Int J Clin Exp Pathol 2016;9(11):11179-11187
www.ijcep.com /ISSN:1936-2625/IJCEP0037550

Original Article

EIF3B silencing inhibits cell proliferation via regulating EGFR/ERK pathway in lung adenocarcinoma

Bo Zhong, Chunyi Zhang, Yefeng Chen, Jixian Tang, Juxin Shen

Department of Respiratory Medicine, Shaoxing People's Hospital, Zhejiang, China

Received August 9, 2016; Accepted September 27, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: Objective: We aimed to investigate the biological role of eukaryotic translation initiation factor 3B (EIF3B) and its mechanism in lung adenocarcinoma (LAD) occurrence. Methods: EIF3B was firstly determined in LAD tissues and cell lines using Western blot and quantitative real-time PCR. EIF3B was then knocked down using RNA interference technology in LAD cell lines, A549 and 95D. Furthermore, function analysis, including CCK-8, colony formation, cell cycle and apoptosis assay were conducted on A549 and 95D cells. Results: EIF3B protein was strongly upregulated in LAD samples compared to normal tissues. The same results were observed in lung cancer cell lines. Knockdown of EIF3B significantly inhibited the proliferation of LAD, induced cell cycle arrest and apoptosis. Furthermore, the proliferation inhibitory effect was associated with downregulation of p-EGFR and p-ERK induced by EIF3B silencing. Conclusion: These data suggest that EIF3B contributes to the proliferation of LAD cells might through regulating EGFR/ERK pathway.

Keywords: Cell proliferation, EIF3B, EGFR, ERK, lung adenocarcinoma

Introduction

Lung cancer is commonly diagnosed as one of the leading causes of cancer-related mortality worldwide [1]. As the most type of lung cancer, non-small cell lung cancer (NSCLC) mainly consists of squamous cell carcinoma, large-cell carcinoma and lung adenocarcinoma (LAD) [2], among which LAD accounts for approximately 40% of NSCLC cases [3]. Despite some diagnostic methods of lung cancer, like pathology, imageology and biochemical techniques can improve the clinical outcome [4], the overall 5-year survival rate of lung cancer patients remains less than 5% [5]. Recently, molecular biological techniques have been widely applied for early diagnosis of lung cancer, which is helpful for us to improve understanding of the pathogenesis of lung cancer. Even though numerous biomarkers, such as carcinoembryonic antigen (CEA) and squamous cell carcinoma antigen (SCC-Ag) have been identified to diagnose lung cancer, critical molecular targets and pathways responsible for lung cancer progress still require further exploration.

As our best knowledge, regulation of eukaryotic protein synthesis at translation initiation level plays a crucial role in normally controlling cell proliferation. It has been reported that eukaryotic translation initiation factors (EIFs) could promote protein synthesis by assembling ribosomes onto mRNA [6]. As the largest factor EIFs, EIF3 consists of 13 subunits (EIF3A-EIF3M) and is essential for cellular initiation of translation and eukaryotic protein synthesis [7, 8]. Accumulated evidences have demonstrated that EIF3 expressed in malignancies is involved in cancer development and progression. For example, EIF3D has been frequently reported to be associated with tumor development, including prostate cancer [9], melanoma [10], colon cancer [11], glioma [12], as well as NSCLC [13]. In addition, increased EIF3A could promote cell proliferation of ovarian cancer cells [14]. Downregulation of EIF3E suppressed cell growth in colon cancer [15]. Furthermore, EIF3B is considered as the major scaffolding subunit of EIF3 complex containing 13 putative subunits in human [16], which is shown to interact with EIF3A, G, I and J with 814-amino acid proteins [17, 18]. Notably, it has emerged that

EIF3B is overexpressed in human bladder and prostate cancer and higher expression has poor prognosis in cancer progression [19]. What's more, knockdown of EIF3B has inhibitory effects on glioma cell proliferation [20]. These previous studies have suggested the oncogenic role of EIF3B in various types of human cancer, but the relevant biological function of EIF3B in LAD remains to be elucidated.

The present study aimed to investigate the effects of EIF3B on LAD using loss-of-function assay. The expression of EIF3B was firstly determined in LAD tissues and cell lines, and a series of functional experiments, including proliferation, cell cycle and apoptosis were conducted on LAD cells. In addition, the possible molecular mechanism underlying the EIF3B silencing was further investigated.

Materials and methods

Human tissue samples

Total 12 pairs of fresh LAD tissues (T) and non-tumor tissue (N) samples were collected from patients after undergo surgery at the Shaoxing People's Hospital of Zhejiang University. All the samples were immediately frozen in liquid nitrogen and stored at -80°C for RNA and protein extraction. This study was approved by the Ethics Committee of the Shaoxing People's Hospital of Zhejiang University. Written informed consent was obtained from all patients prior to surgery.

Cell lines and culture

Human LAD cell lines, A549, 59D and H1299, as well as normal lung epithelial cell lines HBE, were purchased from Shanghai Institute of biological science cell bank. Cell cultures were maintained with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO_2 at 37°C.

