signaling pathways

In order to clarify the possible mechanism of PIK3R1 in lung cancer, we further investigated the role of PIK3R1 play on PI3K/AKT/mTOR signaling pathways. The results of western blot analysis demonstrated that loss of PIK3R1 reduced p-PI3K, p-Akt and p-mTOR expression levels in A549^{sh - PIK3R1} and H1299^{sh - PIK3R1} cell lines (Fig. 5). Moreover, overexpressed PIK3R1 upregulated p-PI3K, p-Akt and p-mTOR expression levels in H1650^{LV - PIK3R1} and H292^{LV - PIK3R1} cells (Fig. 6).

Overexpression of PIK3R1 reversed the effect of sh-PIK3R1 on cell proliferation

LV-PIK3R1 were used to increase PIK3R1 expression level in A549^{sh - PIK3R1} and H1299^{sh - PIK3R1} cells. PIK3R1 expression levels were upregulated in A549 and H1299 cells co-transfection of sh-PIK3R1 + LV-PIK3R1, compared to the transfection with sh-PIK3R1 only (Fig. 7A and 7B). Moreover, cell viability was increased in both A549 (Fig. 7C) and H1299 cells (Fig. 7D) following sh-PIK3R1 + LV-PIK3R1 co-transfection. This indicated that overexpression of PIK3R1 reversed the effect of sh-PIK3R1 on cell proliferation. PIK3R1 thus acted as a promoter in lung cancer progression.

Discussion

Members of the PI3K family proteins are critically regulates many tumour progression and tumorigenesis [16, 17]. In this investigation, we identified the mechanisms of PIK3R1, which encode p85, the regulatory subunit of PI3K in lung cancer. We confirmed the upregulated expression level of PIK3R1 and we found reduction of PIK3R1 expression inhibited proliferation in lung cancer. Overexpression of PIK3R1 promoted lung cancer cell proliferation. In addition, our results identified PIK3R1 promoted lung cancer proliferation via activating PI3K/AKT/mTOR signaling pathways.

We confirmed PIK3R1 exert oncogene properties in lung cancer through positive regulation of PI3K/AKT/mTOR signaling pathways, which consist with many studies. Ai X et al proved increased PIK3R1 expression in HCC tissues, and loss of PIK3R1 led to the colony forming capability inhibition and apoptosis enhancement via inactivating PI3K/AKT signaling [18]. miRNAs have been identified as regulators in post-transcriptional regulation of mRNA expression that affecting tumour growth [19]. Tian F et al found upregulated PIK3R1 expression in NSCLC and miR-486-5p played a crucial role in tumour cell proliferation and invasion by targeting PIK3R1 [20]. Chen S et al found the downregulation of phosphoinositide 3-kinase catalytic subunit gamma led to tumor proliferation inhibition in liver cancer [21]. Zhu Q et al observed that p85alpha and p110beta play roles in prostate cancer cell proliferation [22]. However, other groups displayed PIK3R1 as a tumor suppressor in a variety of tumor development. Yan LX et al identified PIK3R1 could suppressed growth, invasiveness, and metastatic properties of breast cancer by inhibiting PI3K/AKT activation [23]. Wang YD et al found overexpression of PIK3R1 gene inhibits the proliferation and migration in kidney cancer [24]. Altogether, PIK3R1 involved in numerous cancers and might be a potential biomarker for malignant cancers.

PI3K/AKT/mTOR signaling pathways play an important role in many tumors [25, 26]. Cumulative studies demonstrated the effect of PI3K/AKT/mTOR signaling pathways in tumor invasion or metastasis potency, including lung cancer [27, 28]. Ling C et al investigated that miR-4286 expression level was upregulated by PTEN/PI3K/AKT axis in non-small cell lung cancers [29]. MicroRNA-374b accelerated the development of lung cancer via activating PI3K/AKT pathway [30]. LncRNA BC200 regulated the cell proliferation in lung cancer through PI3K/AKT pathway [31]. SHIP1 suppressed cell proliferation, invasion and migration via the PI3K/AKT pathway in lung cancer [32]. PIK3R1 promoted lung cancer proliferation through PI3K/AKT/mTOR signaling pathways in our study.

