HPV16-E6 but not E7 inhibits the antitumor activity of LKB1 in lung cancer cells by down regulating the expression of KIF7

Yue Hu
The First Affiliated Hospital and College of Basic Medical Science, China Medical University
Ming-Zhe Wu
The First Hospital of China Medical University
Na-Jin Gu
The First Affiliated Hospital and College of Basic Medical Sciences, China Medical University
Xue-Shan Qiu
The First Affiliated Hospital and College of Basic Medical Sciences, China Medical University
En-Hua Wang
The First Affiliated Hospital and Colllege of Basic Medical Sciences, China Medical University
Guang-Ping Wu (wug_ping@sina.com)
Southwest Hospital https://orcid.org/0000-0003-3478-6868

Primary research

Keywords: HPV16-E6, HPV16-E7, Kinesin family member 7 (KIF7), p-LKB1, Lung cancer

DOI: https://doi.org/10.21203/rs.3.rs-19692/v2

Abstract

Background: The E6 and E7 proteins in HPV16 are the main oncogenes in the occurrence of lung cancer. In recent studies, we had found that E6 and E7 downregulated the expression of LKB1 in lung cancer cells. However, it is not clear how E6 and E7 regulate LKB1 in lung cancer cells.

Methods: The double directional genetic manipulation and Nuclear plasma separation technology were performed to explore the molecular mechanism of E6 and E7 inhibiting the antitumor activity of LKB1 in well-established lung cancer cell lines.

Results: E6 but not E7 significantly downregulated the expression of tumor suppressor KIF7 at protein level, and the inhibition of KIF7 further reduced the expression of LKB1 both in the nuclei and in the cytoplasm, whereas reduced the expression of p-LKB1 in the cytoplasm only. Therefore, we suggested that HPV16 E6 but not E7 downregulates the antitumor activity of LKB1 by down regulating the expression of p-LKB1 in the cytoplasm only.

Conclusion: We demonstrated for the first time that E6 but not E7 inhibits the antitumor activity of LKB1 in lung cancer cells by downregulating the expression of KIF7. Our findings provided new evidence to support the important role of KIF7 in the pathogenesis of lung cancer and suggested new therapeutic targets.

Background

In 1980, Syrjänen first proposed the hypothesis of the role of HPV infection in the occurrence of bronchosquamous cell carcinoma [1]. With the rapid development of molecular biology technology and its timely application in the study of lung cancer, researchers found that E6 and E7 proteins in HPV16 are the main oncogenes, and long term persistent infection is often related to the occurrence of lung cancer, especially in non-smokers and Asians [2-5]. The E6 protein inhibited cell apoptosis mainly by degrading p53 gene [6], while the E7 protein promoted cell proliferation mainly by inhibiting retinoblastoma protein (pRb) [7]. Therefore, the signaling pathways were regulated by E6 and E7 proteins might not be exactly the same in the occurrence of lung cancer. Recently, we found that the overexpression of both E6 and E7 in HPV 16 downregulated the expression of LKB1 at both protein and mRNA levels in lung cancer cells [8, 9]. However, the potential molecular mechanism of regulation of LKB1 by E6 and E7 is not clear.

Liver kinase B1 (LKB1), also known as serine / threonine kinase 11 (STK11), was first found the tumor suppressor gene in patients with Peutz Jeghers syndrome (PJs) [10, 11]. It has serine / threonine protein kinase activity and plays an important role in the occurrence and development of lung cancer by phosphorylating substrate protein or binding with target protein to regulate gene expression [12, 13]. It had been shown that LKB1 is the key barrier of lung tumorigenesis, which controlled the occurrence, differentiation and metastasis of lung tumorigenesis [14]. The in vitro experiment results showed that the phosphorylation of LKB1 at Ser 428 was involved in the anticancer effect of human oral cancer cells [15].

Recently, Wong et al. found that KIF7 promoted the antitumor activity of LKB1 through up regulation the expression of LKB1 and induction the phosphorylation of LKB1 at Ser 428 [16].

