Research paper

Systematic identification of CDC34 that functions to stabilize EGFR and promote lung carcinogenesis

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\textbf{A B S T R A C T}

\textbf{Background:} How the oncoprotein epidermal growth factor receptor (EGFR) evades proteolytic degradation and accumulates in non-small cell lung cancer (NSCLC) remains unclear, and ubiquitin pathway genes (UPGs) that are critical to NSCLC needs to be systematically identified.

\textbf{Methods:} A total of 696 UPGs (including E1, E2, E3, and deubiquitinases) were silenced by small interfering RNA (siRNA) library in NSCLC cells, the candidates were verified, and their significance were evaluated in patients with NSCLC. The effects of a candidate gene on EGFR were investigated \emph{in vitro} and \emph{in vivo}.

\textbf{Findings:} We report 31 candidates that are required for cell proliferation, with the E2 ubiquitin conjugase CDC34 as the most significant one. CDC34 is elevated in tumor tissues in 76 of 114 (66.7%) NSCLCs and inversely associated with prognosis, is higher in smoker patients than nonsmoker patients, and is induced by tobacco carcinogens in normal human lung epithelial cells. Forced expression of CDC34 promotes, whereas knockdown of CDC34 inhibits, NSCLC cell proliferation \emph{in vitro} and \emph{in vivo}. CDC34 competes with c-Cbl to bind Y1045 to inhibit polyubiquitination and degradation of EGFR. In EGFR-L858R and EGFR-T790M/Del (exon 19)-driven lung tumor growth in mouse models, knockdown of CDC34 significantly inhibits tumor formation.

\textbf{Interpretation:} These results demonstrate that an E2 enzyme is capable of competing with E3 ligase to stabilize substrates, and CDC34 represents an attractive therapeutic target for NSCLCs.

\textbf{Funding:} National Key Research and Development Program of China, National Natural Science Foundation of China, and the CAMS Innovation Fund for Medical Sciences.

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1. Introduction

Hyperactivation of the epidermal growth factor receptor (EGFR) by gain-of-function mutations and overexpression has been found in more than a hal of patients with non-small cell lung cancers (NSCLCs), and inhibition of constitutively activated EGFR significantly benefits lung adenocarcinoma patients with mutant EGFR [1,2]. The proteolysis of EGFR is controlled by an E3 ligase c-Cbl and E2 conjugase Ubc4/5 [3]. c-Cbl binds EGFR in the absence or presence of EGFR [4]; c-Cbl mediates the ubiquitination, endosome fusion, and lysosomal sorting of EGFR [5–7]. The conserved N-terminal of c-Cbl is sufficient to enhance EGFR ubiquitination [8], while the RING finger C-terminal flank controls EGFR fate downstream of receptor ubiquitination [9]. Somatic mutations and loss of heterozygosity (LOH) of c-Cbl had been reported in a proportion...
Research in context

Evidence before this study

The proteolysis of EGFR, an oncprotein that is hyperactivated by somatic mutations or overexpression in more than a half of patients with NSCLC, is controlled by an E3 ligase C-cbl and an E2 ubiquitin-conjugating enzyme Ubc4/5. How EGFR maintains a high level in NSCLCs remains obscure. In addition, the E2 conjugases facilitate proteasomal degradation by transferring the ubiquitin on their conserved cysteine residue to the ε-amino group of lysine residues on substrates. Whether the E2 conjugases have the function to protect substrates from catabolism needs to be determined.

Added value of this study

Our results showed that CDC34 was overexpressed and inversely associated with clinical outcome of the patients, and promoted NSCLC cell proliferation in vitro and in vivo. CDC34 stabilized EGFR by binding with it at Y1045 to dissociate C-cbl and thus prevented subsequent ubiquitination and degradation. Silencing of CDC34 inhibited EGFR-L858R and EGFR-T790M(Del exon 19)-driven lung cancer in mice.

Implications of all the available evidence

Our findings showed for the first time that CDC34 as an E2 enzyme is capable of protecting the protein substrate EGFR from ubiquitination and degradation by competing with E3 ligase C-cbl. Inhibition of CDC34 induced EGFR catabolism and prevented subsequent ubiquitination and degradation. Silencing of CDC34 inhibited EGFR-L858R and EGFR-T790M(Del exon 19)-driven lung cancer in mice.

2. Materials and methods

2.1. Patient samples

The study was approved by the local research ethics committees of Sun Yat-Sen University Cancer Center and the Third Affiliated Hospital of Kunming Medical University. All lung cancer samples were collected with informed consent. The diagnosis of lung cancer was confirmed by at least two pathologists. Tissue samples were taken at the time of surgery and quickly frozen in liquid nitrogen. The tumor samples contained a tumor cellularity of greater than 60% and the matched control samples had no tumor content.

2.2. siRNA library

Four Human siGENOME SMARTpool siRNA Libraries were obtained from Thermo Scientific Dharmacon (Lafayette, CO, USA). These libraries included Human Deubiquitinating Enzymes (G-004,705-05), Human Ubiquitin Conjugation Subset 1 (G-005615-05), Human Ubiquitin Conjugation Subset 2 (G-005625-05), Human Ubiquitin Conjugation Subset 3 (G-005635-05), and siGENOME Controls Complete Kit (K-002800-C2-02). Transfections were performed by using DharmaFECT transfection reagent (T-2001-01) according to the manufacturer’s instructions.

2.3. Cell culture, cell cycle, and cell apoptosis

NSCLC lines A549, H226, H460, H1975, HCC827, human embryonic kidney line HEK293T (obtained from the American Tissue Culture Collection (ATCC; Manassas, VA, USA), and the human normal bronchial epithelial cell line 16HBE (Clonetics, Walkersville, MD) were cultured in Dulbecco Modified Eagle Medium (DMEM) or RPMI 1640 supplemented with 10% fetal bovine serum. Cell viability of A549 and H1975 in siRNA library screening was assayed by the CellTiter-Glo Reagent (Promega, Fitchburg, WI, USA) according to the manufacturer's instructions. The implication of all the available evidence
1:1000 for Western blot), mouse anti-HA (#AE008, Abclonal; 1:2000 for Western blot), rabbit anti-GST (#A-5800, Invitrogen, Frederick, MD, USA; 1:5000 for Western blot), rabbit anti-Ki67 (#ab15580, Abcam, Cambridge, MA, USA; 1:400 for IHC), and rabbit anti-c-Cbl (#ab137375, Abcam; 1:1000 for Western blot). Reagents used included cycloheximide (CHX) (#94271, Amresco Inc., Solon, OH, USA), erlotinib (#HY-12008, MedChemExpress, USA), epoxomicin (#A2606, APEXBio, USA), Universal Tyrosine Kinase Assay Kit (#MK410, Clontech, Palo Alto, CA), MG132 (Sigma, #SML1135), chloroquine (Sigma, #PFR-1258), BaP (Sigma, #B1760), BAA (Sigma, #B2209), and DBA (Sigma, #R19861).