PI3K/AKT/mTOR signaling pathways are potential targets for therapeutic intervention of human cancer [33, 34]. For lung cancer, many groups studied inhibitors of PI3K/AKT/mTOR signaling pathways. Lutein (3,3'-dihydroxy- β , ϵ -carotene) could inhibit cell proliferation and promote apoptosis via the PI3K/AKT/mTOR signaling pathways in lung cancer [35]. Jiang M et al studied the regulation of a safflower chemical compound extraction, hydroxysafflor yellow A (HSYA), and found it worked as a potential candidate in treatment of NSCLC via targeting PI3K/AKT/mTOR signaling pathways [36]. Sapylin (OK-432) suppressed lung cancer cell proliferation and promoted apoptosis through inhibiting PI3K/AKT signaling, providing a new theoretical basis for lung cancer treatment [37]. Wang J et al synthesized a novel compounds of PI3K/AKT pathway inhibitors W934 for lung cancer [38]. We identified PIK3R1 was involved in lung cancer progression and it may provide PIK3R1 as a potential target for the disease.

We found PIK3R1 expression promoted cell proliferation in lung cancer cells. Thereafter, our results showed that PI3K/AKT/mTOR signaling pathways were deactivated by reduction of PIK3R1. These results, taken together, showed that PIK3R1 promoted proliferation of lung cancer by activating the PI3K/AKT/mTOR signaling pathways, thus providing PIK3R1 as a potential therapeutic target for lung

cancer. Further studies with a focus on other tumorigenic properties such as colony forming ability, cell apoptosis, cell migration, cell invasion as well as in vivo experiments are necessary to confirm our results.

In summary, our results investigated that PIK3R1 was upregulated in lung cancer and overexpression of PIK3R1 promoted cell proliferation through activation of the PI3K/AKT/mTOR signaling pathways. Our findings suggested that PIK3R1 play an important role in the development and progression of lung cancer.

Abbreviations

PIK3R1: phosphoinositide-3-kinase, regulatory subunit 1; qPCR: quantitative real-time PCR; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; PI3K: Phosphoinositide-3-OH kinase; ATCC: American Type Culture Collection; HSYA: Hydroxysafflor yellow A.

Declarations

Authors' contributions

WW and HC supervised the experiment. YC, QD, CW, HH and YH carried out the experiment. YC and YH analyzed and discussed the experimental results. Finally, YC, WW and HC wrote the manuscript. All authors approved the final manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Consent for publication

All participants have given consent for publication.

Ethics approval and consent to participate

Ethical approval was given by the Ethics Committee of Huai'an Second People's Hospital, The Affiliated Huai'an Hospital of Xuzhou Medical University.

