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Plakophilin-2 accelerates cell proliferation and migration through activating EGFR signaling in lung adenocarcinoma

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ABSTRACT

Background: Plakophilin 2 (PKP2), encodes a plakophilin protein that belongs to the member of desmosomal proteins. It has been reported that high expression of PKP2 is associated with several types of cancer in humans. However, the role of PKP2 in lung cancer remains obscure.

Methods: PKP2 expression was investigated in non-small cell lung cancer (NSCLC) tissues and non-tumor tissues by performing immunohistochemistry on a tissue microarray and using The Cancer Genome Atlas (TCGA) database. Kaplan-Meier survival analysis and multivariate Cox-regression analysis were performed to identify the clinical significance of PKP2. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), colony formation, Transwell and xenograft tumor growth/ metastasis assays were conducted to evaluate the biological function of PKP2 in vitro and in vivo. Gene set enrichment analysis (GSEA), WB and immunoprecipitation (IP) assay were utilized to explore the potential downstream signaling pathway and molecule mechanism of PKP2 in lung adenocarcinoma (LUAD).

Results: Analysis of PKP2 expression and clinicopathological parameters reveals a significant correlation of PKP2 expression with gender ($n = 1020$, $P < 0.001$) and histological type ($n = 1020$, $P < 0.001$). Subsequently, our results demonstrated that high PKP2 expression is not associated with poor survival in different gender of lung cancer patients, and is an unfavorable and independent prognostic biomarker for LUAD patients, but not for LUSC patients. Gene set enrichment analysis (GSEA) revealed that PKP2 expression is positively associated with EGFR signaling in LUAD. Further, in vitro and in vivo assays revealed that PKP2 promotes cell proliferation, migration and invasion through activating EGFR signaling pathway in LUAD cells.

Conclusion: Our study provides the basis for further investigation of the function and molecular mechanism by which upregulation of PKP2 promotes the development and progression of LUAD. PKP2 may serve as a potential target for anticancer therapies.

1. Introduction

Non-small cell lung cancer (NSCLC), accounting for 85% of all cases, is the main histological subtype of lung cancer and is the first leading cause of cancer death worldwide, with a five-year survival rate of 15% [1]. NSCLC consists of two principal subtypes: lung adenocarcinoma (LUAD) and lung squamous carcinoma (LUSC) [2]. More and more evidence indicated that the biological patterns, fundamental molecular mechanisms and, most importantly, therapeutic strategies between LUAD and LUSC are different, suggesting that early confirmation of histology is key to precise treatment of NSCLC [3,4]. Thus, a clear understanding of the differences of molecular characteristics and mechanisms of these two major subtypes of NSCLC are vital in leading to deeper understanding and identification of novel molecular-targeted

Abbreviations: PKP2, Plakophilin 2; EGFR, epidermal growth factor receptor; EGF, Epidermal growth factor; NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, Lung squamous carcinoma; TCGA, The Cancer Genome Atlas; GSEA, Gene set enrichment analysis; OS, overall survival; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

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strategies for NSCLC therapy.

Desmosome, a cell structure specialized for cell-to-cell adhesion, are composed of desmosome-intermediate filament complexes [5–7]. In recent years, there's increasing studies and focus on the relationship between desmosomal gene and cancer [6,8,9]. In our previous study, we have identified most of desmosomal genes that were differentially expressed between LUAD and LUSC [2]. However, another desmosomal gene PKP2, its expression and clinical implication in NSCLC are still elusive.

In this study, we aim to investigate the expression, clinical relevance and downstream signaling pathway of PKP2 in NSCLC patients using TCGA database. Results indicated that PKP2 was overexpressed in NSCLC tissues and was significantly correlated with gender and histological type of NSCLC patients. Kaplan-Meier survival analysis revealed that high PKP2 expression was not associated with poor OS in different gender of patients. Specifically, Kaplan-Meier survival analysis and multivariate Cox-regression analysis revealed that high PKP2 expression was obviously associated with poor outcome of LUAD patients, but not of LUSC patients. Above all, PKP2 had an unfavorable prognostic impact on LUAD patients, but not on LUSC patients. Additionally, functional analysis revealed that PKP2 had a positive effect on tumor cell proliferation, migration and invasion in LUAD cells. Furthermore, we performed gene set enrichment analysis (GSEA), WB and IP assays to identify the downstream signaling pathway which is associated with PKP2 between LUAD.

2. Materials and methods

2.1. Cell culture

LUAD cell lines H358 and A549 were obtained from the cell bank of the Chinese academy of sciences. H358 and A549 cells were cultured in RPMI-1640 medium (GIBCO, USA) or F12 K medium (GIBCO, USA) supplemented with 10% fetal bovine serum, respectively. All cells were maintained at 37 °C in a 5% CO_2 humidified incubator.

2.2. Patient sample and immunohistochemistry (IHC)

A total of 30 NSCLC specimens including cancerous tissues and noncancerous tissues were obtained from the collaboration (Shanghai Biochip Co Ltd., Shanghai, People's Republic of China). PKP2 mouse monoclonal antibody (1:100; Santa Cruz Biotechnology; SC-393711) that specifically recognizes amino acids 757–881 mapping at the c-terminus of PKP2 was used to perform IHC staining. IHC staining was performed as described previously [10]. The result of IHC have been quantized by two pathologists, and expression levels of PKP2 were defined by the sum of the grades for the percentage of positive staining and intensity. Then, we divided all patients into two groups, the high PKP2 expression group and the low PKP2 expression group, based on the median PKP2 expression level. All experiments and procedures were approved by the Clinical Research Ethics Committee of the Third Military Medical University. Written informed consent was obtained from all patients

2.3. Analysis of publicly available datasets

TCGA data of all 1020 NSCLC patients were directly downloaded from the University of California Santa Cruz database at https://xena.ucsc.edu/. The RNA-Seq data of 1020 NSCLC patients were retained and further analyzed. The mRNA-seq data of PKP2 was added in supplemental Table S1.