2.5. siRNA, shRNA, plasmids and transfections

siRNA or shRNA were purchased from GenePharmaCo. Ltd (Shanghai, China) and the sequences are as follows: GCCUGACCU-CUCUCUACGA (siCDC34-1#); GGAGAGGGGAUCAUAUAC (siCDC34-2#); GAUGCAGGAGUACAGACA (siCDC34-3#); UGAAAGACGCAACACUCC (siCDC34-4#); GCTGCAACCTCTACGAC (human CDC34-shRNA-1#); GAGTGTGATCCTCCTCTGAA (human CDC34-shRNA-2#); CTCTTCTAC-GAGCTACTACTAT (mouse CDC34-shRNA-1#); GAGTGTAATTTCGCTGCT-AC (human CDC34-shRNA-2#); GGUGUUAUGGUCUCAUUU (siEGFR); GGAGCAACAAUUGGCAGUAU (siCDC3). The sequence for CDC34 in CDC34 construct was 5'-GCTGCAACCTCCTACGAA3' (human CDC34-shRNA-1#) and 5'-TGAAGCAACCTACACCTTT-3' (CDC34-shRNA-2#) for CDC34 2#

FLAG-CDC34 vector was constructed based on pCDNA3.1 plasmid; HA-CDC34 vector was constructed based on pCD2 plasmid, and pcDH-CDC34 vector was constructed based on pCDH-GFP plasmid. All CDC34 mutants were subcloned from Flag or HA-tagged CDC34 vectors. FLAG-EGFR was cloned from the pCA-3Flag-HA-EGFR vector and Flag-EGFR intracellular domain (ICD) was cloned from subcloned Flag-EGFR vector. Flag-EGFR vector was kindly provided by Dr. Jianhua Mao (Shanghai Institute of Hematology, Rui Jin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, China), GST or Histagged CDC34, EGFR, CBL, were generated based on the backbone of pGEX-4T-1 and pET28a (kindly provided by Dr. Quan Chen, Institute of Zoology, Chinese Academy of Sciences, Beijing, China), respectively. The shCDC34 constructs were made with PLKO.1 backbone (kindly provided by Dr. Wanzhu Jin, Institute of Zoology, Chinese Academy of Sciences) using Age I and EcoR I sites. Cells were transfected with siRNA, shRNA or plasmids using the Lipofectamine 2000 (Invitrogen) and the phenol-chloroform extraction method according to the manufacturer’s instruction. Total RNA (2 μg) was annealed with random primers at 65 °C for 5 min. The cDNA was synthesized using a 1st-STRAND cDNA Synthesis Kit (Fermentas, Pittsburgh, PA, USA). Quantitative real-time PCR was carried out using SYBR PremixExTaq (Takara Biotechnology, Dalian, China). Primers used for quantitative RT-PCR are as follows: human GAPDH, 5'-GATCAGCAGGATTTGGTCCTG-3' (forward) and 5'-GCAAAGCTTCCCCGTCTCAG-3' (reverse); mouse GAPDH 5'-AGTATGACTCCACTACGGCACA-3' (forward) and 5'-TTCGCTCCTGAGATAGTG-3' (reverse); human CDC34, 5'-GACGAGGGCCGATCTACAACT-3' (forward) and 5'-GAGATG GAGGCGAAACAGCGGAG-3' (reverse); mouse CDC34, 5'-CCCCAACCTACTATGGAGG-3' (forward) and 5'-ACACTTGTTGAAGAACCGGA-3' (reverse); human EGFR, 5'-GGACTCTGAGTCCAGAGGGTC-3' (forward) and 5'-GCTGGC CATCACTGATCTT-3' (reverse); human CND1, 5'-GTCGGACCGGCTGAAAAGA-3' (forward) and 5'-CTCCGCTTCTCGATTGT-3' (reverse). Each sample was analyzed in triplicate for three times.

2.8. Immunofluorescence microscopy

Cells grown on coverslip (24 mm × 24 mm) were fixed with 4% paraformaldehyde for 15 min, washed with 150 mM glycine in PBS, and permeabilized with 0.3% Triton X-100 in PBS for 20 min at room temperature. After blocking with 5% BSA, the cell smears were incubated with indicated primary antibodies overnight at 4 °C, washed, and FITC/PE-labeled secondary antibody in PBS was added to the cell smears. Images were taken by a laser scanning confocal microscopy (Zeiss, Oberkochen, Germany).

2.9. Immunohistochemistry analysis

IHC assay was performed with anti-CDC34, anti-Ki67 and anti-EGFR antibodies. Briefly, formalin-fixed, paraffin-embedded human or mouse lung cancer tissue specimens (5 μm) were deparaffinized through xylene and graded alcohol, and subjected to a heat-induced epitope retrieval step in citrate buffer solution. The sections were then blocked with 5% BSA for 30 min and incubated with indicated antibodies at 4 °C overnight, followed by incubation with secondary antibodies for 90 min at 37 °C. Detection was performed with 3, 3’-diaminobenzidine (DAB, Zhongshan Golden Bridge Biotechnology, Beijing, China) and counterstained with hematoxylin, dehydrated, cleared and mounted as in routine processing. The scoring of immunoreactivity was calculated as IRS (0–12)=RP (0–4) × SI (0–3), where RP is the percentage of staining-positive cells and SI is staining intensity.

2.10. Western blotting

Cells were lysed on ice for 30 min in RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 0.1% SDS, 1% deoxycholate, 1% Triton X-100, 1 mM EDTA, 5 mM NaF, 1 mM sodium vanadate, and protease inhibitors cocktail), and protein extracts were quantified. Proteins (20 mg) were subjected to 8–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electrophoresed and transferred onto a nitrocellulose membrane. After blocking with 5% non-fat milk in Tris-buffered saline, the membrane was washed and incubated with the indicated primary and secondary antibodies and detected by Luminescence Image Analyzer LSA 4000 (GE, Fairfield, CO, USA).