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

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Figures

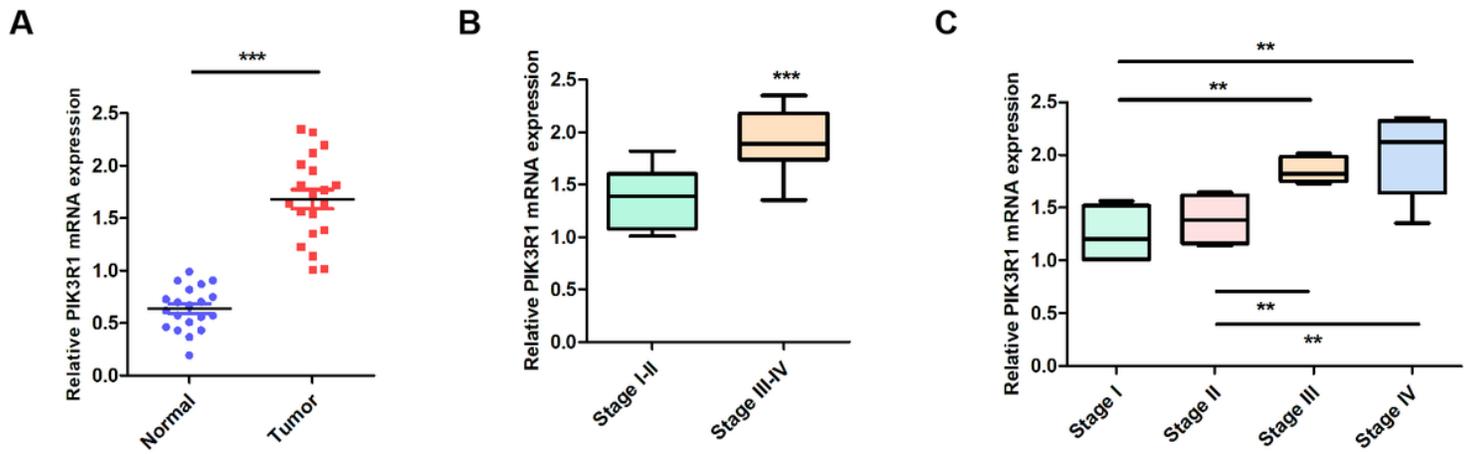


Figure 1

PIK3R1 is upregulated in the lung cancer. (A) The PIK3R1 expression in 20 paired normal-tumour lung cancer patients. (B) (C) PIK3R1 expression levels in different stages of lung cancer patients. ** $P < 0.01$. *** $P < 0.001$.

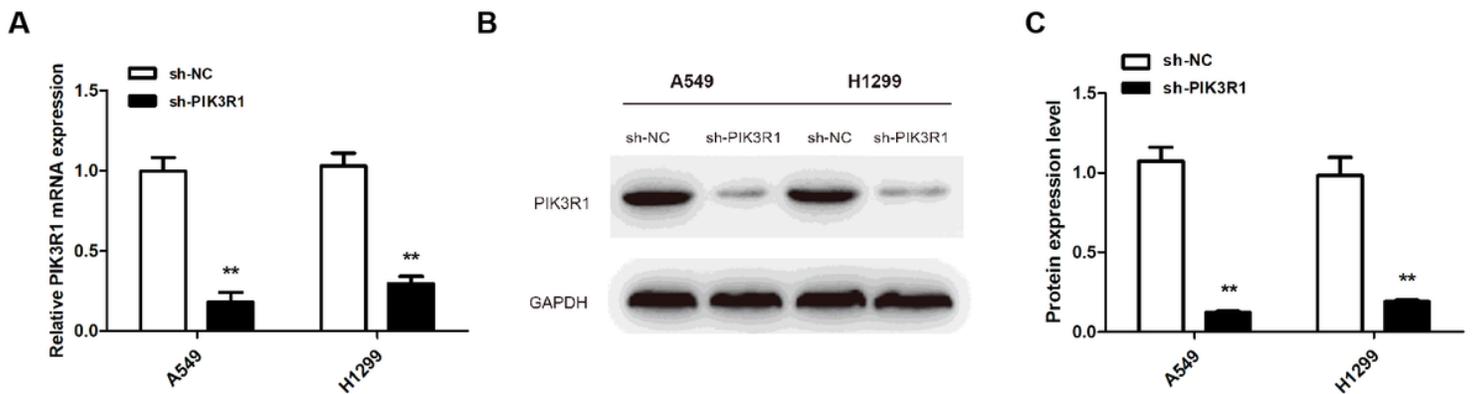


Figure 2

Knockdown of PIK3R1 in lung cancer cells. (A) PIK3R1 mRNA expression level was downregulated in A549sh-PIK3R1 and H1299sh-PIK3R1 cells. (B) Western blot in A549sh-PIK3R1 and H1299sh-PIK3R1 cells. (C) Quantitative PIK3R1 protein level in A549sh-PIK3R1 and H1299sh-PIK3R1 cells. ** $P < 0.01$.