2.4. Plasmid construction and cell transfection

For knockdown, a hairpin precursors presented high efficiency in knocking down PKP2 was constructed as previously described [11]. For overexpression, the full-length open reading frame of human PKP2 was generated by synthesis and subsequent molecular cloning into pIRES2-EGFP. Cells were inoculated in 6-well plates for 24 h. When the cells at 75% confluence, PKP2-shRNA or NC-shRNA and PKP2-overexpression or Vector control, respectively, were transfected into cells using Lipofectamine 2000 Reagent (Invitrogen Preservation, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.5. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay

Cell viability was measured by using a MTS kit (Promega, USA). After PKP2 transfection, a MTS solution was added and mixed with the medium at a ratio of 1:9 and incubated at 37 °C for 1 h. The OD value was measured at 490 nm by a microplate reader (SpectraMax M2, USA).

2.6. Colony formation assay

For colony formation assay, 300 stable transfected cells were re-suspended and seeded into 6-well plates and cultured with RPMI-1640 or F12-K containing 10% FBS at 37 °C. After incubation for two weeks, colonies were fixed in 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet for 5 min. Cell colonies which contain more than 50 cells were then counted.

2.7. Boyden chamber migration/invasion assay

Transwell assays were performed by using transwell plates (Corning). Cells were plated into the upper chamber with serum- and growth factor-free medium. The lower chamber was filled with serum-containing medium. After 24 h incubation at 37 °C, the cells on the upper chamber were removed. Cells that migrated to the lower side were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet. The number of cells that migrated was counted at × 200 magnification in 10 different fields. The results were determined from three repeated experiments.

2.8. Nude mice models

For in vivo tumor growth and metastasis experiment, a total of 1×10^7 stable transfected cells suspended in 150 μl PBS were injected into the right flanks of the nude mice. After 40 days housing, mice were euthanized, the excised tumors were weighed and fixed in Bouin's fluid. The images of tumor were taken. This study was approved by the Committee on the Ethics of Animal Experiments of Third Military Medical University. All experiments on mice were performed at Third Military Medical University, China.

2.9. Gene set enrichment analysis (GSEA)

GSEA is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biologic states. TCGA data can be ordered in a ranked list, according to their differential expression between the phenotype. PKP2 expression level was divided into low and high categories to annotate phenotype, and gene sets from the C2.cp.Reactome v6.2.symbols.gmt (curated) were used.

2.10. WB and co-immunoprecipitation

Lysates of A549 cells were prepared for Western blot analysis. Proteins (40–60 μg) were resolved using 10% sodium polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad, USA). After incubation at 4 °C overnight with primary antibodies for PKP2, EGFR, phosphor-EGFR, ERK1/2 and phosphor-ERK1/2 (Santa Cruz Biotechnology Inc., USA). The membrane was then washed with