DNA extraction and real-time RT-PCR

Total RNA from paired tissues samples or cultured cell lines were extracted with TRIzol (Invitrogen) and reversely transcribed to cDNA according to the manufacturer's protocol. EIF3B gene expression was quantified using 20 μl PCR reaction mixture on BioRad Connet Real-Time PCR platform. Primers for EIF3B and GAPDH were designed as follows: EIF3B-forward: 5'-CGGTGCCTTAGCGTTTGTG-3'; EIF3B-reverse: 5'-CGGTCCTTGTTGTTCTTCTGC-3'; GAPDH-forward: 5'-TGACTTCAACAGCGACACCCA-3'; GAPDH-reverse: 5'-CACCCTGTTGCTGTAGCCAAA-3'. GAPDH was used as an internal control. The PCR extraction procedure were subjected to initially denatured at 95°C for 1 min, followed by 40 cycles of denaturation at 95°C for 5 sec, and last extension at 60°C for 20 sec. All samples were determined in triplicate. The EIF3B expression was evaluated by normalization against GAPDH using the comparative the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Total protein from paired LAD tissues samples or cultured cell lines were extracted with 2 mL ice-cold RIPA butter (Beyotime, Shanghai, China). The concentration of proteins from each sample was determined using BCA Protein Assay Kit (Beyotime, China). Approximately 30 μg were separated on 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). After blocking by 1% bovine serum albumin in TBST for 1 h at room temperature, the membranes were incubated with primary antibodies, including anti-phospho-EGFR, anti-phospho-ERK and GAPDH (Cell Signaling Technology, USA) overnight at 4°C. Then the membranes were washed by TBST and incubated with appropriate secondary antibody (Cell Signaling Technology, USA) for 2 h at room temperature. The signals of proteins were detected with enhanced chemiluminescence kit (Pierce Biotechnology, USA).

Cell transfection of LAD cells

Two small interfering RNAs (siRNAs) targeting human EIF3B gene (siEIF3B-1: 5'-CCGGGGGAGAGAAATTCAAGCAAATTTCAAGAGAATTTGCTTGAATTTCTCTCCCTTTTTG-3' and siEIF3B-2: 5'-AATTCAAAAAGGGAGAGAAATTCAAGCAAATTCTCTTGAAATTTGCTTGAATTTCTCTCCC-3'), as well as a scrambled vector were synthetically purchased from OriGene Technologies, Inc. (Rockville, MD). For transfection, two target cell lines, A549 and 95D, were seeded in six-well plates and then transfected with siRNAs using the Lipofectamine 2000 reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). After transfection for 72 hours, cells were processed for subsequent experiments following determination of knockdown efficacy.

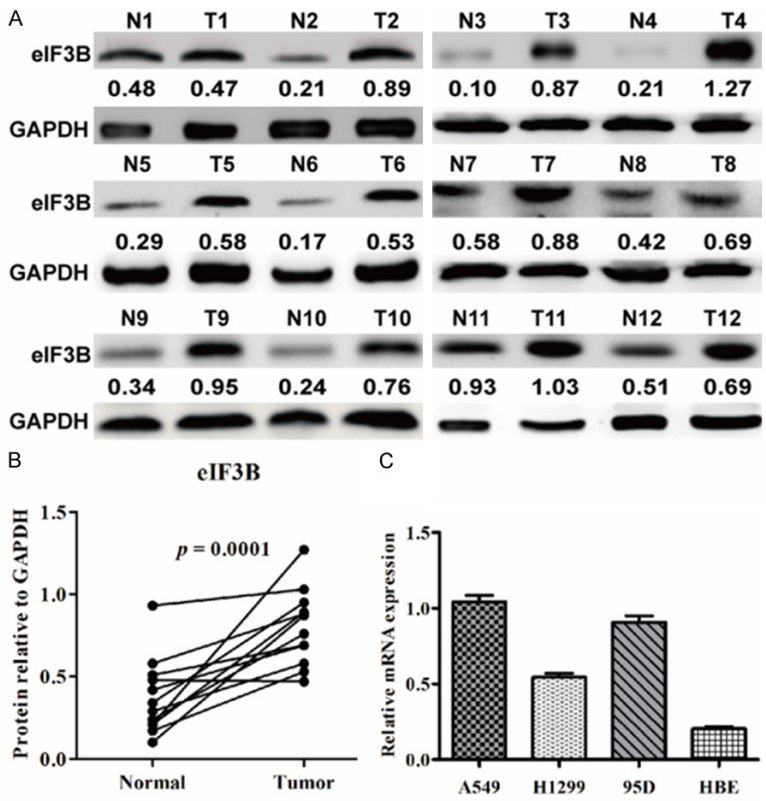

Figure 1. EIF3B is highly expressed in lung adenocarcinoma tissues and cell lines. Western blot (A) analysis were used to determine the protein levels of eIF3B in 12 pairs of lung adenocarcinoma tissues and non-tumor tissues. N, paracarcinoma (normal) lung tissues; T, lung cancer tissues. (B) Quantitative analysis of eIF3B protein in 12 paired lung cancer tissues. (C) qRT-PCR analysis of EIF3B mRNA level in three lung cancer cell lines (A549, 95D and H1299), and a normal lung epithelial cell line HBE. GAPDH was used as internal control. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, as compared with normal tissues or cell line.