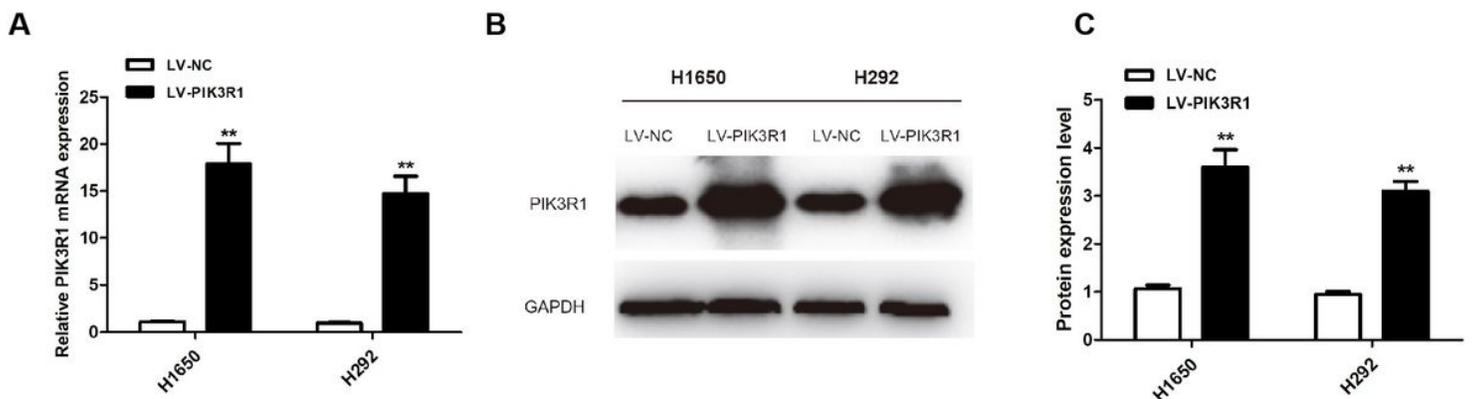


Figure 3

Overexpression of PIK3R1 in lung cancer cells. (A) PIK3R1 mRNA expression level was upregulated in H1650LV-PIK3R1 and H292LV-PIK3R1 cells. (B) Western blot analysis in H1650LV-PIK3R1 and H292LV-PIK3R1 cells. (C) Quantitative PIK3R1 protein level in H1650LV-PIK3R1 and H292LV-PIK3R1 cells. $^{**}P < 0.01$.