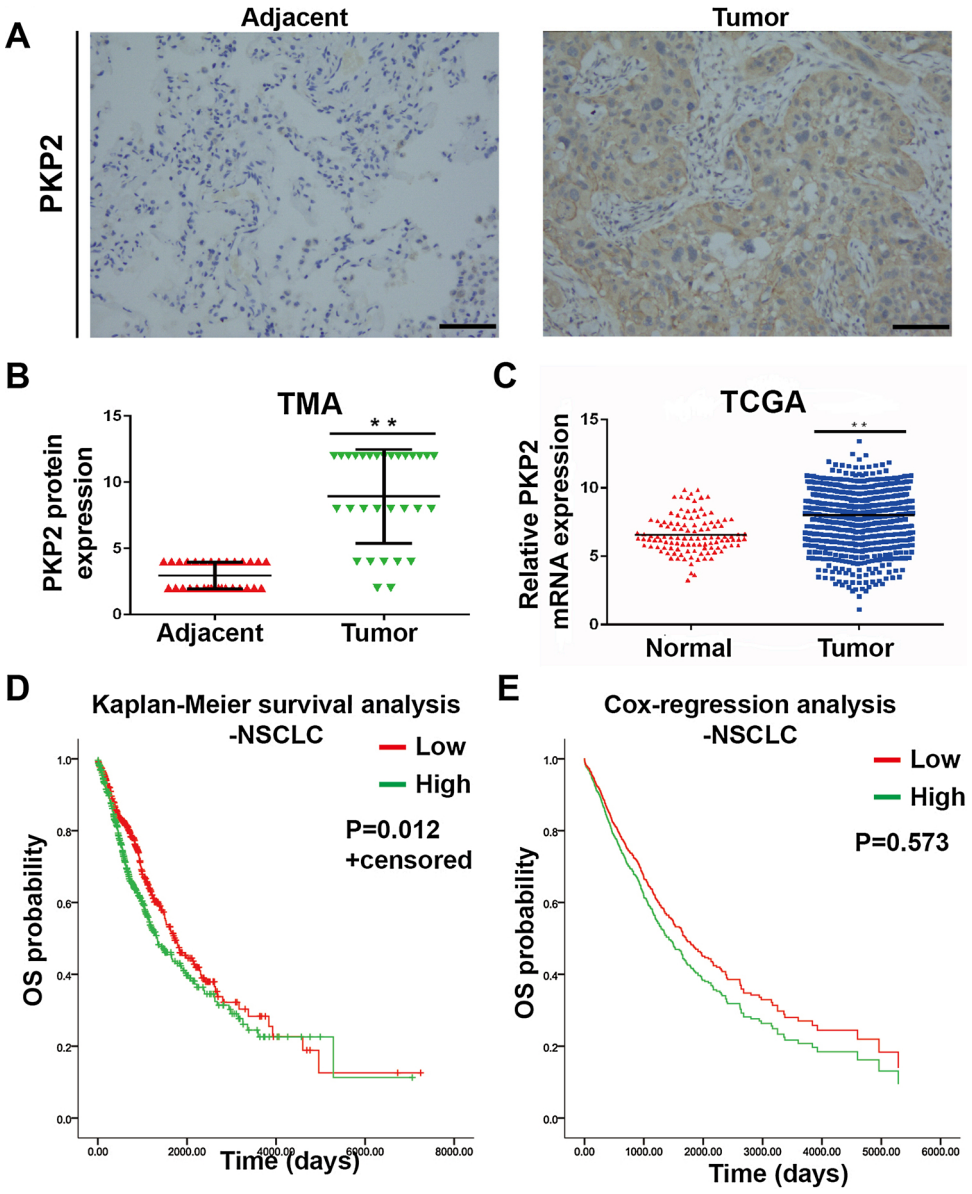


Fig. 1. PKP2 is upregulated in NSCLC. (A and B) The protein expression of PKP2 in tumor and adjacent samples were detected by immunohistochemistry (IHC) on a tissue array. The anti-PKP2 antibody was employed for IHC. Scale bar represents 50 mm. (C) The mRNA level of PKP2 was investigated in NSCLC tissue and normal tissue using TCGA database. (D) Kaplan-Meier analysis of PKP2 expression in patients with NSCLC. (E) Cox-regression analysis of PKP2 expression in patients with NSCLC.

Table 1
Association of PKP2 expression with clinicopathological characteristics in human NSCLC patients (Chi-square test).

Variable	Category	PKP2 expression		P value
		High (n = 512)	LOW (n = 508)	
Age (years)	< 60	64	73	0.115
	≥ 60	226	248	
Gender	Male	341	270	< **0.001**
	Female	169	239	
Histologic type	LUAD	182	302	< **0.001**
	LUSC	315	186	
Clinical stage (AJCC)	I	251	271	0.461
	II	146	138	
	III	90	78	
	IV	19	14	

Table 2
Multivariate analysis of diff ;erent prognostic factors in NSCLC patients (n = 1020) (Cox-regression analysis). Abbreviations: HR, hazard ratio; CI, confidence interval.

Variables	Multivariate analysis		
	HR	95%CI	P
PKP2 expression	1.064	0.858-1.318	0.573
Age	0.842	0.715-0.992	**0.040**
Gender	0.882	0.704-1.104	0.273
Clinical stage	1.444	1.187-1.757	< **0.001**
Histological type	0.953	0.762-1.191	0.670

Bolded values indicate statistical significance, P < 0.05.

phosphate-buffered saline 3–5 times and then reacting with a HRP-conjugated secondary antibody (Beyotime, China). An enhanced chemiluminescence kit (Beyotime, China) was used to detect the signals.