CCK-8 assay

A549 and 95D cells were seed onto a 96-well plate at a density of 2,000 cells after transfection with targeted siRNA or scrambled vector, and cultured for 72 hours. Cell Count Kit-8 (CCK-8, Beyotime) assay was used to evaluate cell viability in A549 and 95D cells after EIF3B knockdown at days 1, 2, 3, 4 and 5, respectively. Briefly, 10% CCK-8 solution (v/v) was added into each well at each time point and incubation was continued for another 1 h. The OD value in each well was measured with an ELISA reader (Bio-Rad, USA) at a wavelength of 450 nm.

Colony formation assay

To evaluate the effect of EIF3B on monolayer colony formation, stably transfected A549 and 95D cells were reseeded into six-well plates at a density of 400 cells per well cultured until the colonies were visible. After cultured for 2 weeks, colonies were fixed in 4% paraformaldehyde for 30 min and stained with 0.05% crystal violet for 20 min. All visible colonies in each well was counted under a light microscope and photographed. Each colony consisted of more than 50 cells.

Cell cycle analysis

Flow cytometer combined with propidium iodide (PI) staining was used to determine the cell cycle progression following EIF3B knockdown in A549 and 95D cells. Briefly, cells were reseeded on 6-cm dishes at a density of 2×10^5 cells per dish and fixed in 70% cold ethanol overnight at 4°C, followed by incubation with 300 μl PBS containing PI for 30 min at 37°C in dark. The DNA content was analyzed using flow cytometry (Cell Lab Quanta, Beckman Coulter).

Apoptosis analysis

Annexin V-APC/PI double staining Kit (KeyGEN, Nanjing, China) was used to detect the cell apoptosis as described by the manufacturer's instruction. In brief, A549 and 95D cells were reseeded on 6-cm dishes at a density of 2×10^5 cells. Then cells were harvested in complete RPMI-1640 medium and centrifuged at 1000 rpm for 5 min. Each of the cells were washed with 1 × PBS and subjected to Annexin V-APC/7-PI double staining. Data acquisition and analysis were performed by flow cytometer (Cell Lab Quanta, Beckman Coulter).

Statistical analysis

Data are statistically analyzed using SPSS 13.0 software and presented as mean ± standard deviations (SD) from at least three independent experiments. Graphical representations were

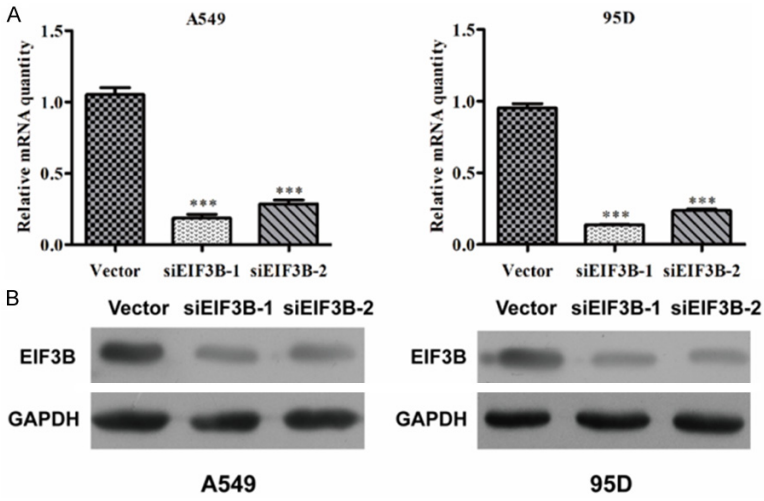

Figure 2. Knockdown of EIF3B expression in lung adenocarcinoma cell lines. (A) RT-PCR and (B) Western blot analysis were used to validate the knockdown efficiency of siEIF3B-1 and siEIF3B-2 in A549 and 95D cells. GAPDH was used as internal control. ***$P < 0.001$, as compared with empty vector groups.

performed with GraphPad Prism 5 (San Diego, CA) software. Student's t-test was used to evaluate the differences between two groups. A *P*-value of less than 0.05 was considered statistically significant.

Result

EIF3B is up-regulated in LAD tissues and cell lines

To investigate the expression level of EIF3B in LAD, paired tissues of 5 patients were collected for Western blot analysis. As shown in **Figure 1A**, clearly increased levels of EIF3B expression were detected among 11 of 12 LAD tissues with paired normal tissues (**Figure 1A** and **1B**). Subsequently, several LAD cells lines were selected to analyze the expression of EIF3B using RT-PCR analysis. As depicted in **Figure 1C**, compared with the normal lung epithelial cell lines HBE, the mRNA level of EIF3B was commonly elevated in A549, 95D and H1299 cells. To conduct a series of biological function assays, A549 and 95D cell with relative higher EIF3B expression were chosen for loss-of-function study.

Knockdown of EIF3B expression by siRNA in LAD cells

To explore the role of EIF3B in LAD development, two EIF3B-siRNAs (siEIF3B-1 and siEIF3B-2) and non-silencing scrambled vector were produced and infected A549 and 95D cells. After transfection, the EIF3B mRNA and protein levels were determined by RT-PCR and Western blot analysis, respectively. As shown in **Figure 2A**, the EIF3B mRNA level was significantly decreased after two siEIF3B infection compared with the empty vector infection in A549 and 95D cells. Consistent with this result, both siEIF3B-1 and siEIF3B-2 could obviously down regulated the EIF3B protein level in A549 and 95D cells (**Figure 2B**). Therefore, we successfully constructed EIF3B-silencing LAD cell models.