2.11. CO-immunoprecipitation

Cells were treated either with or without 10 μM MG132 for 3–4 h before lysis. After wash with cold PBS for two times, cells were suspended in IP lysis buffer (40 mM Tris–HCl, pH 7.4, 137 mM NaCl, 1.5 mM MgCl₂, 0.2% sodium deoxycholate, 1% Nonidet P-40, 2 mM EDTA, 1 mM PMSF, complete protease inhibitors cocktail) and cleared by centrifugation. Indicated antibody was added and incubated overnight with each cell lysate at 4 °C. Protein A/G PLUS-Agarose beads (Santa Cruz) were added after washing for 3 times with lysis buffer. After 2-h of incubation, beads were washed four times, 5 min per wash in IP wash buffer (40 mM Tris–HCl, pH 7.4, 137 mM NaCl, 1.5 mM MgCl₂, 2 mM EDTA, 0.2% Nonidet P-40).
2.12. Protein purification, GST or His pull-down assay, and in vitro kinase assay

GST or His-tagged CDC34, EGRF\textsuperscript{CD}, c-Cbl proteins were expressed in E. coli Rosetta (DE3). GST fusion proteins were purified on glutathione-Sepharose 4 Fast Flow beads (GE Health Science, Pittsburgh, PA, USA) and His fusion proteins were purified on HisPur Cobalt Resin (Thermo Scientific, Basingstoke, UK), respectively. The GST was removed with thrombin (Amresco). For the GST pull-down, 2 μg of GST-fusion protein was incubated with cell lysates or purified proteins for 2 h at 4 °C and then washed 5 times with 1 mL PBS buffer. The precipitate complex was boiled with sample buffer containing 1% SDS for 5 min at 95 °C and subjected to SDS-PAGE. The nitrocellulose membrane was stained with Ponceau S and followed by immunoblotting with indicated antibodies. \textit{In vitro} EGFR kinase activity was determined using Universal Tyrosine Kinase Assay Kit (TaKaRa Biotechnology), following the manufacturer’s instructions.

2.13. Animal studies

The animal studies were approved by the Institutional Review Board of Institute of Zoology, Chinese Academy of Sciences, and the methods were carried out in accordance with the approved guidelines. The mice were numbered, injected with indicated cells or virus particles, and randomized into groups. For xenograft tumor models, six-week-old SCID beige mice were maintained in the pathogen-free (SPF) conditions. A549-luciferase cells stably expressing the shCDC34 (1 \times 10\textsuperscript{6}) or pCDH—CDC34 (2 \times 10\textsuperscript{6}) were injected into the lateral tail veins of the mice, and the tumors were monitored by IVIS Spectrum Imaging System 30, 45, or 55 days after cell inoculation (Caliper Life Sciences, Hopkinton, MA, USA). For EGFR\textsuperscript{L858R} or EGRF\textsuperscript{T790M} (exon 19)-driven lung tumors, 6-week-old Tet-op-EGFR\textsuperscript{L858R} (CCSP-tTA FVB mice (kindly provided by Professor Liang Chen, Jinan University, Guangzhou, China) were intranasally administered with shCDC34 or shNC lentiviral particles once a day for 3 days. One week after the first lentiviral administration, DOX was added to feed the mice and the lung tumors were analysed by micro-CT (PerkinElmer, Waltham, MA, USA) scanning, and tumor volume was quantitated by the Analyze 12.0 (PerkinElmer) Caliper microCT Analysis Tools according to the manufacturer’s instruction. The AJ mice were exposed to cigarette smoke generated by DSI’s Buxco Smoke Generator (Buxco, NC, USA) inside a perspex box, at a frequency of 5 cigarettes per day, 5 days per week for 2 months. Whole body cigarette smoke exposure per cigarette was 3 min followed by a 15-min period of fresh air [22]. Mice were anesthetized by mixture of oxygen/iso-flurane inhalation and positioned with legs fully extended, and assayed according to manufacturers’ instruction. Survival of the mice was evaluated from the first day of DOX treatment until death or became moribund, at which time points the mice were sacrificed.

2.14. Statistical analysis

All experiments were repeated at least three times and the data were presented as the mean ± SD unless noted otherwise. Differences between data groups were evaluated for significance using Student’s \textit{t}-test of unpaired data or one-way analysis of variance. \textit{P} values less than 0.05 indicate statistical significance.

2.15. Data availability

Cancer microarray data was downloaded from the Oncomine datasets Okayama Lung (can also be found at the Gene Expression Omnibus, GSE31210), Selamat Lung (the Gene Expression Omnibus, GSE32867), Stearman Lung (the Gene Expression Omnibus, GSE2415), Landi Lung (the Gene Expression Omnibus, GSE10072), and Su Lung (the Gene Expression Omnibus, GSE7670). The data of the reverse phase protein array of 235 lung adenocarcinomas were downloaded from the Cancer Genomics Hub (CGHub) [https://cgub.ucsd.edu/] with approval by the National Institutes of Health (NIH; approval number #24437-4). All the remaining data supporting the findings of this study are available within this paper and its supplementary information.

3. Results

3.1. A systematic silencing of UPGs in lung cancer cells

All the 696 UPGs found in human genome (Supplementary Table 1) were silenced by transfection of small interfering RNA (siRNA) of the Dharmacon human siGENOME SMARTpool library into the NSCLC lines A549 (with wild type EGRF) and H1975 (harboring L858R/T790M EGRF mutation). Cell viability was measured 72 h after transfection, and the Z-scores from duplicate experiments for each SMARTpool (Fig. 1a) were determined [21]. To validate the results, a secondary screen was performed in A549 and the results showed that the robust Z scores of most of the genes across repeats were strongly correlated (\textit{r} = 0.86; Fig. 1b). A SMARTpool was considered a hit if the Z-score was \textless{} –2 in both A549 and H1975 (Fig. 1c and Supplementary Table 1). Eighty hits (11.5%) were identified in A549 and 72 hits (10.3%) were uncovered in H1975 cells (Supplementary Fig. 1), with 31 hits discovered in both cells (Supplementary Fig. 1 and Fig. 1c). These hits include two E2 enzymes (UBE2T, CDC34), twenty E3 ligases (UBE3A, WWP2, TRIM68, and others), and nine others (e.g. USP48, PSMD14, OUTD5).