Kinesin family member 7 (KIF7) is on 15q26.1 and a Kinesin-4 family member that has been shown to play critical roles in primary cilia formation and Hedgehog (Hh) signaling in embryonic development [17, 18]. KIF7 was a novel tumor suppressor in prostate cancer that acts by suppressing proliferation, migration, invasion and tumorigenesis through LKB1/PTEN/AKT signaling pathway [16], the low expression of KIF7 indicated poor prognosis in epithelial ovarian cancer [19] and facilitated cell survival and migration of choriocarcinoma cells [20].

In this study, we investigated that HPV16 E6 up-regulated the expression of p-LKB1 through HPV- KIF7-LKB1 axis. Here, we report that HPV16-E6 but not E7 inhibits the antitumor activity of LKB1 in lung cancer cells by down regulating the expression of KIF7

Materials And Methods

Cell culture

Three human non-small-cell lung carcinomas (NSCLC) cell lines H460, H1299, A549, and normal human bronchial epithelial (HBE) cell line were used in this study. HBE, H460, H1299, and A549 cell lines were obtained from the ATCC (Manassas, VA, USA) and cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS, Cellmax, Beijing, China) at 37˚C in a 5% CO2 humidified atmosphere.

1) Transfection and interference

Based on the previous study results, H1299 cells were E6 and E7 low-expression cell lines, while A549 cells were E6 and E7 high-expression cell lines [9]. The plasmid of pEGFP-N1-HPV16 E6, pEGFP-N1-HPV16 E7, and pEGFP-N1 were kindly provided by Prof Xudong Tang, Institute of Biochemistry and Molecular Biology, Guangdong Medical College, China. HPV16 E6 SiRNA and HPV16 E7 SiRNA were purchased from RIBOBIO (Guangzhou, China), KIF7 siRNA was purchased from General Biosystems (Anhui, China). Disordered siRNA was used as a nonspecific siRNA control.

The cells were performed in 6-well plates using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) as per instructions from the manufacturer. Transfection with empty vector and mock transfection were used as controls. The protein analysis was assessed 48 hours after transfection by western blotting. The mRNA analysis was assessed 24 hours after transfection by quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR).

Western-blot assays

The assays were performed as previously described [9]. Information about primary antibodies is as follows: HPV16 E6 (1:100, Bioss Biotechnology Co., Ltd, Beijing, China), HPV16 E7 (1:100, Bioss Biotechnology Co., Ltd, Beijing, China), KIF7 (1:1000, Proteintech, Wuhan, China), LKB1 (1:800,

Proteintech, Wuhan, China), p-LKB1 (Phospho-Ser428) (1:800, Sangon Biotech, Shanghai, China), GAPDH (1:1000, Cell Signaling Technology, Danvers, MA, USA), and Lamin B (1:800, WanLeibio, Shenyang, China).

Quantitative real-time PCR

Total RNA was extracted from cells with Trizol solution (TaKaRa, Dalian, China) and RNase RNA isolation kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. A total of 1μg RNA was subjected to reverse transcription reaction to obtain cDNAs by using a Prime Script TM RT reagent Kit (TaKaRa, Dalian, China). qRT-PCR was performed using SYBR® Premix Ex Taq II (TaKaRa, Dalian, China) on 7900HT Fast Real-Time PCR System (Applied Biosystems). Non-template controls were carried out every time for each primer pair to detect nonspecific amplification. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as the internal control. All reactions were run in triplicate. The fold change of mRNA expression was calculated using the $2^{-\Delta\Delta ct}$ method. The detailed information of the primers is given in Table 1.

Nuclear plasma separation technology

H1299 cells were interference with SiKIF7. Nuclear and Cytoplasmic Protein Extraction Kit (P0028, Beyotime, Shanghai, China) was used to perform nuclei isolation. All the experiments were performed on ice, Cytoplasmic protein extraction reagent A with 1mM PMSF was added to the cell suspension, and then 10-15 minutes of ice bath after 5 seconds of violent oscillation at the highest speed, cytoplasmic protein was added to cytoplasmic protein extraction reagent B. After 5 seconds of violent oscillation at the highest speed, the supernatant was centrifuged at 4℃ at 12000g for 5 minutes. The nuclear protein extraction reagent with 1mM PMSF was added to the remaining precipitate, which was intensely vortex at the highest speed for 15-30 seconds and then placed in an ice bath for 30 minutes, during which time the nuclear protein was intensely vortex for 15-30 seconds every 1-2 minutes. After centrifugation at 4℃ and 12000g for 10 minutes, the supernatant was absorbed into the precooled plastic tube, which was nuclear protein.