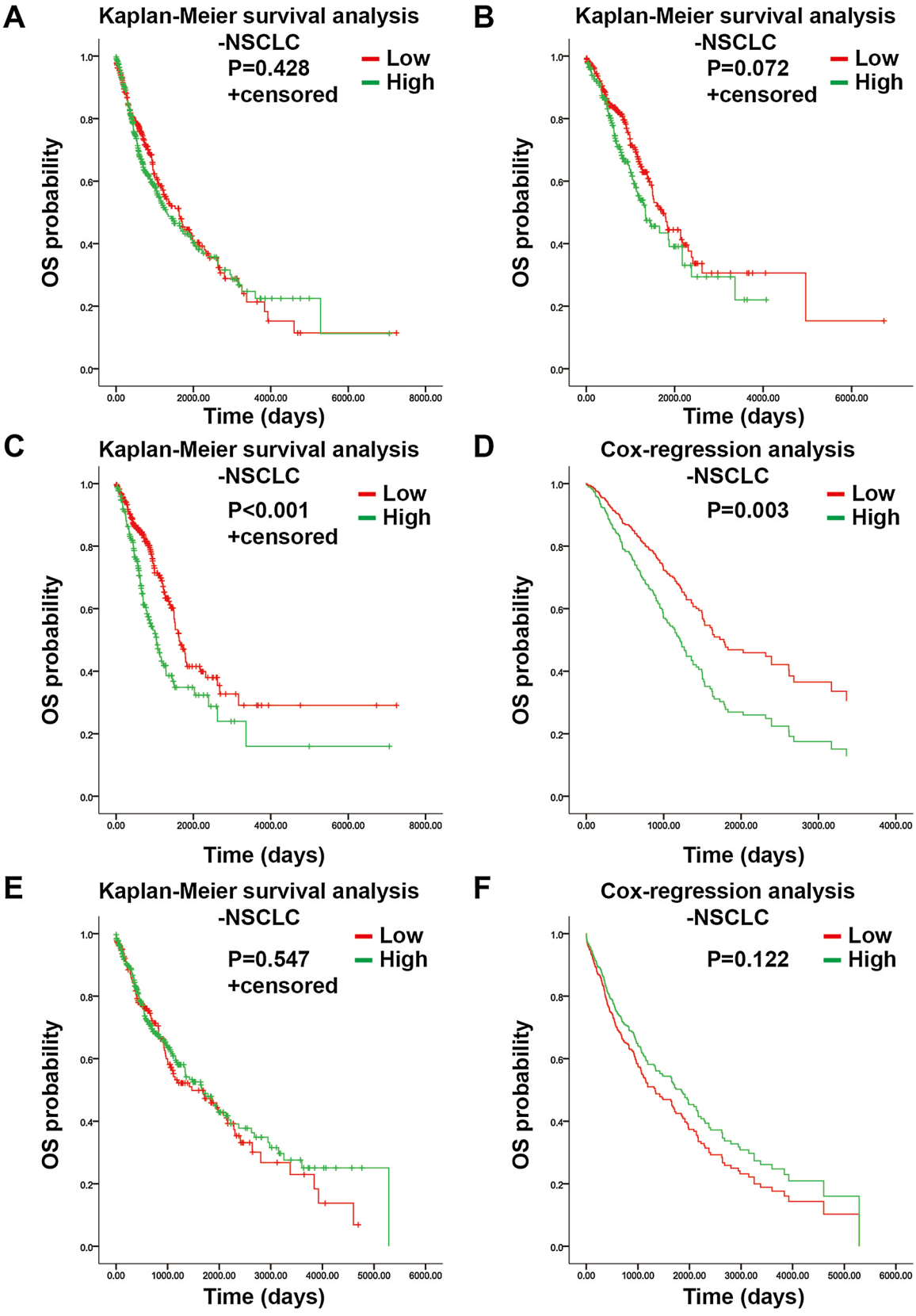


Fig. 2. PKP2 expression has a negative prognostic significance in LUAD patients, but not in LUSC patients. (A) Kaplan-Meier analysis of PKP2 expression in male patients. (B) Kaplan-Meier analysis of PKP2 expression in female patients. (C) Kaplan-Meier analysis of PKP2 expression in patients with LUAD. (D) Cox-regression analysis of PKP2 expression in patients with LUAD. (E) Kaplan-Meier analysis of PKP2 expression in patients with LUSC. (F) Cox-regression analysis of PKP2 expression in patients with LUSC.

Table 3
Multivariate analysis of diff ;erent prognostic factors in LUAD (n = 484) and LUSC (n = 501) patients (Cox-regression analysis). Abbreviations: HR, hazard ratio; CI, confidence interval.

Variables	Multivariate analysis LUAD			Multivariate analysis LUSC		
	HR	95%CI	P	HR	95%CI	P
PKP2 expression	1.631	1.183-2.248	**0.003**	0.803	0.609-1.060	0.122
Age	0.865	0.681-1.097	**0.231**	0.842	0.670-1.057	0.138
Gender	1.238	0.896-1.709	**0.195**	0.668	0.474-0.940	0.021
Clinical stage	1.639	1.149-2.337	**0.006**	1.371	1.072-1.753	0.012

Bolded values indicate statistical significance, P < 0.05.

Equal loading of proteins on the gel was verified by reprobing the membrane with β-actin antibody.

IP was carried out as previously described [12]. Cell lysates were prepared and immunoprecipitated with the indicated antibody or mouse IgG using the Co-IP Kit (Pierce, USA) according to the manufacturer's instructions. The precipitated protein was assessed by WB using the indicated antibody.

2.11. Statistical analysis

All the analyses were performed with R version 3.4.3 and Graphpad prism 5.0 software. Data are presented as mean ± SD. The expression comparison of PKP2 between tumor and normal samples were calculated using student's t-test. The correlation between the expression of PKP2 and the clinical pathologic feature of NSCLC patients was analyzed by the Pearson's chi-squared test or Fisher's exact test. The Kaplan Meier method was used to assess the relationship between PKP2 and OS in NSCLC patients. Multivariate analysis of prognostic predictors was performed by using Cox proportional hazard models. All P values were two-tailed and considered statistically significant when less than 0.05.

3. Results

3.1. PKP2 is overexpressed in NSCLC tissues and its high expression correlates with poor outcome of patients

To determine the role of PKP2 in lung cancer, we first detected the protein expression of PKP2 by immunohistochemistry (IHC) on a tissue microarray containing 30 NSCLC tissues and 30 paired adjacent non-tumor tissues. We found that PKP2 is significantly upregulated in tumor samples compared with the normal control in NSCLC (Fig. 1A and B). To further confirm this result, the mRNA level of PKP2 was analyzed in paired NSCLC tissues and adjacent normal tissues using TCGA data. Consistently, the results showed that the mRNA expression of PKP2 in NSCLC tissues are higher than that in adjacent normal tissues (Fig. 1C). We next to evaluate the clinical implication of PKP2 in NSCLC. As shown in Table 1, PKP2 expression levels were evidently correlated to gender (n = 1020, P < 0.001) and histological type (n = 1020, P < 0.001) of NSCLC patients.

To evaluate whether PKP2 expression is associated with the prognosis of NSCLC patients, Kaplan-Meier survival analysis and multivariate Cox-regression analysis were performed using TCGA data. Kaplan-Meier survival analysis revealed that higher expression of PKP2 was associated with markedly shorter overall survival (OS) of NSCLC patients (Fig. 1E, P = 0.012). However, in addition to clinical stage (HR = 1.444, P < 0.001) and age (HR = 0.842, P = 0.040), PKP2 expression was not an independent prognostic factor (HR = 1.064, P = 0.573) for the OS of NSCLC patients (Fig. 1E, Table 2).