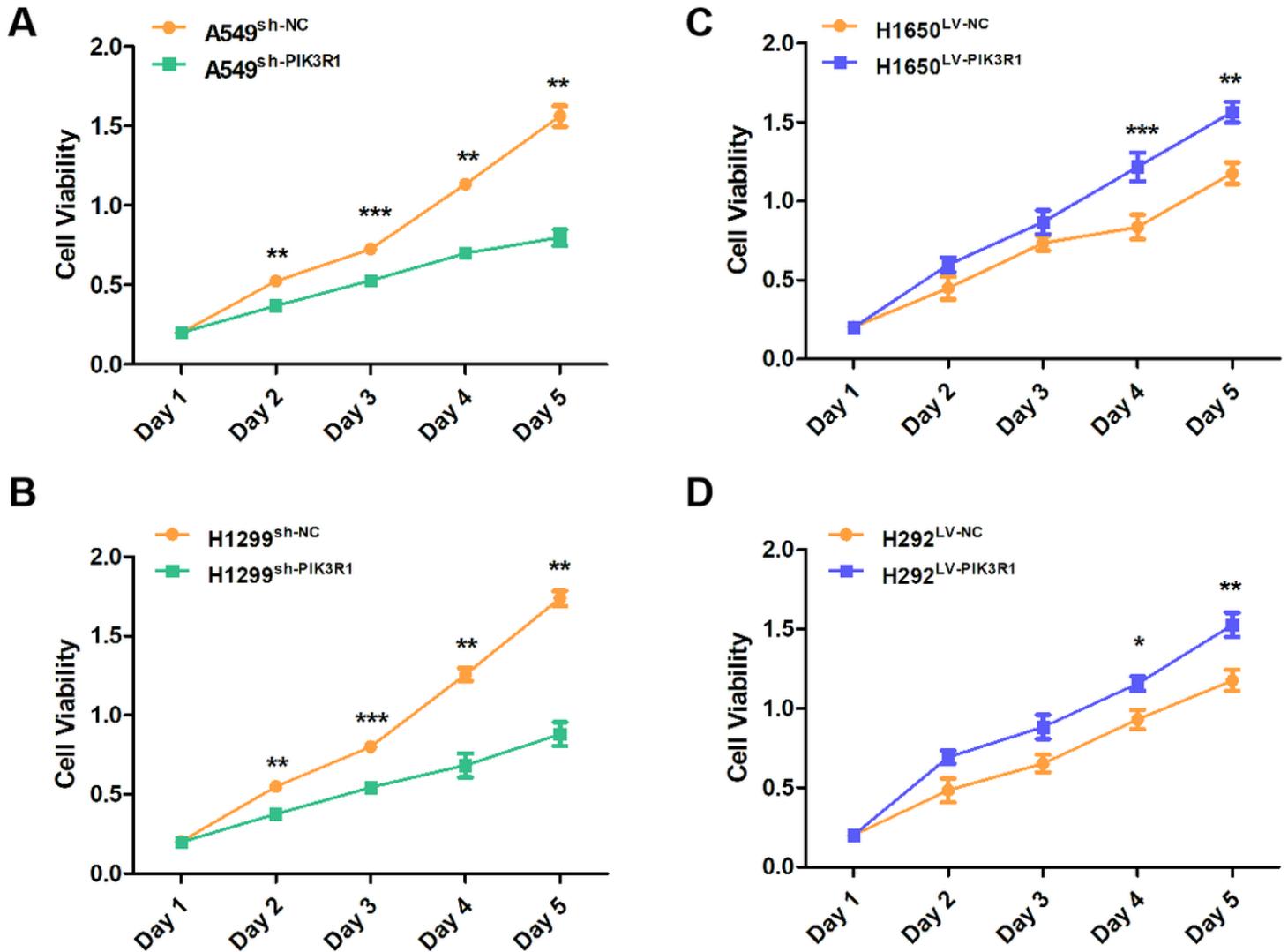


Figure 4

Upregulated PIK3R1 promotes lung cancer cells proliferation. (A) Cell viability assay of A549sh-PIK3R1 cells compared to A549sh-NC cells. (B) Cell viability assay of H1299sh-PIK3R1 cells compared to H1299sh-NC cells. (C) Cell viability assay of H1650LV-PIK3R1 cells compared to H1650LV-NC cells. (D) Cell viability assay of H292LV-PIK3R1 cells compared to H292LV-NC cells. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