Statistical analysis

SPSS 22.0 software was utilized for statistical analyses in this study. Each assay was performed at least 3 times. The data were expressed as mean ± SD. Statistical significance was determined by Student's t-test, and a p value<0.05 was considered significant.

Results

The screening of lung cancer cell lines

Based on our previous results, H1299 cell line was low E6 and E7 expression cell line, and A549 cell line was high E6 and E7 expression cell line [21]. The expression of KIF7 was screened in normal bronchial

epithelial cell line HBE and lung cancer lines H1299, H460, and A549, respectively. As shown in S1Fig, the high expression level of KIF7 was found in HBE and H1299, whereas low expression level of KIF7 was observed in H460 and A549 cell lines. Further assays were designed and performed based on these results.

The overexpression of E6 significantly downregulated the expression of KIF7 and LKB1, whereas the overexpression of E7 significantly downregulated the expression of LKB1 only.

The pEGFP-N1-E6 or E7 vectors were transiently transfected into the low expression H1299 cell line, and the E6 or E7 empty vectors and mock transfections served as controls. The results showed that the overexpression of E6 significantly downregulated the expression of KIF7 at protein level only and the expression of LKB1 at both protein and mRNA levels, whereas the overexpression of E7 significantly downregulated the the expression of LKB1 at both protein and mRNA levels and had no regulatory effect on KIF7. Results are presented in Figure1A.

The inhibition of E6 evidently upregulated the expression of KIF7 and LKB1, whereas the inhibition of E7 evidently upregulated the expression of LKB1 only

To further verify the regulatory roles of E6 or E7 on KIF7 and LKB1, we applied E6 or E7-specific siRNA to knockdown the expression of E6 or E7 in the A549 cell line. E6 or E7-nonspecific siRNA and mock specific siRNA were used to serve as the controls. The results indicated that the inhibition of E6 evidently upregulated the expression of KIF7 at protein level only and the expression of LKB1 at both protein and mRNA levels, whereas the inhibition of E7 evidently upregulated the expression of LKB1 at both protein and mRNA levels and had no regulatory effect on KIF7. The results were presented in Fig.1 B.

The inhibition of KIF7 obviously downregulated the expression levels of LKB1 and p-LKB1

To further verify the regulatory roles of KIF7 on LKB1 and p-LKB1, we applied KIF7-specific siRNA to knockdown the expression of KIF7 in the H1299 cell line. KIF7-nonspecific siRNA and mock specific siRNA were used to serve as the controls. The results indicated that the inhibition of KIF7 obviously downregulated the expression of LKB1 at both protein and mRNA levels and the expression of p-LKB1 at protein level. The results were presented in Fig.2 A.

The loss of KIF7 reduced the expression of LKB1 both in the nuclei and in the cytoplasm, whereas reduced the expression of p-LKB1 in cytoplasm only.

To determine whether the localization of LKB1 or p-LKB1 is related to the regulation of KIF7, we used western blotting to detect the expression of LKB1 or p-LKB1 in nucleic and cytoplasmic fractions. We knocked KIF7 in H1299 cells and separated the proteins in the nucleus and in the cytoplasm by using nuclear plasma separation technology. We found that the expression level of LKB1 was downregulated in both the nuclei and the cytoplasm, whereas the expression level of p-LKB1 was downregulated in cytoplasm only. The results were presented in Fig.2B.

Discussion

In our previous work, we found that the overexpression of both E6 and E7 in HPV 16 downregulated the expression of LKB1 at both protein and mRNA levels in lung cancer cells [8, 9]. However, the underlying molecular mechanism of the regulation of LKB1 by HPV16 is not clear. In this study, we found that the overexpression of E6 significantly downregulated the expression of KIF7 at protein level only and the expression of LKB1 at both protein and mRNA levels. Conversely, the knockdown of E6 evidently upregulated the expression of KIF7 at protein levels only and the expression of LKB1 at both protein and mRNA levels. We believed that the regulation mechanism of E6 on KIF7 might be through translational or post-translational pathways. However, we found that whether by transfection or interference, E7 had no regulatory effect on KIF7. Although E7 could also downregulate the expression of LKB1, it was not achieved by downregulating KIF7. Therefore, we speculate that the regulation of LKB1 by E7 may be accomplished by other genes. To our knowledge this is the first time found that KIF7 was involved in HPV16 E6 but not E7 downregulate the expression of LKB1 in lung cancer cells. The detailed regulatory mechanism between E6 and KIF7 needs to be further studied in the future.