To identify genes that are critical to lung carcinogenesis, the association between the expression levels of the 31 genes and the clinical outcome of NSCLC patients was analyzed using the Online Survival Analysis Software [23] (http://kmplot.com/analysis/index.php?p=service&cancer=lung). We reported that the expression of six genes, CDC34 (Fig. 1d, upper panel), UBE2T, RNF152, TRIM17, CBL, and RNF17D, was inversely associated with overall survival of the patients. The prognostic significance of CDC34 was further analyzed using stage-matched samples. For stage 1 patients (n = 577), those with higher expression of CDC34 had much shorter survival time than cases with lower level of CDC34 (Fig. 1d, lower panel). For stage 2 (Supplementary Fig. 2a) and 3 (Supplementary Fig. 2b) NSCLCs, patients with higher expression of CDC34 had slightly, but not statistically significantly, shorter overall survival than cases with lower CDC34. The significance of CDC34 in stage 4 NSCLCs was not analyzed due to small sample size of the patients (n = 4). The data [24,25] of the cancer microarray database Oncomine [26] (http://www.oncomine.org) showed that, of these 6 genes, CDC34 was most significantly upregulated in lung tumors compared to normal tissues (Fig. 1e). CDC34 was therefore chosen for further study.

3.2. Overexpression of CDC34 in NSCLCs

We tested the expression of CDC34 in 114 NSCLCs (Table 1) by quantitative reverse transcription polymerase chain reaction (\textit{qRT-PCR}) (Fig. 1f), Western blot (Fig. 1g and h) and IHC (Fig. 1i and j), and showed that in 76 (66.7%) of the patients the expression of CDC34 was significantly elevated in tumor samples than the counterpart normal lung tissues (Table 1). A two-sided Fisher exact test showed that the expression of CDC34 in smoker NSCLCs was significantly higher than in non-smoker patients (\textit{p} = 0.027; Table 1). In works of Oncomine database [24, 25, 27–30], CDC34 expression in tumor samples was higher than in their paired normal lung tissues (Fig. 1k), and smokers [25] had higher CDC34 expression in tumor tissues than nonsmokers (Fig. 1l). We then tested the effects of several tobacco carcinogens on normal human lung epithelial cell line 16HBE, and showed that benzo[a]pyrene (BaP), benzo[a]anthracene (BAA), and dibenzo[a,h]anthracene (DBA) induced upregulation of CDC34 in the
Fig. 1. Identification of CDC34 as a crucial oncogene in NSCLCs by siGenome screening. (a) A549 and H1975 cells were treated with 50 nM siRNAs from the siGenome library (containing 696 UPGs) for 72 h and the cell viability was measured. The z score was calculated as described in materials and methods. (b) A second replicated screening was conducted in A549 to validate the results of the initial screening. (c) Heat map showing 31 candidates with Z score ≤ -2 in both A549 and H1975. (d) Overall survival of NSCLCs of all stages (upper) and stage 1 (lower) with high or low expression of CDC34. (e) The expression of 6 candidate genes in Okayama and Selamat cohorts (Tumor/Normal ≥ 1.5). (f-j) The expression of CDC34 was tested by qRT-PCR (f), Western blot (g, h), and IHC (i, j) assays of NSCLCs of our cohort. Molecular weight (kDa) of the protein bands was listed on the right side (g). Size bar, 1 mm. The densitometry analysis of the Western blot results (h) and the immunoreactivity score of CDC34 (j) was calculated. P values, Student’s t-test. (k) CDC34 expression was detected by microarrays in tumor samples and normal lung tissues in Oncomine datasets. AD, adenocarcinoma; SC, squamous cell carcinoma; A+S, adenocarcinoma and squamous cell carcinoma; Ger, Germany; Ca, Canada; It, Italy; Jp, Japan; Tw, Taiwan, China. *** P < 0.001. (l) CDC34 in never smoker and smoker NSCLCs of the work of Selamat et al. [25] in Oncomine datasets. P value, Student’s t-test. (m) 16HBE cells were treated with BaP, BAA, and DBA at indicated concentration for 24 h, and lysed for Western blot assay. (n) IHC assays of CDC34 in the lungs of A/J mice exposed to tobacco smoke for two months. Scale bar, 250 μm. (o) Western blot analyses of lysates of lung tissues of A/J mice exposed to tobacco smoke for two months.
cells (Fig. 1m). Upregulation of CDC34 was also seen in A/J mice exposed to tobacco smoke for two months (Fig. 1n and o).

3.3. CDC34 is required for lung cancer cell proliferation

To evaluate the role of CDC34 in lung cancer, siRNA against CDC34 (siCDC34) was transfected into A549 and H1975 cells, which resulted in a reduction of CDC34 protein (Fig. 2a, left) and cell viability (Fig. 2a, right) of the cells, and even modest knockdown had significant effects. Silencing of CDC34 led to a significant inhibition of cell growth (Fig. 2b) and suppression of colony forming activity (Fig. 2c) of the NSCLC cells. On the contrary, exogenous expression of CDC34 (by transient transfection) significantly increased the proliferation (Fig. 2d), growth (Fig. 2e), and colony forming activity (Fig. 2f) of the cells.

3.4. CDC34 positively regulates EGFR

We aimed to identify the downstream target of CDC34 in lung cancer. By using the data of the reverse phase protein array of 235 lung adenocarcinomas from a previous work [31], we analyzed the potential association between CDC34 and some driver proteins of lung cancer such as EGFR, N-RAS, MEK1, and LKB1, as well as CDC34 substrates p27 [32]. We reported that the expression of CDC34 was inversely associated with p27 expression (Fig. 3b, Table 2). We reported that the expression of CDC34 (Fig. 3b, Table 2). We reported that the expression of CDC34 in tumors is at least 1.5 times higher than in counterpart normal lung tissues.

P values were calculated using a two-sided Fisher’s exact test.