3.2. High PKP2 expression suggests worse prognosis of LUAD patients, but not of LUSC patients

PKP2 expression is obviously correlated with clinicopathological characteristic of lung cancer patients, suggesting a diff ;erent clinicopathological significance of PKP2 in different gender and subtypes of lung cancer patients. However, Kaplan-Meier survival analysis indicated that high PKP2 expression was not linked to poor OS in male patients (P = 0.428) and female patients (P = 0.072), respectively (Fig. 2A and B).

We next to investigate the relationships between PKP2 expression and survival of LUAD and LUSC patients respectively. Univariate and multivariate Cox-regression analysis indicated that high PKP2 expression was linked to poor OS (P < 0.001) and as an independent predictor (HR = 1.631, P = 0.003) in LUAD patients (Fig. 2C and D, Table 3), but not in LUSC patients (Fig. 2E and F, Table 3). Moreover, we further assessed the association of PKP2 expression with outcomes of LUAD and LUSC patients by using the Kaplan-Meier Plotter tool. Consistently, high expression of PKP2 was associated with unfavorable prognosis in LUAD (n = 720, HR = 1.35, 95% CI = 1.07–1.70, P = 0.011) (Supplemental Fig. 1A), but not in LUSC (n = 524, HR = 0.92, 95% CI = 0.73–1.17, P = 0.500) (Supplemental Fig. 1B).

3.3. Overexpression of PKP2 promotes LUAD cell proliferation, migration and invasion in vitro

The clinical significance of PKP2 in LUAD spurs us on to explore the potential role of PKP2 in LUAD. We chose two classic LUAD cell lines, H358 and A549 for further study. Then, we examined the protein expression of PKP2 in these cells and found that PKP2 is overexpressed in H358 cell line and under expressed in A549 cell line (Fig. 3A). In order to achieve a more pronounced effect, the PKP2 was knocked-down in H358 cells (high expression of endogenous PKP2) or overexpressed in A549 cells (low expression of endogenous PKP2), and the expression of PKP2 was verified by WB (Fig. 3B and C). Subsequently, MTS assay revealed that knockdown of PKP2 weakened proliferative ability and colony forming ability of H358 cells, while overexpression of PKP2 enhanced proliferative ability and colony forming ability of A549 cells (Fig. 3D −G). Further Transwell assay was carried out to investigate the effect of PKP2 on cell migration and invasion. The results showed that PKP2 also can strengthen migration and invasion abilities of LUAD cells (Fig. 3H and I).

3.4. Overexpression of PKP2 accelerates tumor growth and metastasis in vivo

To further confirm the functional role of PKP2 in vivo, the nude mice subcutaneous xenograft models were conducted. H358-NC and H358-shRNA stable cells were subcutaneously injected into the right posterior flanks of nude mice, respectively. We found that the nude mice received H358-shRNA cells formed smaller and lighter tumors than those received vector control cells (Fig. 4A and B). Furthermore, the effect of PKP2 silencing on pulmonary metastasis was examined by H&E staining. Noticeably, H&E staining showed that low PKP2 expression resulted in a significant decrease in the number and size of lung metastasis loci (Fig. 4C).

3.5. PKP2 activates epidermal growth factor receptor (EGFR) signaling in LUAD cells

According to previous reports, upregulation of PKP2 leads to activation of EGFR signaling. So, we speculate that EGFR signaling may contribute to PKP2-mediated cell proliferation and migration. To verify this hypothesis, GSEA assay was first performed using RNA-Seq gene expression data from the TCGA database. The results indicated that the high expression of PKP2 was significantly correlated with EGFR