The increased expression of protein and mRNA of LKB1 does not mean that its antitumor activity is increased. Wong et al. had speculated that KIF7 upregulated the antitumor activity of LKB1 in two ways, one was phosphorylation, and the other was subcellular localization; and they also found that KIF7 significantly upregulated the expression of LKB1 in both the nuclei and the cytoplasm and promoted the phosphorylation of LKB1 at Ser 428 [16]. However, they did not find that the expression level of LKB1 phosphorylation induced by KIF7 in the nucleus was completely different from that in the cytoplasm. In the present study, we found that inhibition of KIF7 not only significantly downregulated the expression of LKB1, but also downregulated the expression of p-LKB1. Our results from nuclear-cytoplasmic protein separation western blot analysis showed that the inhibition of KIF7 significantly decreased the expression level of p-LKB1 at Ser 428 in the cytoplasm, but the expression level in the nucleus was basically unchanged. These results indicated that the antitumor activity of LKB1 was determined by two factors, one was phosphorylation of LKB1, the other was that p-LKB1 must be located in the cytoplasm, both were indispensable. It is not clear which of the two conditions occurs first, the phosphorylation or the subcellular translocation. The detailed molecular mechanism needs further study in the future.

We had carried out a series of studies on the role of HPV16 in the carcinogenesis of lung cancer in the past, and the carcinogenic effects of E6 and E7 proteins were completely consistent [8, 9, 21]. However, Liu et al. had found that E6 and E7 in HPV16 played different roles in the regulation of ERK signaling pathway [22]. For the first time in this study, we found that HPV16-E6 but not E7 inhibits the antitumor activity of LKB1 in lung cancer cells by down regulating the expression of KIF7.

Conclusion

We demonstrated for the first time that E6 but not E7 inhibits the antitumor activity of LKB1 in lung cancer cells by downregulating the expression of KIF7 and the antitumor activity of LKB1 was determined

by two factors, one was phosphorylation of LKB1, the other was that p-LKB1 must be located in the cytoplasm, both were indispensable. Our findings provided new evidence to support the important role of KIF7 in the pathogenesis of lung cancer and suggested new therapeutic targets.

Abbreviations

HPV: Human papillomavirus; KIF7: Kinesin family member 7; LKB1: Liver kinase B1; STK11: serine / threonine kinase 11.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Wu GP and Hu Y designed the study; Hu Y, Wu MZ and Gu NJ performed the study and wrote the manuscript; Wu GP, Qiu XS and Wang EH analysed and generated the images. All authors read and approved the final manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China to Guang-Ping Wu, Grants No.81171650 and 81672082.

Availability of data and materials

Not applicable.

Ethical Approval

Ethical approval was obtained for the experimental procedures by the Ethics Committee of the First Hospital of China Medical University (APPROVAL NUMBER/2016-125), Shenyang, China.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID ID