Table 1
Summary of baseline demographic characteristics of the 114 patients.

| Characteristics | Cases, n | CDC34 high* | n (%) | P values* |
|-----------------|----------|-------------|-------|-----------|
| Total number    | 114      | 76 (66.7)   |       | 0.264     |
| Age             |          |             |       |           |
| <65             | 74       | 51 (68.9)   |       |           |
| ≥65             | 28       | 16 (57.1)   |       |           |
| not determined  | 12       | 9 (75)      |       |           |
| Gender          |          |             |       |           |
| male            | 71       | 50 (70.4)   |       | 0.127     |
| female          | 31       | 17 (54.8)   |       |           |
| not determined  | 12       | 9 (75)      |       |           |
| Smoking         |          |             |       |           |
| smoker          | 59       | 44 (74.6)   |       | 0.027     |
| non-smoker      | 43       | 23 (53.5)   |       |           |
| not determined  | 12       | 9 (75)      |       |           |
| Histology       |          |             |       |           |
| adenocarcinoma  | 61       | 36 (59)     |       | 0.093     |
| squamous-cell carcinoma | 37 | 28 (75.7) |           |           |
| not determined  | 16       | 12 (75)     |       |           |
| TNM stage       |          |             |       |           |
| I-II            | 58       | 35 (60.3)   |       | 0.219     |
| III-IV          | 43       | 31 (72.1)   |       |           |
| not determined  | 13       | 10 (76.9)   |       |           |

* The standard of CDC34 high is that CDC34 in tumors is at least 1.5 times higher than in counterpart normal lung tissues.

3.5. Effects of CDC34 on lung cancer growth in vivo

To study the effect of CDC34 on lung cancer cell proliferation in vivo, A549-luciferase cells transfected with shCDC34 (Supplementary Fig. 3f) were inoculated into SCID-beige mice via tail vein, and the results showed that the luciferase signal in CDC34 knockdown groups was significantly lower than in control group (Fig. 4a). Hematoxylin-eosin (HE) staining showed that the lungs from the control group were almost full of tumor cells, but lungs from the CDC34 knockdown mice had markedly less tumor cells (Fig. 4b). The expression levels of CDC34 and EGFR were tested in the tumor tissues by IHC (Fig. 4c) and Western blot assays (Fig. 4d), and the results showed that in the lungs of the mice inoculated with A549-luciferase-shCDC34 cells, the protein level of the two molecules was markedly lower than the control group. Moreover, the overall survival of the mice harboring the CDC34-knockdown cells was significantly longer than the control group (Fig. 4e).

To further test the role of CDC34 in tumor growth in vivo, pCDH-CDC34 plasmid was transfected into A549-luciferase cells, which were then injected into SCID-beige mice via tail vein. To reflect the growth of the xenografted tumor, luciferase intensity of the mice was measured by IVIS Spectrum 30, 45, and 55 days after cell inoculation. We found that while A549-luciferase cells propagated gradually, CDC34 significantly increased tumor burden (Fig. 4f and g) and shortened life-span of the mice (Fig. 4h). HE and Ki67 staining of lung sections confirmed the tumor facilitating effect of CDC34 (Fig. 4i), while Western blot assays of tumor lysates indicated the upregulation of EGFR and Cyclin D1 in CDC34-expressing cells in vivo (Fig. 4j).

3.6. CDC34 interacts with EGFR

To elucidate the mechanism of CDC34 in regulating EGFR, we performed co-immunoprecipitation (CO-IP) assay and found that EGFR was precipitated by CDC34 (Fig. 3c, Supplementary Fig. 3b). In CDC34-expressing cells, the expression level of the two molecules was markedly higher than in control group (Fig. 3c). The immuno-precipitation analysis showed that CDC34 colocalized with EGFR mainly in intracellular region near the cell membrane (Fig. 3b). In H460 cells with EGFR-silenced by siEGFR, ectopic expressed Flag-EGFR interacted with HA-CDC34 (Fig. 3c). To confirm these findings, purified GST-CDC34 was co-precipitated with Flag-cDC34 from H460 cell lysates (Fig. 3d). To obtain purified GST-EGFR (from bacteria E.Coli) precipitated CDC34 from H460 cell lysates (Fig. 3e), in H460 and 293T cells, CDC34 was capable of interacting with EGFR in the presence and absence of EGF stimulation (Supplementary Fig. 4a
Fig. 2. CDC34 is required for proliferation of NSCLC cells in vitro and in vivo. (a) A549 and H1975 cells were transfected with siCDC34 (1# to 4#) and lysed 72 h later for the detection of CDC34 expression by Western blot assays (left). Cell viability was assessed by the CellTiter-Glo Reagent (right). Numbers under the Western blot bands are the relative expression values to Actin determined by densitometry analysis. Error bars, sd. (b) A549, H1975, and H460 cells were transfected with siCDC34 (1# and 4#), and cell proliferation was assessed by trypan blue exclusion analysis. The relative expression of CDC34 was detected by Western blot, and the numbers under the Western blot bands are the relative expression values to Actin determined by densitometry analysis. Error bars, sd; P values, Student’s t-test. (c) Foci formation and soft-agar assays of A549, H460, and H1975 cells transfected with siCDC34. Error bars, sd; P values, Student’s t-test. (d) The H460 (d, f) or A549 (e) cells were transfected with Flag- or HA-tagged CDC34 and cell proliferation was assessed by trypan blue exclusion analysis (d, e) or colony formation assays (f). The ectopic expression of exogenous proteins was shown by Western blot bands. Inat: inactive; NC, negative control. Error bars, sd; P values, Student’s t-test. *P< 0.05, **P< 0.01.
Fig. 3. CDC34 positively regulates EGFR. (a) A scatter diagram of reverse phase protein array (RPPA) data showing a negative correlation between the mRNA level of CDC34 and protein level of p27, and a positive correlation between the mRNA level of CDC34 and protein level of EGFR. The data are from TCGA datasets. P values, Student’s t-test. (b) IHC analysis of CDC34 and EGFR in NSCLCs of our setting. (c) The expression of CDC34 in EGFR WT and mutant NSCLCs in datasets and our setting. P values, Student’s t-test. (d) Western blot analysis of EGFR/pEGFR in cells transfected with siCDC34 in H460, H226, and H1975 cells with or without EGF co-incubation. Numbers under the Western blot bands are the relative expression values to Actin determined by densitometry analysis. (e) H460 cells transfected with siCDC34 were lysed, the lysates were immunoprecipitated with an anti-EGFR antibody and assayed for tyrosine kinase activity. Error bars, sd. P values, Student’s t-test. (f) Western blot analysis of EGFR/pEGFR and downstream molecules in EGF-stimulated, siCDC34-transfected H460 cells. (g) Immunoblotting of lysates of Flag-CDC34-expressing A549 (upper) or HA-CDC34-transfected H226 (lower) cells co-incubated with or without EGF. Numbers under the Western blot bands are the relative expression values to Actin determined by densitometry analysis. (h) H460 cells were transfected withFlag-CDC34, co-incubated with or without EGF, lysed, and the cytoplasm and nucleus proteins were harvested for immnunoblotting using indicated antibodies. (i) H460 cells were transfected with Flag-CDC34, lysed 24 h later, and the lysates were immunoprecipitated with an anti-EGFR antibody and assayed for tyrosine kinase activity. Error bars, sd. P values, Student’s t-test. (j) H460 cells were transfected with siCDC34 and/or CDC34 res, which is mutated to resist siCDC34 targeting. Immunoblot assays were conducted using indicated antibodies (j), cell viability was analyzed by MTT assay (k), and colony forming activity was evaluated by foci formation and soft-agar assays (l). Error bars, sd. P values, Student’s t-test. P values for indicated comparison: Group 1 vs group 2: < 0.05; group 1 vs group 3: < 0.01; group 2 vs group 4: < 0.05; group 3 vs group 5: < 0.05.
and b), suggesting that CDC34-EGFR interaction was independent of the phosphorylation status of EGFR.