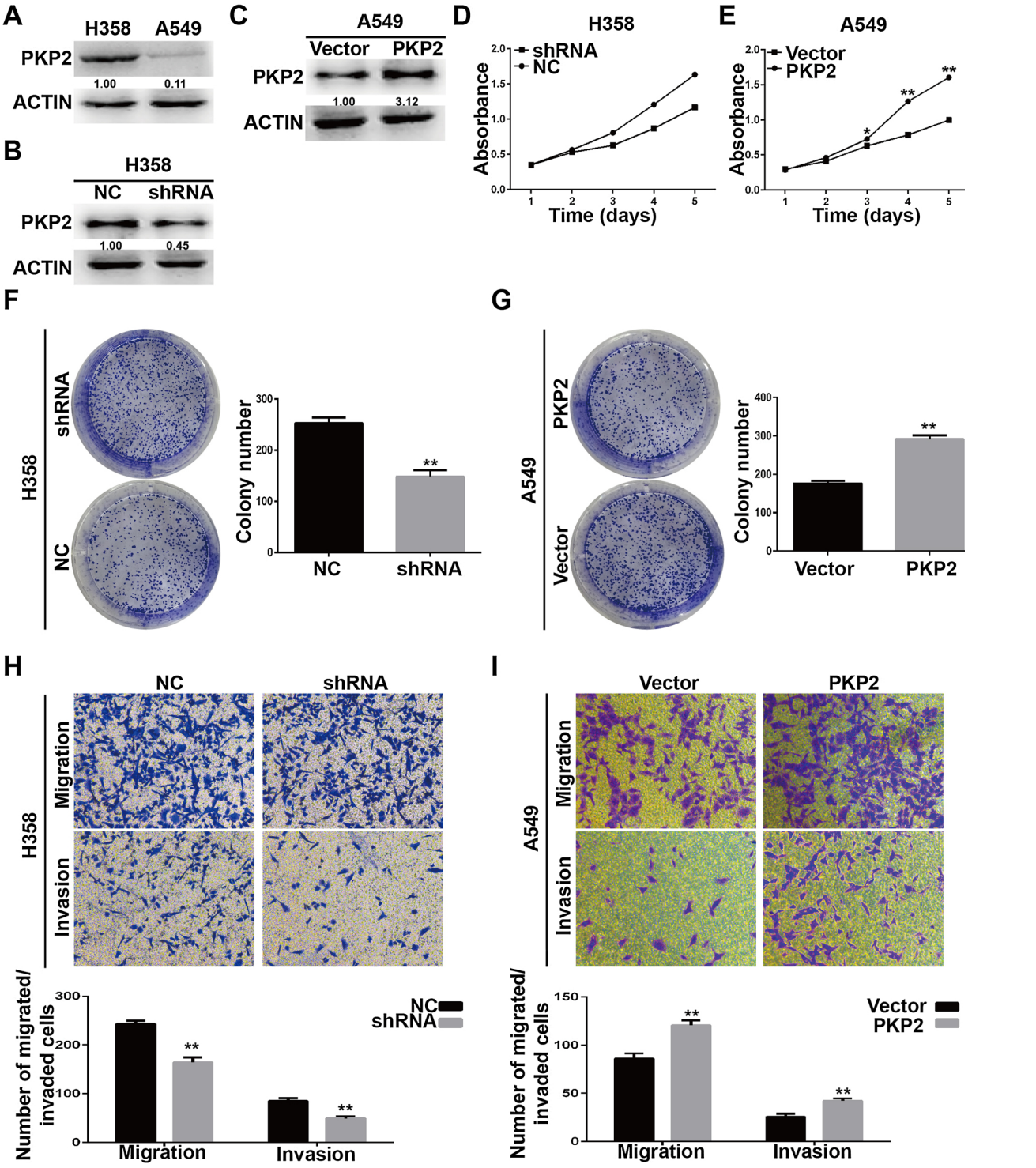
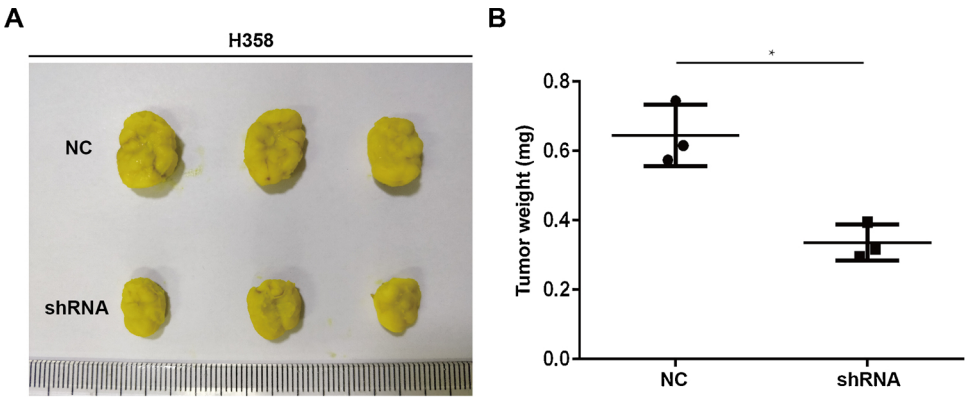


Fig. 3. PKP2 promotes cell proliferation, migration and invasion in vitro. (A) The protein expression of PKP2 was detected by WB. (B and C) Knockdown or overexpression of PKP2 expression in two lung cancer cell lines were examined by WB. (D and E) PKP2 promotes proliferation of LUAD cells. (F and G) PKP2 promotes colony formation of LUAD cells. (H and I) PKP2 promotes migration and invasion of LUAD cells.

signaling pathway in LUAD (Fig. 5A, Table 4). Next, the downstream EGFR signaling was detected in A549 cells with ectopic expression of PKP2 by WB. Consistently, PKP2 can activate EGFR signaling in A549 cell line (Fig. 5B). Due to the fact that the interaction between PKP2 and EGFR was thought to be essential for EGFR autophosphorylation and EGFR-mediated signal transduction. Thus, we utilized IP assay to identify this interaction and found this interaction can be detected in A549 cells (Fig. 5C).

3.6. Ablation of EGFR signaling abrogates the effect of PKP2 over expression on cell proliferation and migration

Encouraged by the above results, we next examined whether PKP2 promotes cell proliferation and migration through regulating EGFR signaling. The activity of EGFR signaling was blocked by Lapatinib, an inhibitor of EGFR signaling pathway, when overexpression of PKP2 (Fig. 6A). The results of MTS, colony formation and Transwell assays



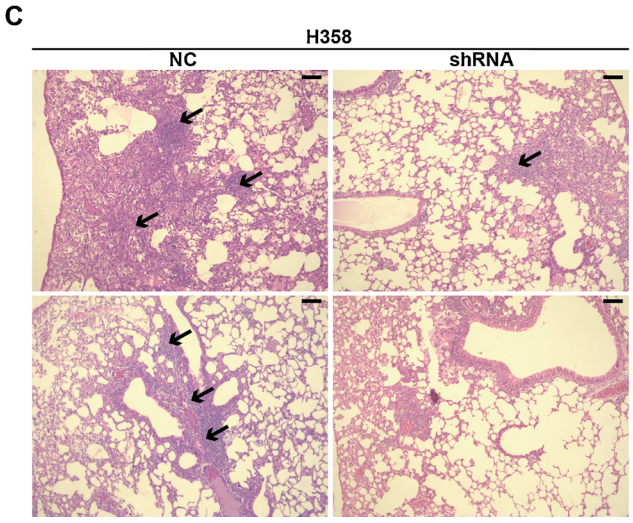


Fig. 4. PKP2 promotes tumor growth and metastasis in vivo. (A) Xenograft tumors were removed from mice and fixed with Bouin's solution. The photograph of tumors were taken. (B) Tumor weights from nude mice were measured. *P < 0.05. (C) Lung invaded by tumor cells were detected by H&E staining. Scale bar represent 100 μm.