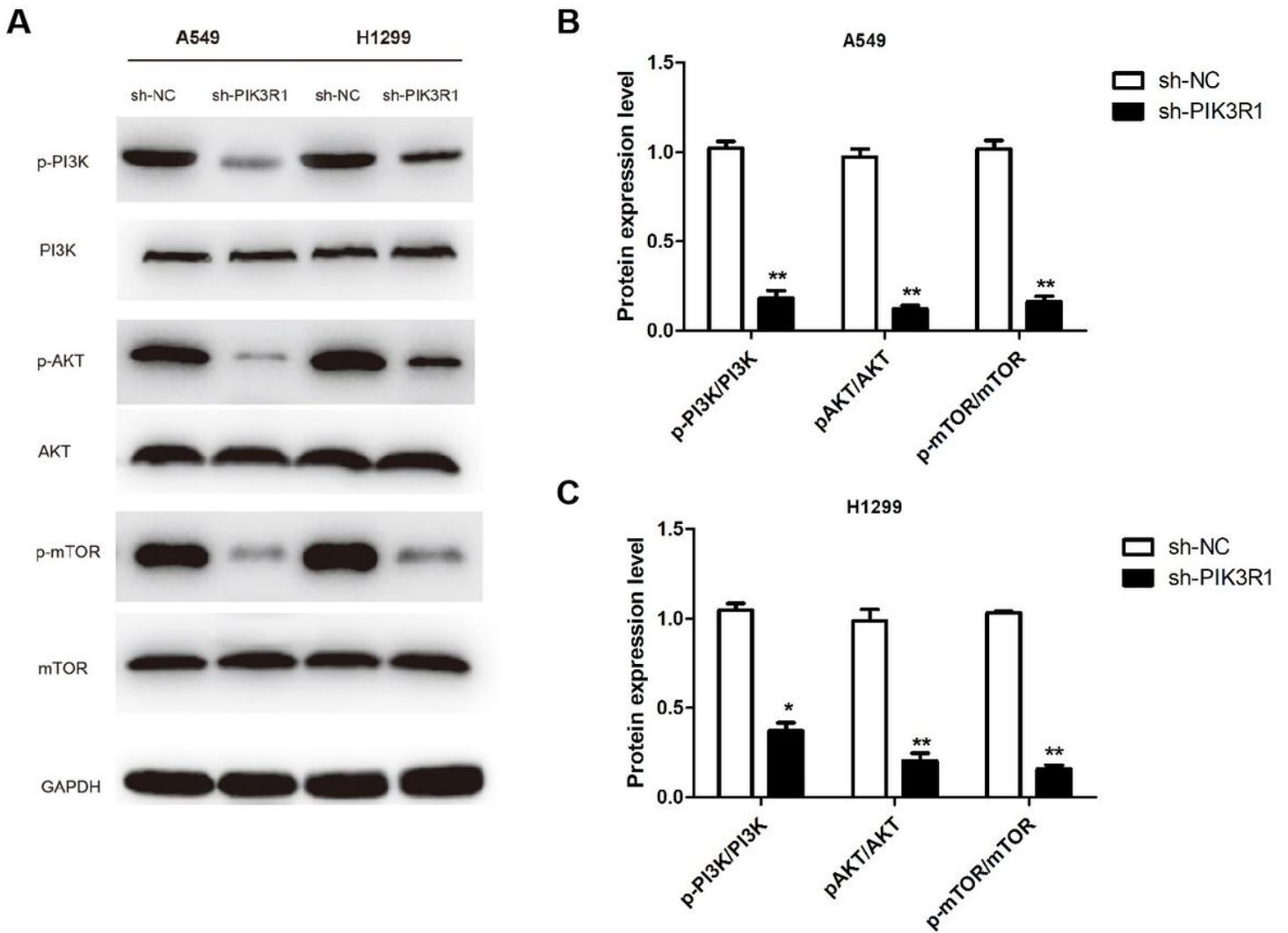


Figure 5

Knockdown of PIK3R1 suppresses the PI3K/AKT/mTOR signaling pathways. (A) Western blot analysis of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR protein levels in A549sh-PIK3R1 and H1299sh-PIK3R1 cells. (B) Quantitative protein level normalized to GAPDH in A549sh-PIK3R1 and H1299sh-PIK3R1 cells. (C) Quantitative protein level of p-PI3K/PI3K, pAKT/AKT and p-mTOR/mTOR in A549sh-PIK3R1 and H1299sh-PIK3R1 cells. *P < 0.05. **P < 0.01.