Cell proliferation was significantly suppressed following siEIF3B transfection in LAD cells

To elucidate the possible role of EIF3B in LAD, cell viability was firstly examined in A549 and 95D cells after EIF3B knockdown using CCK-8 assay. As shown in **Figure 3A**, the growth curves of EIF3B knockdown cells were obviously lower than that of control groups after continuously culture for 5 days in A549 and 95D cells. Statistical analysis further indicated inhibition of EIF3B significantly reduced the growth rate of the A549 and 95D cells following siRNA transfection compared with the control groups ($P < 0.001$). Furthermore, the effect of EIF3B on colony formation ability was evaluated in cells. As shown in **Figure 3B**, both siEIF3B-1 and siEIF3B-2 remarkably reduced the size of single colony and the number of colonies formed in A549 and 95D cells ($P < 0.001$). These results strongly suggest that EIF3B acts as a potential tumor gene in LAD. In addition, siEIF3B-1 had more obvious inhibitory effects than siEIF3B-2, which was thus selected for the following assays.

EIF3B knockdown induced cell cycle arrest at G2/M phase in LAD cells

As widely accepted, cell cycle regulation plays an important role in controlling cell proliferation, especially in cancer occurrence. Therefore, we further investigated the effect of EIF3B on cell cycle distribution in A549 and 95D cells

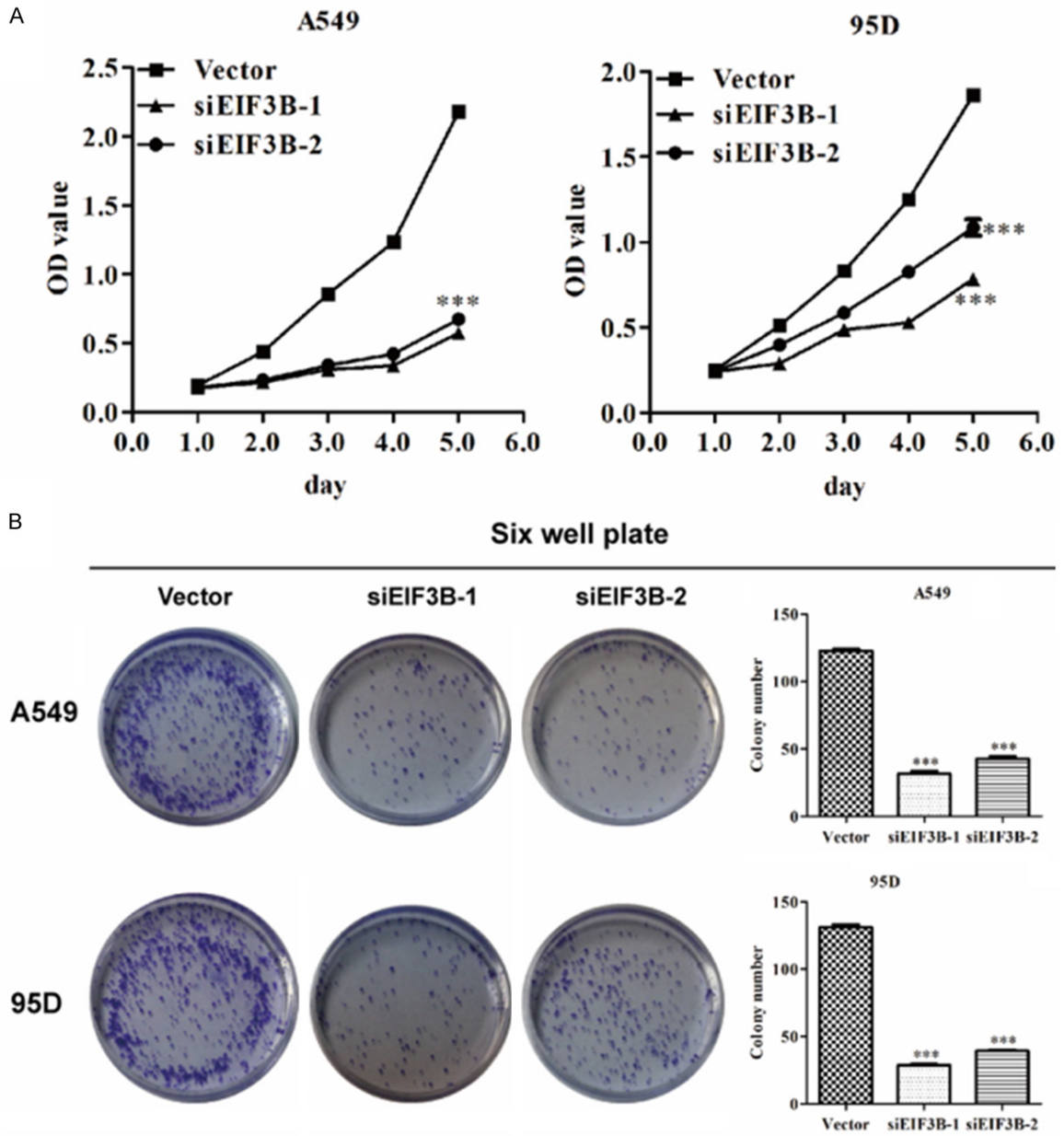

Figure 3. Knockdown of EIF3B impaired cell viability and colony formation ability. A. CCK-8 assay was used to measure cell viability in A549 and 95D cells after transfected with siEIF3B-1, siEIF3B-2 or empty vector. B. Colony formation assay was used to evaluate the proliferative ability in A549 and 95D cells after transfected with siEIF3B-1, siEIF3B-2 or empty vector. ***$P < 0.001$, as compared with empty vector groups.