To determine the CDC34-binding region within EGFR, several deletion mutants were constructed, and the results showed that the intracellular domain (EGFRICD) could bind CDC34 in cells (Fig. 5f). In vitro assays using purified GST-CDC34 and His-EGFRICD (Fig. 5g) or GST-EGFRICD/His-CDC34 (Fig. 5h) harvested from bacteria E. coli confirmed the direct binding of the two proteins, while GST itself did not interact with EGFR or CDC34. To unveil the region of CDC34 that binds to EGFR, deletion mutants were designed (Fig. 5i) and transfected into E. coli, and in vitro His-pull down and immunoblotting assays were performed. We found that while the C-terminal (162–236 amino acids) or the mutant lacking E2 catalytic domain could not bind, the full-length and the N-terminal of CDC34 (1–162 amino acids) bound EGFR (Fig. 5j). The active site residue Cys93 was not required for interaction with EGFR, because mutations in this amino acid (C93A) did not affect CDC34-EGFR interaction (Supplementary Fig. 4c).

### 3.7. CDC34 protects EGFR from proteolytic degradation

We noticed that knockdown of CDC34 in A549 and H1975 cells resulted in downregulation of EGFR at protein (Fig. 3d, f, Supplementary Fig. 3) but not mRNA level (Fig. 6a), suggestive of a role for CDC34 in stabilizing EGFR. The cycloheximide (CHX) chase assay was conducted in H460 cells in the absence and presence of EGFR, and the half-life of EGFR was calculated [33]. We reported that knockdown of CDC34 led to downregulation of EGFR (Fig. 6b), and the half-life of EGFR in the absence of EGFR was 3.9 and 2.6 h for siNC and siCDC34 treatment groups, respectively. In the presence of EGFR, the half-life of EGFR was 2.9 and 1.5 h for siNC and siCDC34 treatment groups, respectively, Knockdown of CDC34 also reduced EGFR half-life in H1975 cells (Fig. 6b; 3.15 and 1.75 h for siNC and siCDC34 treatment groups, respectively). In HCC827 cells co-incubated with EGFR, further showed that forced expression of CDC34 increased the stabilization of c-Cbl by EGFR; upon erlotinib treatment, knockdown of CDC34 reduced EGFR/c-Cbl interaction (Supplementary Fig. 5a). We reported that knockdown of endogenous CDC34 increased the ubiquitination of EGFR (Fig. 6l, right panel). In in vitro experiments using His-CDC34 purified from E. coli and Flag-EGFR from 293T cells, we found that increased levels of c-Cbl supressed CDC34-EGFR interaction (Fig. 6m), while increased dosages of CDC34 diminished c-Cbl-EGFR binding (Fig. 6n). In vitro experiments using GST-EGFR and His-CDC34/His-c-Cbl from E. coli confirmed that c-Cbl competed with CDC34 to bind EGFR (Supplementary Fig. 5 g), c-Cbl binds to Y1045 and Y1045F mutation in EGFR abrogated binding between EGFR and c-Cbl tyrosine kinase binding (TKB) domain [37]. We found that Y1045F mutation in EGFR also impaired the interaction between EGFR and CDC34 (Fig. 6o), indicating that Y1045 is the binding site for both c-Cbl and CDC34, siCDC34-mediated loss of EGFR was rescued by knockdown of c-Cbl (Fig. 6p), indicating that c-Cbl is critical for CDC34-mediated EGFR stabilization.

### 3.8. EGFR is critical to CDC34-induced cell proliferation

CDC34 is a key regulator of cell cycle. We tested the effects of CDC34 knockdown on NSCLC cells, and reported that shCDC34 treatment of H1975 and A549 cells blocked cell cycle progression at G1 phase (Fig. 7a) but did not induced significant apoptosis (Supplementary Fig. 6a). Lin et al. showed that nuclear localized EGFR functions as a transcription factor to activate genes such as Cyclin D1 that are required for highly proliferating activities [38]. EGFR also negatively regulates p27 [39]. We showed that silencing of CDC34 resulted in downregulation of Cyclin D1 and upregulation of p27 in the cells (Fig. 7b). Indeed, knockdown of CDC34 in H1975 (Fig. 7c) and HCC827 (Supplementary Fig. 6b) cells led to downregulation of EGFR and Cyclin D1 in both the cytoplasm and nucleus compartments. Transfection of siCDC34 into the cells reduced CDC34 and Cyclin D1 (CCND1) at mRNA level (Fig. 7d and Supplementary Fig. 6c). Silencing of CDC34 or EGFR by siRNA also induced downregulation of Cyclin D1 and upregulation of p27 (Fig. 7e), arrested cell cycle at G1 phase (Fig. 7f), inhibited cell viability (Fig. 7g), and suppressed colony forming activity (Fig. 7h and i) of H460 cells. Interestingly, transfection of EGFR into siCDC34/shEGFR-treated H460 cells rescued the above inhibitory effects (Fig. 7f–i), indicating that downregulation of EGFR has a critical role in the anti-proliferative effects of CDC34 silencing.