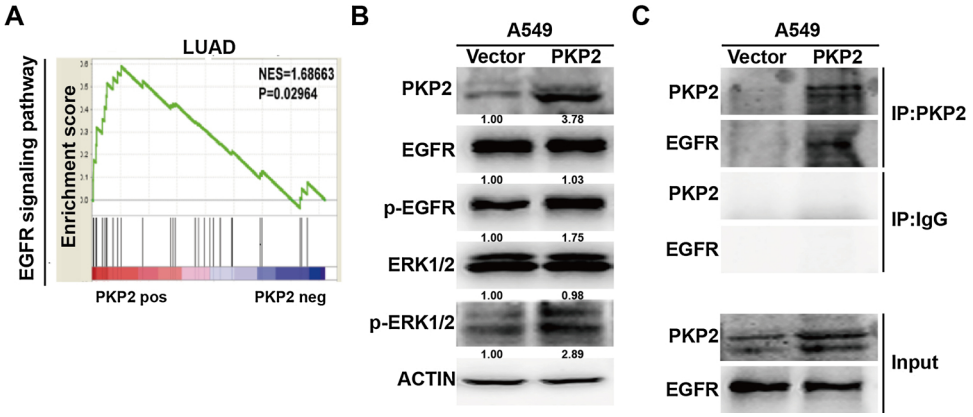


Fig. 5. PKP2 activates EGFR signaling pathway in LUAD cells.
(A) GSEA showed that high PKP2 expression was positively correlated with EGFR signal in LUAD. (B) The effect of PKP2 overexpression on EGFR signaling was monitored by WB. (C) The interaction between PKP2 and EGFR was monitored by WB.

Table 4
Data of GSEA assay for the correlation of PKP2 levels and EGFR signal sets in Reactome.

Upregulated biological processes	ES	NES	NOM P-value	FDR q-value	FWER P-value
EGFR signaling in LUAD	0.58542	1.68663	0.02964	0.20571	0.900
EGFR signaling in LUSC	0.49149	1.41015	0.10505	0.32430	0.999

Abbreviations: ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate; FWER, Family wise error rate.

showed that blockade of EGFR diminished the proliferation, migration and invasion of PKP2-overexpressing A549 cells (Fig. 6B and D).

4. Discussion

In this study, we take the lead in unmask the differences in PKP2 expression between NSCLC tissues and non-tumor tissues. Through IHC assay and analysis of TCGA data, we demonstrated that PKP2 expression in tumor tissues is higher than that in corresponding non-tumor tissues. In addition, we have observed that the expression of PKP2 in LUSC is higher than in LUAD (Table 1), indicating that upstream