Guang-Ping Wu https://orcid.org/0000-0003-3478-6868

References

1. Syrjänen KJ. Epithelial lesions suggestive of a condylomatous origin found closely associated with invasive bronchial squamous cell carcinomas. Respiration. 1980; 40(3): 150-60.
2. Corrales L, Rosell R, Cardona AF, Martín C, Zatarain-Barrón ZL, Arrieta O. Lung cancerin never smokers: The role of different risk factors other than tobacco smoking. Crit Rev Oncol Hematol. 2020; 148:102895. [Epub ahead of print]
3. Liu J, Huang B, Xiu Z, Zhou Z, Liu J, Li X, Tang X. PI3K/Akt/HIF-1α signaling pathway mediates HPV-16 oncoprotein-induced expression of EMT-related transcription factors in non-small cell lung cancercells. J Cancer. 2018; 9(19):3 456-3466.
4. de Oliveira THA, do Amaral CM, de França São Marcos B, Nascimento KCG, de Miranda Rios AC, Quixabeira DCA, Muniz MTC, Silva Neto JDC, de Freitas AC. Presence and activity of HPVin primary lung cancer. J CancerRes Clin Oncol. 2018; 144(12): 2367-2376.
5. Zhang E, Feng X, Liu F, Zhang P, Liang J, Tang X. Roles of PI3K/Akt and c-Junsignalingpathways in human papillomavirus type 16 oncoprotein-induced HIF-1α, VEGF, and IL-8 expression and in vitro angiogenesis in non-small cell lung cancer cells. PLoS One. 2014; 9(7): e103440.
6. Chlon TM, Hoskins EE, Mayhew CN, Wikenheiser-Brokamp KA, Davies SM, Mehta P, Myers KC, Wells JM, Wells SI. High-risk human papillomavirus E6 proteinpromotesreprogramming of Fanconi anemia patient cells through repression of p53 but does not allow for sustained growth of induced pluripotent stem cells. J Virol. 2014; 88(19): 11315-26.
7. Todorovic B, Hung K, Massimi P, Avvakumov N, Dick FA, Shaw GS, Banks L, Mymryk JS. Conservedregion3 of human papillomavirus 16 E7 contributes to deregulation of the retinoblastoma tumor suppressor. J Virol. 2012; 86(24): 13313-23.
8. Yang JH, Li XY, Wang X, Hou WJ, Qiu XS, Wang EH, Wu GP. Long-term persistent infection of HPV 16 E6 up-regulate SP1 and hTERT by inhibiting LKB1 in lung cancer cells. PLoS One. 2017; 12(8): e0182775.
9. Shao JS, Sun J, Wang S, Chung K, Du JT, Wang J, Qiu XS, Wang EH, Wu GP. HPV16 E6/E7 upregulates HIF-2α and VEGF by inhibiting LKB1 in lung cancer cells. Tumour Biol. 2017; 39(7): 1010428317717137.
10. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Höglund P, Järvinen H, Kristo P, Pelin K, Ridanpää M, Salovaara R, Toro T, Bodmer W, Olschwang S, Olsen AS, Stratton MR, de la Chapelle A, Aaltonen LA. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature. 1998; 391(6663): 184-7.
11. Mehenni H, Resta N, Park JG, Miyaki M, Guanti G, Costanza MC. Cancer risks in LKB1 germline mutation carriers. Gut. 2006; 55(7): 984-90.