### 3.9. Inhibition of CDC34 suppresses EGFR L858R-driven lung cancer

To evaluate the therapeutic potential of CDC34 inhibition, an EGFRL858R-driven lung cancer mouse model was established as described [40], and the lentiviral particles containing short hairpin RNA targeting CDC34 (shCDC34-1#) were generated and intranasally administrated into the lungs of the mice before they were treated with doxycycline (DOX) to induce lung cancer [40]. One month after the initiation of DOX treatment, the mice were detected by microscopic computed tomography (micro-CT). We found disseminated tumors in the lungs of control group mice, but only small tumors were seen in the CDC34 knockdown group mice (Fig. 8a and Supplementary Fig. 7a). Tumor volume of the mice was quantitated by the Analyze 12.0 (PerkinElmer) Caliper microCT Analysis Tools, and the results showed that shCDC34 treatment significantly reduced tumor volume of the mice.
Fig. 4. Effects of CDC34 on tumor growth in vivo. (a) SCID beige mice were injected with $1 \times 10^6$ A549-luciferase cells via tail vein, detected by the IVIS Spectrum system 30 days later (left), and the relative luciferase intensity in the mice was analyzed (right). Error bars, sd; $P$ values, Student’s $t$-test. (b) Hematoxylin-eosin (HE) staining of the lung sections from mice of each group. (c) IHC analysis of CDC34, EGFR and Ki67 in tumors from mice of each group. Scale bar, 1 mm. (d) The expression of CDC34 and EGFR in the tumor samples from the mice of each group. (e) Kaplan–Meier survival curve of the mice. $P$ value, log-rank test. *, $P < 0.05$; **, $P < 0.01$. (f–i) pCDH–CDC34-expressing A549-luciferase cells ($2 \times 10^5$) were injected into SCID-beige mice, and detected for relative luciferase intensity by IVIS Spectrum at indicated time points (f, g). Error bars, sd; $P$ values, Student’s $t$-test. Kaplan–Meier survival curve of the mice was shown (h). $P$ value, log-rank test. The mice were sacrificed, the lung tissues were subjected to hematoxylin-eosin (HE) or IHC staining (i; scale bar, 0.5 mm), or lysed for Western blot assays using indicated antibodies (j). *, $P < 0.05$; **, $P < 0.01$. 

$10^6$
Histological examination of the lungs demonstrated that the shCDC34-treated mice developed lesions in alveoli, whereas the shNC group mice harbored disseminated adenocarcinomas (Fig. 8a). qRT-PCR, immunoblot, and IHC assays of the lung specimens revealed the downregulation of CDC34 at both mRNA and protein levels (Fig. 8b and c) and the decrease in EGFR as well as Ki67 (Fig. 8c) in shCDC34-treated mice. Western blot analysis further showed that DOX administration induced the expression of EGFR, and silencing of CDC34 downregulated EGFR, pEGFR, pAKT, pERK, and Cyclin D1, and upregulated p27 in lungs of the mice (Fig. 8d). In addition, the overall survival of the shCDC34 group mice was significantly prolonged as compared to shNC-treated mice (Fig. 8e).

### 3.10 Knockdown of CDC34 inhibits EGFR T790M/Del (E746-A750)-driven lung cancer

The EGFR T790M mutation is associated with acquired resistance to erlotinib in NSCLCs. We tested the effects of shCDC34 on Tet-op-
Fig. 6. CDC34 inhibits the ubiquitination of EGFR and protects it from proteolytic degradation. (a) qRT-PCR analysis of CDC34 and EGFR in A549 and H1975 cells 72 h after siCDC34 transfection. Error bars, sd; P values, Student’s t-test. (b) Cycloheximide (CHX) chase assay to measure EGFR half-life in H460 (in the absence and presence of EGF) and H1975 cells transfected with siCDC34-1# (lower panel). The relative expression values of EGFR were the results determined by densitometry analysis normalized to Actin. Error bars, sd. (c) HCC827 cells were transfected with 50 nM siCDC34-1# for 24 h, treated with erlotinib for 24 h, and lysed for Western blot assays using indicated antibodies. (d) Western blot analysis of the EGFR expression in H460 cells transfected with siCDC34 and HA-Ub, lysed, and the lysates were subjected to immunoprecipitation (IP) and immunoblot using indicated antibodies. (e) A549 and H1975 cells were transfected with siCDC34 and HA-Ub, lysed for IP and immunoblot assays. (g) H460 cells were transfected with indicated constructs, treated with MG132, and lysed for IP and immunoblot assays. (h) The cells were transfected with CDC34 and HA-Ub, and lysed for IP and immunoblot assays using indicated antibodies. (i) Western blot analysis of EGFR in #1 siCDC34-transfected H460 cells in the presence or absence of chloroquine (CQ). (j) HCC827 cells were transfected with siCDC34 and HA-Ub for 24 h, treated with or without chloropuine for additional 24 h, lysed, and subjected to IP and immunoblot using indicated antibodies. (k) H460 cells were transfected with CDC34 (left) or CBL (right) in the absence or presence of EGF for 48 h, and lysed for IP and immunoblotting using indicated antibodies. (l) 293T cells were transfected with the wild type or mutant (Y1045F) Flag-EGFR ICD for 48 h, and lysed for IP and immunoblotting using indicated antibodies. (m) In vitro Co-IP experiments using Flag-EGFR purified from 293T cells, His-c-Cbl (from E. coli), and increasing amount of purified His-c-Cbl (from E. coli) proteins and indicated antibodies. (n) In vitro Co-IP experiments using Flag-EGFR purified from 293T cells, His-c-Cbl (from E. coli), and increasing amount of purified His-c-Cbl (from E. coli) proteins and indicated antibodies. (o) 293T cells were transfected with the wild type or mutant (Y1045F) Flag-EGFR ICD for 48 h, and lysed, subjected to IP and immunoblotting using indicated antibodies. (p) siCDC34-mediated loss of EGFR is rescued by knockdown of CBL. A549 cells were transfected with siCDC34 and/or siCBL for 72 h. Before lysis for immunoblot assays, the cells were serum-starved for 3 h and then stimulated by 50 ng/mL EGF for 15 min.
EGFR790M/Del (E746-A750) /CCSP-rtTA transgenic mice [41]. Interestingly, we found that silencing of CDC34 (Fig. 8f) led to alleviated lung carcinogenesis in the mice, reflected by micro-CT (Fig. 8g, Supplementary Fig. 7b) and histological examination (Fig. 8h). Knockdown of CDC34 also reduced Ki67 (Fig. 8i) and downregulated the expression of EGFR, pEGFR, pAKT, pERK, and Cyclin D1, and upregulated p27 (Fig. 8j) in tumor tissues of the mice. The overall survival of the shCDC34 treatment group mice was also significantly prolonged compared to shNC-treated mice (Fig. 8k).