using flow cytometry and PI staining. As illustrated in **Figure 4A** and **4C**, the profiles of cell cycle distribution were quite different between siEIF3B-1 groups and vector groups in both A549 and 95D cells. As shown in **Figure 4B**, the percentage of cells in G0/G1 phase was significantly lower than that in vector infected cells (58.62% vs 74.75%, $P < 0.001$) in A549 cells, whereas the percentage of S and G2/M phase in siEIF3B-1 transfected cells were remarkably higher than those in vector infected cells (23.58% vs 15.9%, 17.8% vs 9.35%, respectively, $P < 0.001$). A similar tendency was also found in 95D cells (**Figure 4D**, $P < 0.001$) except there was no significant differences in the percentage of S phase in siEIF3B-1 and controls. Collectively, EIF3B knockdown could arrest cell cycle at G2/M phase in LAD cells.

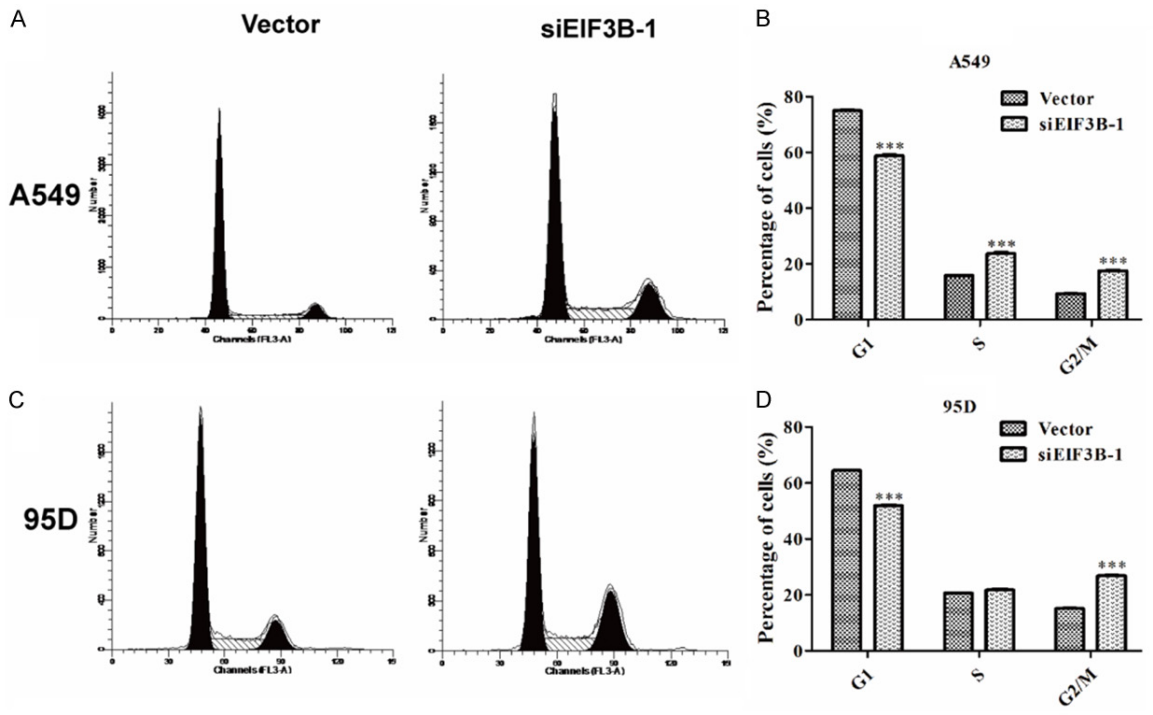

Figure 4. Knockdown of EIF3B arrested cell cycle at G2/M phase in lung adenocarcinoma. A and C. Cell cycle distribution of A549 and 95D was measured by flow cytometric analysis after PI staining. B and D. Quantification of the percentage of cells in G0/G1, S and G2/M phases in A549 and 95D cells after transfected with siEIF3B-1 or empty vector. $***P < 0.001$, as compared with empty vector groups.