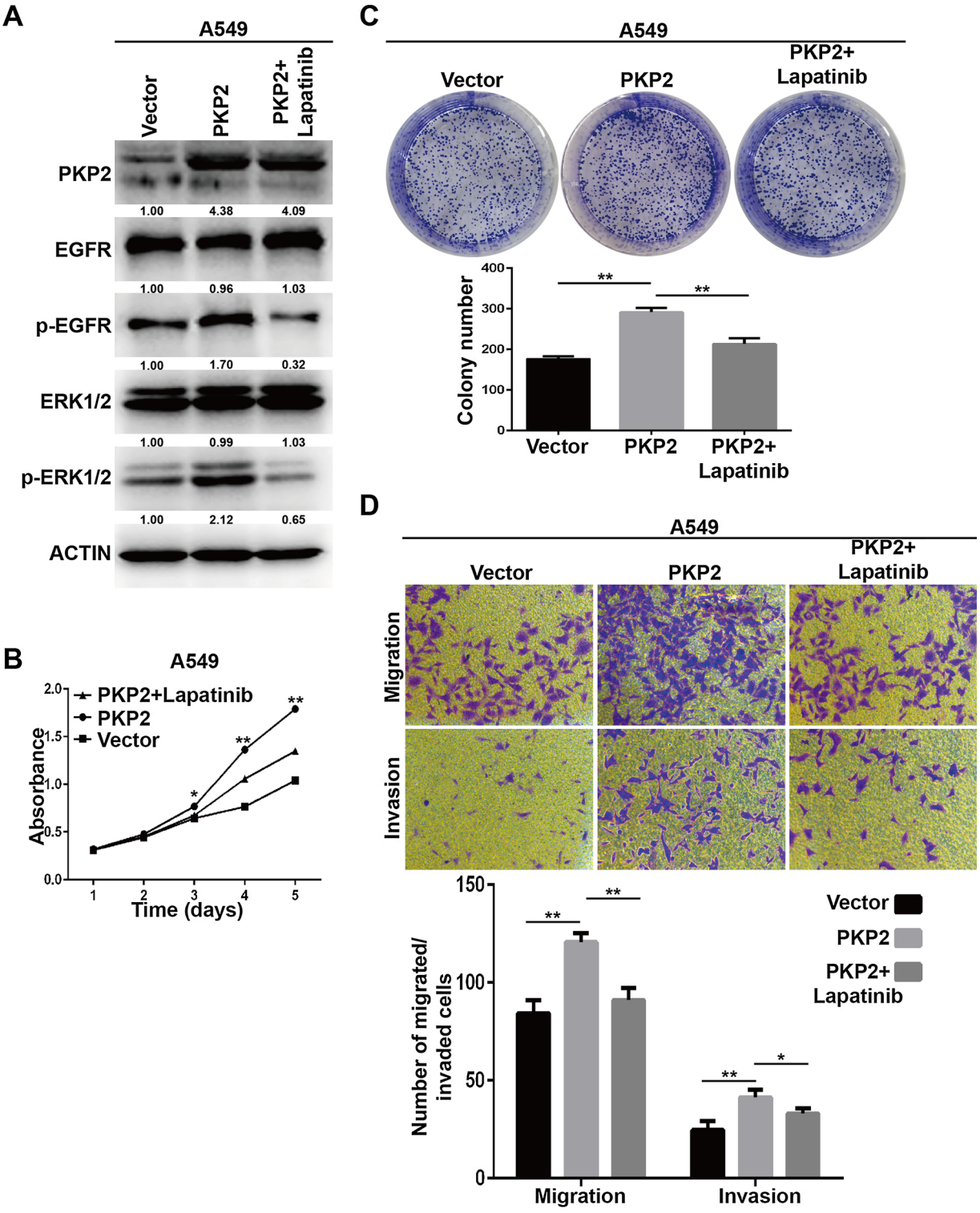


Fig. 6. Inhibition of EGFR signaling can block the acceleration effect of PKP2 overexpression on cell proliferation and migration. (A) The protein level of PKP2 and EGFR signaling-related gene were detected by WB. (B) MTS assay was used to examine the effect of EGFR signaling ablation on cell proliferation of A549-PKP2 cells. (C) Colony formation assay was used to examine the effect of EGFR signaling ablation on colony formation of A549-PKP2 cells. (D) Transwell assay was used to examine the effect of EGFR signaling ablation on cell migration and invasion of A549-PKP2 cells.

regulatory mechanisms of PKP2 gene between them are different. While coincidence, based on TCGA lung cancer datasets, we found that the amplification rate of PKP2 gene in LUSC (3.08%) is higher than in LUAD (2.3%). So, we speculate the expression differences of PKP2 in LUAD and LUSC may be due to the different amplification rate. All the above data gives us a good indication of that whether PKP2 is correlated with the clinicopathological factors of NSCLC patients. Indeed, PKP2 expression is obviously correlated with gender and histological type of NSCLC patients, suggesting a diff ;erent clinicopathological significance of PKP2 in different subtypes and gender of lung cancer patients. To investigate the clinical significance of PKP2, we next to explore the association between PKP2 expression and

prognostic outcomes in different subtypes and gender patients respectively. The results showed that high PKP2 expression was only correlated with LUAD patients' outcome. Further multivariate analysis demonstrated that PKP2 is also an independent prognostic marker for LUAD patients, but not for LUSC patients. So, we speculate that the different clinical implication and biological properties of PKP2 between LUAD and LUSC might result from different molecular mechanisms.

EGFR, a transmembrane tyrosine kinase receptor for members of the epidermal growth factor (EGF) family of extracellular protein ligands, is widely expressed in several types of cancer [13–18]. Under normal conditions, EGFR is critical to maintain cell growth and development [19]. When EGFR gene contains amplification, or mutation, it is the hallmark of cancer and contributes to the uncontrolled division of cells [20]. In our study, GSEA revealed that PKP2 was related to EGFR signaling in LUAD. Indeed, PKP2 can promote cell proliferation and migration through regulating EGFR signaling via binding to EGFR in A549 cells, a canonical LUAD cell line. From previous studies, it is known that mutation or amplification of EGFR are implicated in about 48-80% of all epithelial cancers, including NSCLC [21]. However, in the above experiment, the status of EGFR in A549 cell line is wild-type. These data spurs us to explore whether PKP2 can bind to mutant EGFR. Previous research has shown that the cytoplasmic (aa 644–957; C-terminal) domain of EGFR is the key for interaction with PKP2. Combining with mutations and deletions of EGFR occurs most frequently in exon 18–21 (aa 688–874; C-terminal) which are contained in the cytoplasmic domain of EGFR in NSCLC patients, we considered PKP2 could not interact with mutant EGFR followed by EGFR autophosphorylation. These data still cannot explain why PKP2 associates with EGFR signaling of LUAD patients in clinic. Then, it deserves to be further investigated.

It is worth mentioning that there is no link between PKP2 expression and EGFR signaling in LUSC patients (Table 4). Notably, the mutation rate of EGFR in patients with LUAD was markedly higher than patients with LUSC [20]. Therefore, EGFR always serves as a therapeutic target for LUAD patients with EGFR mutation, but not for LUSC. On the basis of our above results and these previous data, we suspect that there is no association between PKP2 expression and outcome of LUSC patients may be due to PKP2 cannot activate EGFR signaling in LUSC cells. Of course, this hypothesis remains to be tested empirically. Further studies are required to test this.

In summary, we demonstrated that the abnormally high expression of PKP2 is associated with unfavorable prognosis and EGFR activation of LUAD patients, but not of LUSC patients. The precise role of PKP2 in LUAD may provide specifically effective therapies against tumor development and progression for improving LUAD patients' survival.

Ethical approval

All procedures performed in studies involving human/animal participants were in accordance with the ethical standards of the institutional and/or national research committee (the Clinical Research Ethics Committee of the Third Military Medical University) and with the 1964 Declaration of Helsinki and its later amendments.

Competing interests

The authors declare that they have no competing interests.

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No.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152438.

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