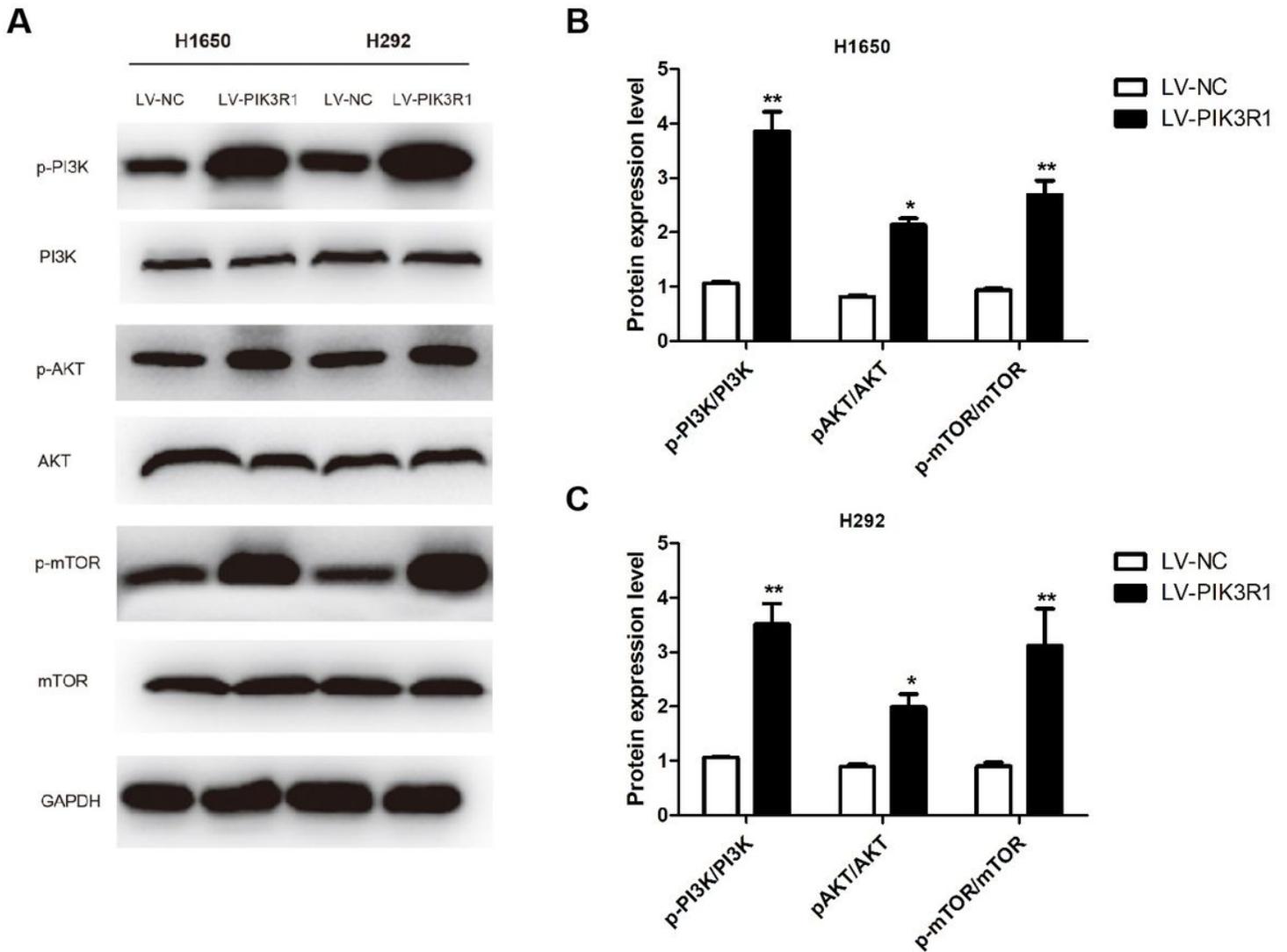


Figure 6

Increased PIK3R1 activates the PI3K/AKT/mTOR signaling pathways. (A) Western blot analysis of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR protein levels in H1650LV-PIK3R1 and H292LV-PIK3R1 cells. (B) Quantitative protein level normalized to GAPDH in H1650LV-PIK3R1 and H292LV-PIK3R1 cells. (C) Quantitative protein level of p-PI3K/PI3K, pAKT/AKT and p-mTOR/mTOR in H1650LV-PIK3R1 and H292LV-PIK3R1 cells. *P < 0.05. **P < 0.01.