EIF3B knockdown promotes cell apoptosis in LAD cells through inhibit EGFR/ERK pathway

Flow cytometry and Annexin V/PI double staining were used to determine whether silencing EIF3B had an accelerated effect on the apoptosis of A549 and 95D cells (**Figure 5A**). As shown in **Figure 5B**, statistical analysis demonstrated EIF3B knockdown significantly increased the percentage of apoptotic cells, including early apoptosis (Annexin V+/PI-) and late apoptosis (Annexin V+/PI+) in A549 and 95D cells following siEIF3B-1 transfection, as compared to the empty vector groups ($P < 0.001$). It has been reported that therapeutic approaches against EGFR, as well as its downstream signaling, like MEK/ERK pathway is a promising direction for lung cancer therapy [21, 22]. To further investigate the molecular mechanism underlying the inhibitory effects on proliferation induced by EIF3B silencing, we analyzed the expression of p-EGFR and p-ERK using Western blot analysis. As shown in **Figure 5D** and **5E**, the p-EGFR and p-ERK protein levels were significantly down regulated in siEIF3B-1 groups compared to that in empty vector groups in both A549 and 95D cells. These results suggest EIF3B knockdown induced apoptosis might through EGFR/ERK pathway regulation in LAD.

Discussion

Uncontrolled cell proliferation is usually the main character of malignant tumor occurrence. Recent studies demonstrate eukaryotic mRNA translation and protein synthesis play a key role in the tumorigenesis pathways. EIF3B, as a subunit of EIF3 complex, has been reported to participate in nearly all stages of translation and protein synthesis and promote various cancer progressions. However, its biological function in LAD has not been fully elucidated. Here, we demonstrated that EIF3B was upregulated in LAD fresh biopsy tissues and several LAD cell lines, thus indicating that EIF3B may exhibit increased expression in LAD.

The present study further revealed that knockdown of EIF3B by siRNA suppressed malignant cell proliferation of A549 and 95D, induced cell cycle arrest at G2/M phase and promoted cell apoptosis. These results indicated that EIF3B