4. Discussion

In this study, we used a large-scale siRNA screening to identify UPGs that are critical to lung carcinogenesis, and unveiled 31 candidates that were required for proliferation of A549 and H1975 cells (Fig. 1, Supplementary Fig. 1). Among them, CDC34 represented the most significant one, which was elevated in 76 of 114 (66.7%) NSCLCs and was inversely associated with clinical outcome of the patients (Table 1, Fig. 1). CDC34 was overexpressed in acute lymphoblastic leukemia [42], multiple myeloma [43], breast cancer [44], hepatocellular carcinomas [45,46], and prostate cancer [47], indicating that this E2 conjugase plays a critical role in tumorigenesis.

Cigarette smoking is responsible for more than 1.44 million lung cancer deaths each year worldwide [48]. Tobacco smoke causes genomic mutations in tumors [49] and counterpart normal controls [50] [51], promotes cell proliferation, inhibits programmed cell death, facilitates angiogenesis, invasion and metastasis potentials, and enhances tumor promoting inflammation [52-54]. We found that CDC34 was overexpressed in 44 of 59 (74.6%) smoker NSCLCs and in 23 of 43 (53.5%) nonsmoker patients (P = 0.027; Table 1). In another cohort [25], CDC34 expression in smokers was significantly higher than in nonsmokers (Fig. 1). These results suggest that tobacco smoke may induce CDC34 expression to maintain hyperactivation of EGFR oncoprotein, thus contributing to lung carcinogenesis. This possibility was confirmed by the findings that tobacco smoke caused elevation of CDC34 in A/J mice, and tobacco carcinogens BaP, BAA, and DBA induced upregulation of CDC34 in the cells (Fig. 1). Therefore, tobacco may induce lung carcinogenesis in an unexpected way by perturbing the expression of genes like CDC34, IRX5 [55], and RFWD3 [56], which were identified by large-scale screening (Supplementary Table 1).

CDC34 functions as a K48 Ub chain-building enzyme and cognate E2 of SCF E3 ligases, and collaborates with HECT-, RING-, and RING-between-RING (RBR)-E3s to control proteolysis of substrates [57]. So far, no evidence suggests a role for CDC34 in EGFR turnover, which is tightly controlled by Ubc4/5/c-Cbl cascade [3]. We showed that knockdown of CDC34 resulted in downregulation of EGFR, whereas ectopic expression of CDC34 led to upregulation of EGFR and increase in its tyrosine kinase activity (Figs. 3 and 6). The downstream signaling molecules, pAKT and pERK, were accordingly affected (Fig. 3). Mechanistically, the E2 catalytic domain of CDC34 bound EGFR at its ICD region, and competed with c-Cbl to bind EGFR at Y1045 and inhibited the K48-linked polyubiquitination and subsequent...
Fig. 8. Knockdown of CDC34 significantly suppresses L858R- and T790M-EGFR-driven lung cancer. (a) The EGFR<sup>L858R</sup>-transgenic mice were intranasally infected with virus particles containing shNC or shCDC34-1#, treated with DOX, scanned by micro-CT (left), and tumor volume was quantitated by microCT Analysis Tools (right). *P* values, Student’s t-test. ***, *P* < 0.001. (b) Hematoxylin-eosin staining of the lung sections from mice of each group. The expression of CDC34 at both mRNA and protein levels in the lung tissues was tested by qRT-PCR (left panel) and Western blot (right panel), respectively, and the results were shown. Error bars, sd; *P* values, Student’s t-test. **, *P* < 0.01. (c) IHC analysis of CDC34, Ki67, and EGFR in tumors from mice of each group. Scale bar, 1 mm. (d) The expression of indicated proteins in the tumor samples from the mice of each group was detected by Western blot. (e) Kaplan–Meier survival curve of the mice. (f) The EGFR<sup>T790M/Del (exon19)</sup>-transgenic mice were intranasally infected with viral particles containing shNC or shCDC34-1#, treated with DOX, sacrificed one month later, and the lung tissue lysates were subjected to immunoblot to detect the expression of CDC34 and EGFR. *P* values, Student’s t-test. ***, *P* < 0.001. (g) Hematoxylin-eosin staining of the lung sections from mice of each group. Scale bar, 1 mm. (h) IHC analysis of CDC34, Ki67, and EGFR in lung tumors from the mice. Scale bar, 1 mm. (i) The expression of indicated proteins in the tumor samples from the mice of each group. (j) Kaplan–Meier survival curve of the mice. (k) Schematic representation of CDC34 in lung epithelial cells.
degradation of the substrate (Fig. 8). In cellular and animal models, silencing of CDC34 suppressed, while overexpression of CDC34 promoted, lung cancer cell proliferation (Figs. 2, 3, 4). The inhibitory effects of CDC34 on NSCLC cells was mediated by EGFRT790M, since ectopic expression of EGFRT790M rescued siCDC34-induced suppression of the cells (Fig. 7). These results demonstrate previously unreported functions of CDC34, and indicate that an E2 Ub conjugase can compete with E3 ligases to protect proteolysis of substrates.

CDC34 exerts oncogenic or tumor-promoting functions, and has been used as a therapeutic target for drug development [58]. Inhibition of CDC34 enhances anti-myeloma activity of proteasome inhibitor bortezomib [43] and contributes to the chemopreventive activity of Chinese herbs (anti-tumor B, ATB) in mouse model of lung cancer [59]. We showed that silencing of CDC34 inhibited cell proliferation and colony forming activity of NSCLC cells in vitro (Figs. 2 and 3), and suppressed tumor growth and prolonged lifespan of xenograft NSCLC mouse models in vivo (Fig. 4). In an EGFRT790M-driven mouse lung adenocarcinoma model, shCDC34 treatment significantly inhibited cancer progression and prolonged survival time of the mice (Fig. 8). These results indicate that CDC34 represents a rational drug target for NSCLC. Moreover, treatment of the patients with gefitinib and erlotinib will fail because of the development of EGFRT790M mutation [60], which affects the gatekeeper residue in the kinase catalytic domain. Thus, the gatekeeper of the inhibitor is expected to be a new target in lung cancer. The results of the study suggest that CDC34 is a potential therapeutic target for drug development.

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