12. Jiang S, Shi F, Lin H, Ying Y, Luo L, Huang D, Luo Z. Inonotus obliquus polysaccharides induces apoptosis of lung cancercells and alters energy metabolism via the LKB1/AMPK axis. Int J Biol Macromol. 2019 Nov 18. pii: S0141-8130(19)32725-4.
13. Park C, Lee Y, Je S, Chang S, Kim N, Jeong E, Yoon S. Overexpression and Selective Anticancer Efficacy of ENO3 in *STK11*Mutant LungCancers. Mol Cells. 2019; 42(11): 804-809.
14. Ji H, Ramsey MR, Hayes DN, Fan C, McNamara K, Kozlowski P, Torrice C, Wu MC, Shimamura T, Perera SA, Liang MC, Cai D, Naumov GN, Bao L, Contreras CM, Li D, Chen L, Krishnamurthy J, Koivunen J, Chirieac LR, Padera RF, Bronson RT, Lindeman NI, Christiani DC, Lin X, Shapiro GI, Jänne PA, Johnson BE, Meyerson M, Kwiatkowski DJ, Castrillon DH, Bardeesy N, Sharpless NE, Wong KK. LKB1modulateslung cancer differentiation and metastasis. Nature. 2007; 448(7155): 807-10
15. Tsai SC, Tsai MH, Chiu CF, Lu CC, Kuo SC, Chang NW, Yang JS. AMPK-dependent signaling modulates the suppression of invasion and migration by fenofibrate in CAL 27 oral cancer cells through NF-κB pathway. Environ Toxicol. 2016; 31(7): 866-76.
16. Wong KY, Liu J, Chan KW. KIF7attenuates prostate tumor growth through LKB1-mediated AKT inhibition. Oncotarget. 2017; 8(33): 54558-54571.
17. He M, Subramanian R, Bangs F, Omelchenko T, Liem KF Jr, Kapoor TM, Anderson KV. The kinesin-4 protein Kif7 regulates mammalian Hedgehog signaling by organizing the cilium tip compartment. Nat Cell Biol. 2014; 16(7): 663-72.
18. Cheung HO, Zhang X, Ribeiro A, Mo R, Makino S, Puviindran V, Law KK, Briscoe J, Hui CC. The kinesin protein Kif7 is a critical regulator of Gli transcription factors in mammalian hedgehog signaling. Sci Signal. 2009; 2(76): ra29.
19. Yao Y, Liu L, He W, Lin X, Zhang X, Lin Z, Zeng Z, Guo S. Low expression of KIF7indicates poor prognosis in epithelial ovarian cancer. Cancer Biomark. 2019; 26(4): 481-489.
20. Ho J, Du Y, Wong OG, Siu MK, Chan KK, Cheung AN. Downregulation of the gli transcription factors regulator Kif7facilitates cell survival and migration of choriocarcinoma cells. PLoS One. 2014; 9(9): e108248.
21. Fan R, Hou WJ, Zhao YJ, Liu SL, Qiu XS, Wang EH, Wu GP. Overexpression of HPV16 E6/E7 mediated HIF-1α upregulation of GLUT1 expression in lung cancer cells. Tumour Biol. 2016, 37(4): 4655-63.
22. Liu F, Lin B, Liu X, Zhang W, Zhang E, Hu L, Ma Y, Li X, Tang X. ERK Signaling Pathway Is Involved in HPV-16 but not E7 Oncoprotein-Induced HIF-1α Protein Accumulation in NSCLC Cells. Oncol Res. 2016; 23(3): 109-18.

Table

Table 1. Sequencesand features of primers used for qRT-PCR

Gene	Forward/ Reverse	Sequence	Size(bp)	mRNA
E6	270	GTATGGAACAACAT TAGAACAGCAA	79	KX545363
	349	GTGGCTTTTGACAG TTAATACACC		
E7	482	GCATGGAGATACAC CTACATTG	273	KX545363
	754	TGGTTTCTGAGAAC AGATGG		
LKB1	223	AGGGCCGTCAAGAT CCTCAA	187	KU178339
	409	GCATGCCACACACG CAGTA		
KIF7	2496	GGAGAAGAAGCAGG CTACGG	374	NM_198525.3
	2869	GCTGCTGTAGCACC TTCTCC		
GAPDH	50	TTCTTTTGCGTCGCC	71	XM_019023188.1
	120	AGCCGAG CCAGGCGCCCAATA CGACCAAA		

mRNA: messenger RNA; qRT-PCR: quantitative real-time reverse transcriptase-polymerase chain reaction

Supplemental Information Note

Figure Supplement 1. Detection of the expression of KIF7 was in lung cancer cell lines (H1299, H460, and A549) using Western blotting; HBE, a normal bronchial epithelial cell line, served as the positive control, and GAPDH served as the internal control.