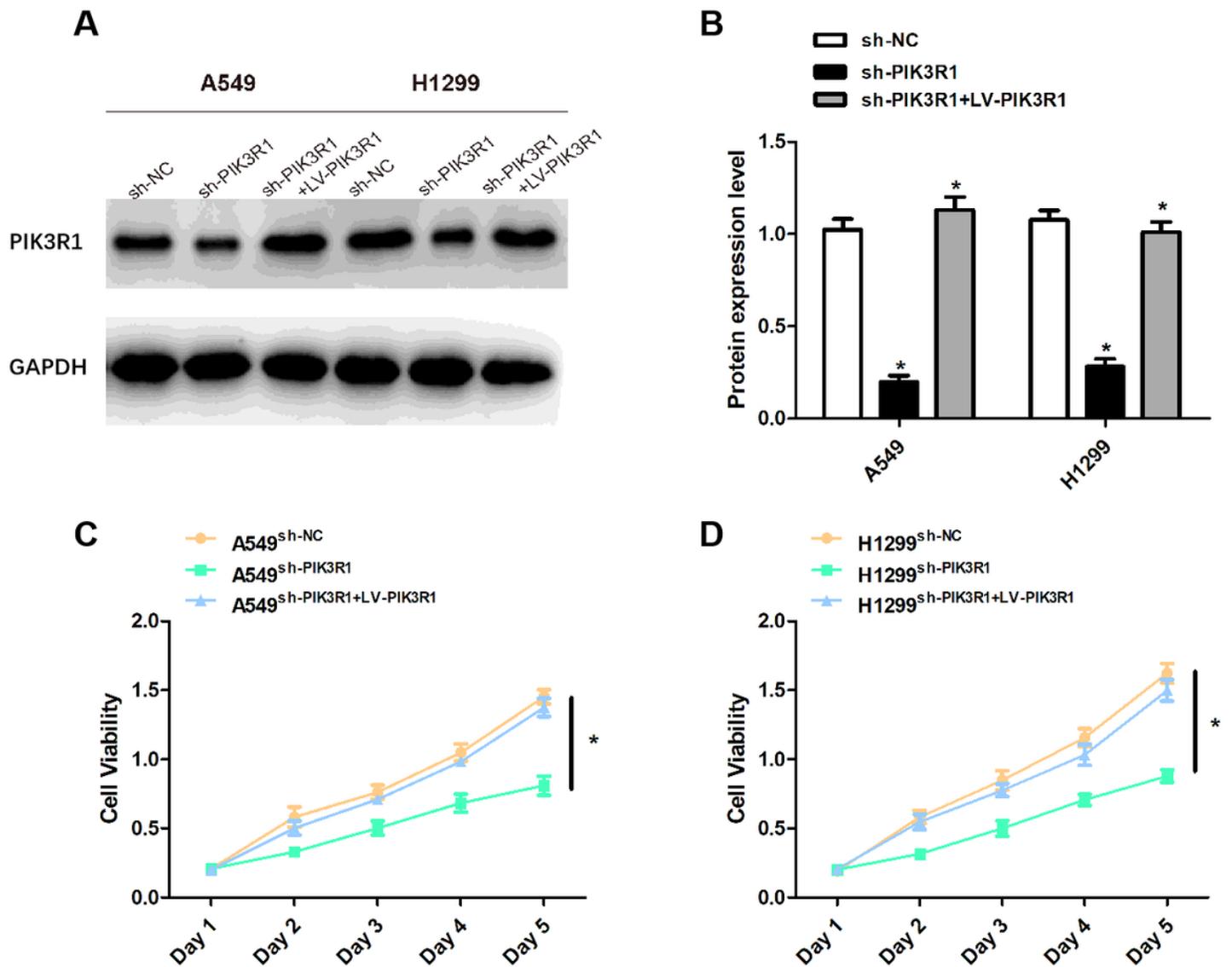


Figure 7

Overexpression of PIK3R1 reversed the effect of sh-PIK3R1 on cell proliferation. (A) (B) Western blot analysis and quantitative protein level of PIK3R1 in A549sh-NC, A549sh-PIK3R1, A549sh-PIK3R1+LV-PIK3R1, H1299sh-NC, H1299sh-PIK3R1, and H1299sh-PIK3R1+LV-PIK3R1 cells. (C) (D) Cell viability were measured by CCK-8 assay in A549sh-NC, A549sh-PIK3R1, A549sh-PIK3R1+LV-PIK3R1, H1299sh-NC, H1299sh-PIK3R1, and H1299sh-PIK3R1+LV-PIK3R1 cells. *P < 0.05.

Supplementary Files

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