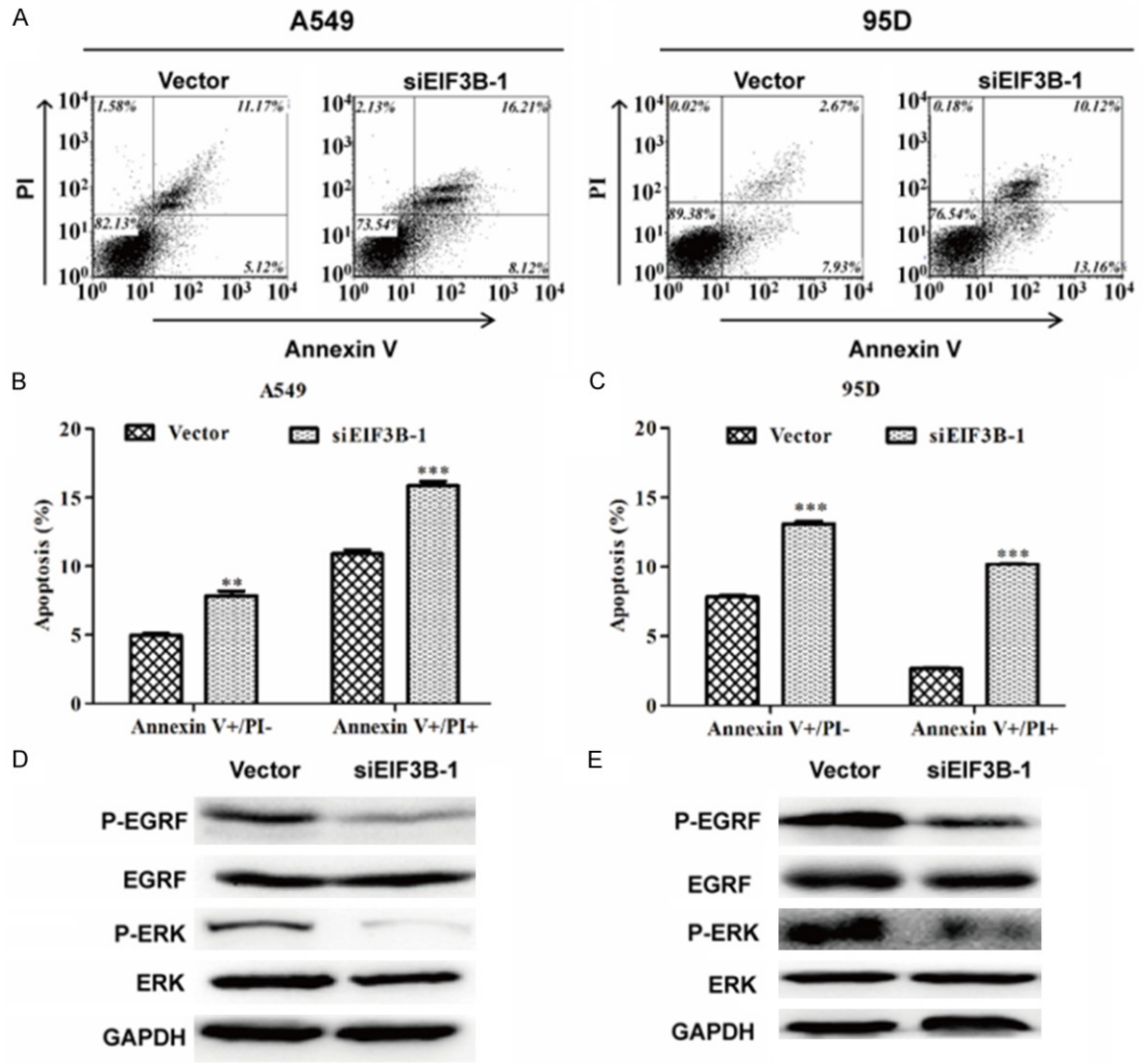

Figure 5. EIF3B silencing promoted cell apoptosis through inhibiting EGFR/ERK pathway. A. Cell apoptosis analysis was conducted by flow cytometric analysis after Annexin V/PI double staining in A549 and 95D following transfection with siEIF3B-1 or empty vector. B and C. Quantification of the early apoptosis (Annexin V+/PI-) and late apoptosis (Annexin V+/PI+) in A549 and 95D cells following siEIF3B-1 transfection or empty vector. D and E. The protein levels of p-EGFR, EGFR, ERK and p-ERK were determined by Western blot analysis in A549 and 95D cells following siEIF3B-1 transfection or empty vector. GAPDH was used as internal control. ***$P < 0.001$, as compared with empty vector groups.

might be associated with an inhibition in DNA replication, which cause a reduced cell growth rate found in cell growth analysis. Consistent with our results, EIF3B silencing inhibits the cell growth of colon cancer [23] and glioma [20]. It has been shown that the main function of EIF3 is to adjust the interaction between mRNA and ribosome, often the initial process of protein synthesis. Therefore, it not hard to understand EIF3B promoted cell cycle progression through regulation of protein synthesis.

In addition to cell cycle regulation, promoting cell apoptosis is also an important aspect of antitumor therapy on the growth of tumor cells [24]. Our results showed knockdown of EIF3B remarkably promoted cell apoptosis. Similar findings have also been described in other tumor types, including colon cancer [23] and glioma [20]. Furthermore, we found knockdown of EIF3B could down regulate expression of p-EGFR and p-ERK. The increased EGFR plays an important role in different types of human tumors and correlates with the enhanced cellular proliferation [25]. Moreover, its downregulation promotes the development of resistance to different targeted therapies [26, 27]. Our results suggest possible distinct interactions

between EIF3B and EGFR expression in LAD. EGFR regulation of cell proliferation and survival is also involved in its downstream pathways, like ERK1/2, Akt and STAT3 pathways [28]. Consistently, EIF3B silencing effectively inhibited the EGFR/ERK pathway, which is of significance because the inactivation of EGFR/ERK signaling suppresses cell proliferation.

In summary, our studies reveal for the firstly time the involvement of EIF3B in LAD development and the potential molecular mechanisms underlying its oncogenic function. These results indicate EIF3B might be an oncogene in LAD and its knockdown is a promising anti-LAD therapy.

Acknowledgements

This work is supported by grants from Public welfare Technology Application Research Project of Shaoxing, China (No. 2014B70065).

Disclosure of conflict of interest

None.

Address correspondence to: Chunyi Zhang, Department of Respiratory Medicine, Shaoxing People's Hospital, 568 North Zhongxing Road, Yuecheng District, 312000, Zhejiang, China. Tel: +86-575-88229042; E-mail: zhangchuyi_112@163.com

References

[1] Cancer statistics. JAMA 2013; 310: 982.
[2] Charloux A, Quoix E, Wolkove N, Small D, Pauli G and Kreisman H. The increasing incidence of lung adenocarcinoma: reality or artefact? A review of the epidemiology of lung adenocarcinoma. Int J Epidemiol 1997; 26: 14-23.
[3] Hsu CH, Hsu CW, Hsueh C, Wang CL, Wu YC, Wu CC, Liu CC, Yu JS, Chang YS and Yu CJ. Identification and characterization of potential biomarkers by quantitative tissue proteomics of primary lung adenocarcinoma. Mol Cell Proteomics 2016; 15: 2396-410.
[4] Li HM, Guo K, Yu XY, Yu Z and Xu P. Expression and clinical significance of A-kinase anchor protein 4 in lung adenocarcinoma tissue. Thorac Cancer 2016; 7: 273-278.
[5] Ni L, Zhu X, Gong C, Luo Y, Wang L, Zhou W, Zhu S and Li Y. Trichosanthes kirilowii Fruits Inhibit Non-Small Cell Lung Cancer Cell Growth Through Mitotic Cell-Cycle Arrest. Am J Chin Med 2015; 43: 349-64.
[6] Pestova TV, Kolupaeva VG, Lomakin IB, Pilipenko EV, Shatsky IN, Agol VI and Hellen CU. Molecular mechanisms of translation initiation in eukaryotes. Proc Natl Acad Sci U S A 2001; 98: 7029-7036.
[7] Spilka R, Ernst C, Mehta AK and Haybaeck J. Eukaryotic translation initiation factors in cancer development and progression. Cancer Lett 2013; 340: 9-21.
[8] Mayeur GL, Fraser CS, Peiretti F, Block KL and Hershey JW. Characterization of eIF3k: a newly discovered subunit of mammalian translation initiation factor eIF3. Eur J Biochem 2003; 270: 4133-4139.
[9] Gao Y, Teng J, Hong Y, Qu F, Ren J, Li L, Pan X, Chen L, Yin L and Xu D. The oncogenic role of EIF3D is associated with increased cell cycle progression and motility in prostate cancer. Med Oncol 2015; 32: 1-8.
[10] Hui L, Zhou F, Wang H, Da L, Gang C, Zuo X, Sun L, Zhang X and Yang S. Knockdown of EIF3D suppresses proliferation of human melanoma cells through G2/M phase arrest. Biotechnol Appl Biochem 2015; 62: 615-620.
[11] Yu X, Zheng B and Chai R. Lentivirus-mediated knockdown of eukaryotic translation initiation factor 3 subunit D inhibits proliferation of HCT116 colon cancer cells. Biosci Rep 2014; 34: e00161.
[12] Ren M, Zhou C, Liang H, Wang X and Xu L. RNAi-Mediated Silencing of EIF3D Alleviates Proliferation and Migration of Glioma U251 and U87MG Cells. Chem Biol Drug Des 2015; 86: 715-722.
[13] Lin Z, Xiong L and Lin Q. Knockdown of eIF3d inhibits cell proliferation through G2/M phase arrest in non-small cell lung cancer. Med Oncol 2015; 32: 1-8.
[14] Zhang Y, Yu JJ, Tian Y, Li ZZ, Zhang CY, Zhang SF, Cao LQ, Zhang Y, Qian CY, Zhang W, Zhou HH, Yin JY and Liu ZQ. eIF3a improve cisplatin sensitivity in ovarian cancer by regulating XPC and p27Kip1 translation. Oncotarget 2015; 6: 25441-25451.
[15] Li Z, Lin S, Jiang T, Wang J, Lu H, Tang H, Teng M, Fan J. Overexpression of eIF3e is correlated with colon tumor development and poor prognosis. Int J Clin Exp Pathol 2014; 7: 6462-6474.
[16] Hinnebusch AG. eIF3: a versatile scaffold for translation initiation complexes. Trends Biochem Sci 2006; 31: 553-562.
[17] Zhou M, Sandercock AM, Fraser CS, Ridlova G, Stephens E, Schenauer MR, Yokoi-Fong T, Barsky D, Leary JA, Hershey JW, Doudna JA and Robinson CV. Mass spectrometry reveals modularity and a complete subunit interaction map of the eukaryotic translation factor eIF3. Proc Natl Acad Sci U S A 2008; 105: 18139-18144.