Figures

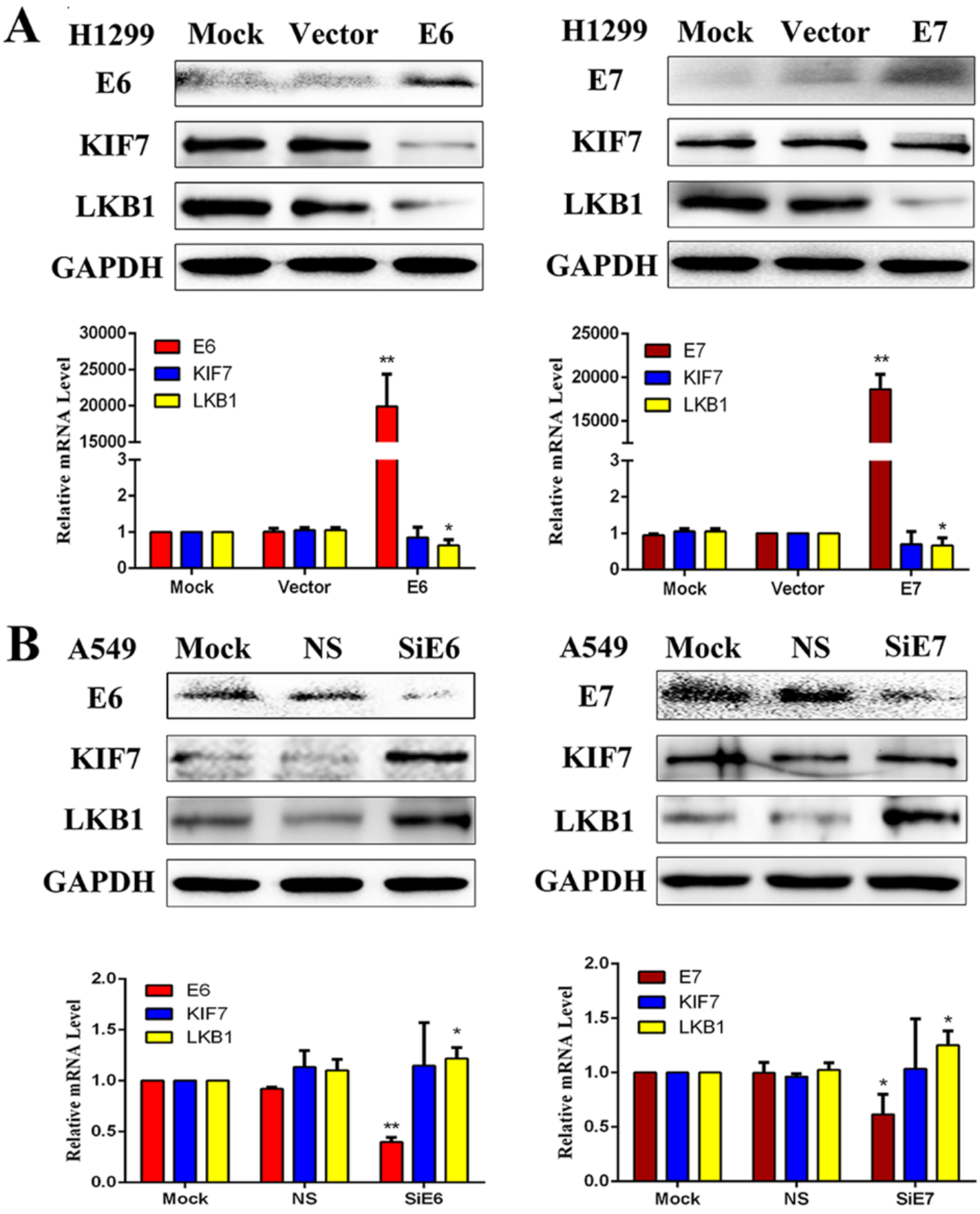
A
H1299
Mock
Vector
E6
E6
KIF7
LKB1
GAPDH
Relative mRNA Level
30000
25000
20000
15000
3
2
1
0
E6
KIF7
LKB1
**
*
Mock
Vector
E6
H1299
Mock
Vector
E7
E7
KIF7
LKB1
GAPDH
Relative mRNA Level
25000
20000
15000
3
2
1
0
E7
KIF7
LKB1
**
*
Mock
Vector
E7
B
A549
Mock
NS
SiE6
E6
KIF7
LKB1
GAPDH
Relative mRNA Level
2.0
1.5
1.0
0.5
0.0
E6
KIF7
LKB1
**
*
Mock
NS
SiE6
A549
Mock
NS
SiE7
E7
KIF7
LKB1
GAPDH
Relative mRNA Level
2.0
1.5
1.0
0.5
0.0
E7
KIF7
LKB1
*
*
Mock
NS
SiE7

Figure 1

The effects of E6 and E7 on the regulation of KIF7 and LKB1 expression by transfection (A) and interference (B) in lung cancer cells. E6, E7, KIF7, and LKB1 were demonstrated by western blotting and RT-qPCR in lung cancer cells. Mock: mock transfection; Vector: empty vector; NS: no significance (*p<0.05; **p<0.0l).