[18] ElAntak L, Tzakos AG, Locker N and Lukavsky PJ. Structure of eIF3b RNA recognition motif and its interaction with eIF3j: structural insights into the recruitment of eIF3b to the 40 S ribosomal subunit. J Biol Chem 2007; 282: 8165-8174.

[19] Wang H, Ru Y, Sanchez-Carbayo M, Wang X, Kieft JS and Theodorescu D. Translation initiation factor eIF3b expression in human cancer and its role in tumor growth and lung colonization. Clin Cancer Res 2013; 19: 2850-2860.

[20] Liang H, Ding X, Zhou C, Zhang Y, Xu M, Zhang C and Xu L. Knockdown of eukaryotic translation initiation factors 3B (EIF3B) inhibits proliferation and promotes apoptosis in glioblastoma cells. Neurol Sci 2012; 33: 1057-1062.

[21] Antonicelli A, Cafarotti S, Indini A, Galli A, Russo A, Cesario A, Lococo FM, Russo P, Mainini AF, Bonifati LG, Nosotti M, Santambrogio L, Margaritora S, Granone PM and Dutly AE. EGFR-targeted therapy for non-small cell lung cancer: focus on EGFR oncogenic mutation. Int J Med Sci 2013; 10: 320-330.

[22] Citri A and Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 2006; 7: 505-516.

[23] Wang Z, Chen J, Sun J, Cui Z and Wu H. RNA interference-mediated silencing of eukaryotic translation initiation factor 3, subunit B (EIF3B) gene expression inhibits proliferation of colon cancer cells. World J Surg Oncol 2012; 10: 119.

[24] Abaza MS, Orabi KY, Al-Quattan E and Al-Attiyah RJ. Growth inhibitory and chemo-sensitization effects of naringenin, a natural flavanone purified from Thymus vulgaris, on human breast and colorectal cancer. Cancer Cell Int 2015; 15: 46.

[25] Spaulding DC and Spaulding BO. Epidermal growth factor receptor expression and measurement in solid tumors. Semin Oncol 2002; 29: 45-54.

[26] Chae YK, Gagliato Dde M, Pai SG, Carneiro B, Mohindra N, Giles FJ, Ramakrishnan-Geethakumari P, Sohn J, Liu S, Chen H, Ueno N, Hortobagyi G, Gonzalez-Angulo AM. The Association between EGFR and cMET Expression and Phosphorylation and Its Prognostic Implication in Patients with Breast Cancer. PLoS One 2016; 11: e0152585.

[27] Lin MC, Huang MJ, Liu CH, Yang TL and Huang MC. GALNT2 enhances migration and invasion of oral squamous cell carcinoma by regulating EGFR glycosylation and activity. Oral Oncol 2014; 50: 478-484.

[28] Tsien CI, Nyati MK, Ahsan A, Ramanand SG, Chepeha DB, Worden FP, Helman JI, D'Silva N, Bradford CR, Wolf GT, Lawrence TS and Eisbruch A. Effect of erlotinib on epidermal growth factor receptor and downstream signaling in oral cavity squamous cell carcinoma. Head Neck 2013; 35: 1323-1330.