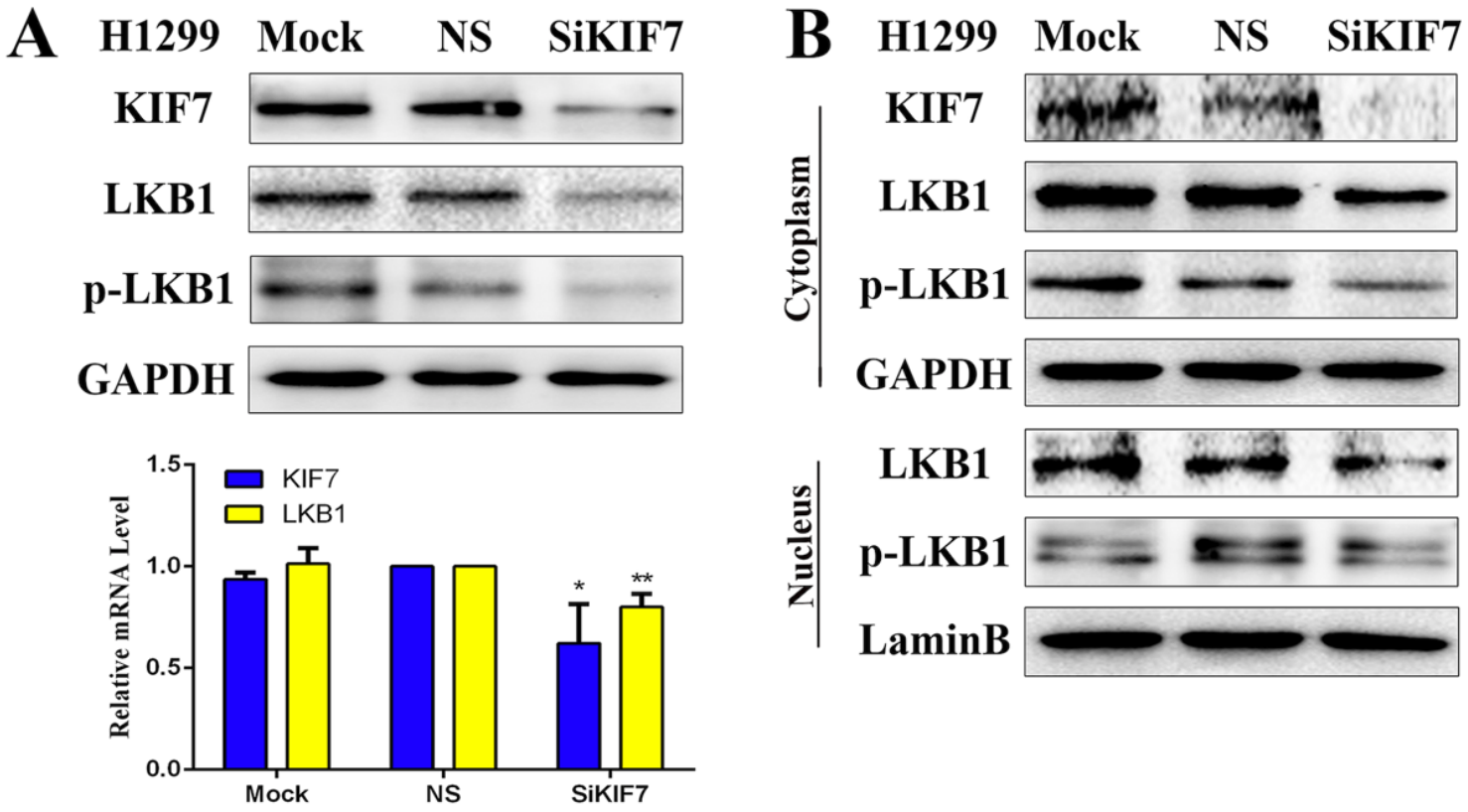

Figure 2

The effects of KIF7 on the regulation of LKB1 and p-LKB1 expression by interference, KIF7, LKB1, and p-LKB1 were demonstrated by western blotting in H1299 cells (A). The inhibition of KIF7 downregulated the expression levels of LKB1 in both the cytoplasmic and the nuclear and the expression level of p-LKB1 in the cytoplasmic only. KIF7-specific siRNA was performed by western blotting in H1299 cells (B). Mock: mock transfection; NS: no significance (*p<0.05; **p<0.0l).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementFigure.tif