

Received: 2019.09.11
Accepted: 2019.11.04
Published: 2019.11.20

Epidermal Growth Factor Receptor Mutations in Resectable Non-Small Cell Lung Cancer Patients and their Potential Role in the Immune Landscape

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

CDE 1 **Wei Xia**
ADEG 2 **Wenjun Mao**
BDF 2 **Ruo Chen**
BD 2 **Rongguo Lu**
DF 2 **Feng Liu**
DF 2 **Yijun He**
F 2 **Shengfei Wang**
ADEG 3 **Xiaomin Li**
ADEG 2 **Mingfeng Zheng**

1 Department of Intensive Care Unit, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, Jiangsu, P.R. China
2 Department of Cardiothoracic Surgery, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, Jiangsu, P.R. China
3 Emergency Department, The Affiliated First People's Hospital of Lianyungang of Nanjing Medical University, Lianyungang Clinical College of Nanjing Medical University, Lianyungang, Jiangsu, P.R. China

Corresponding Authors: Wenjun Mao, e-mail: maowjmedical@126.com, Xiaomin Li, e-mail: lixiaominlyg@163.com, Mingfeng Zheng, e-mail: zhengmfmedical@126.com

Source of support: This work was supported by grants from the Young Medical Key Talents Project in Jiangsu province (QNRC2016193) and the Precision Medicine Project of the Wuxi Municipal Commission of Health and Family Planning (J201805)

Background: The epidermal growth factor receptor (EGFR) is a therapeutic target for non-small cell lung cancer (NSCLC), but knowledge on gene mutations that contribute to NSCLC development and persistence is lacking. In this study, we investigated genetic variations in EGFR and their association with the clinical and pathological factors of NSCLC.


Material/Methods: Clinical cases (331 patients) and The Cancer Genome Atlas (TCGA) cases (1040 patients) were selected and analyzed using the refractory mutation systems cBioPortal and the Tumor Immune Estimation Resource (TIMER).

Results: EGFR mutation frequencies were 54.4% (180 of 331 patients) and 8.0% (83 of 1040 patients) in the clinical and TCGA cohorts, respectively. EGFR mutations were strongly associated with smoking and pathology ($P \leq 0.05$) in the clinical cohort, and with gender, smoking, and pathology ($P = 0.001$, $P < 0.001$, and $P < 0.001$, respectively) in TCGA cohort. In cases of lung squamous carcinoma (LUSC), EGFR was overexpressed as a result of DNA amplification, but this amplified expression showed no association with the overall survival (OS) or progression-free survival of LUSC patients. EGFR gene alterations were, however, associated with worse OS in lung adenocarcinoma (LUAD) patients. Immune cell infiltrates from LUAD and LUSC tumors differed according to EGFR expression. EGFR mutations resulted in a decline of immune infiltration or a lack of infiltrating immune cells in the NSCLC microenvironment.

Conclusions: Mutational profiles of the EGFR in NSCLC patients provide useful information for the use of tyrosine kinase inhibitors for adjuvant or neoadjuvant therapy and immunotherapy.

MeSH Keywords: **Carcinoma, Non-Small-Cell Lung • Mutation • Protein-Tyrosine Kinases • Receptor, Epidermal Growth Factor**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/920042>

 3127

 2

 10

 44



Background

As one of the most lethal tumors globally, lung cancer ranks first and second in terms of mortality among males and females in China, respectively [1], with 5-year survival rates as low as 4% [2]. A range of driver gene mutations have been implicated in lung cancer development including epidermal growth factor receptor (EGFR) and ROS1 mutations [3–5]. Precision therapy using tyrosine kinase inhibitors (TKIs) can improve prognosis in those patients harboring specific genetic alterations [6], producing response rates of up to 80% in non-small cell lung cancer (NSCLC) patients with TKI-sensitive EGFR mutations [7,8].

The majority of studies have focused on TKI administration during early disease stages, and impressive responses and improved patient outcomes have been documented [9,10]. In addition, EGFR TKI neoadjuvant therapy in resectable NSCLC can diminish the surgical rate [9]. When used as adjuvant therapy for stage II-III A (N1-N2) NSCLC patients after complete resection (R0), EGFR TKI therapy can yield longer disease-free survival when compared with traditional platinum-based chemotherapy [10]. Thus, the clinical benefits of EGFR TKIs in resectable NSCLC are promising.

The mutation profile of the EGFR has been investigated widely in metastatic NSCLC. However, EGFR mutation profiles in resectable NSCLC are rare. Understanding the EGFR mutation profile and its correlation with clinicopathological factors will help guide EGFR TKI therapy in resectable NSCLC precisely in the future.

The recent discovery of immune checkpoints, including programmed cell death 1 ligand (PDL1) and its receptor (PD1), represents a breakthrough in lung cancer immunotherapy [11]. Inhibiting the PD1/PDL1 interaction is efficacious in NSCLC immunotherapy owing to immune cell effector reactivity on NSCLC [12]. Despite the promise of immunotherapy during cancer treatment [13,14], there is limited information on the immune signature of EGFR in NSCLC [15]. Furthermore, the relationship between EGFR-mediated signaling and the immune checkpoint molecules, PD1/PDL1, has not been studied in detail [16].

Herein, we investigated the altered EGFR profiles in resectable NSCLC patients and their potential role in shaping the tumor immune microenvironment to unveil the potential clinical importance of the EGFR immune signature and its association with PD1/PDL1.

Material and Methods

Clinical and The Cancer Genome Atlas (TCGA) cohort analyses of EGFR mutations

A single-center retrospective analysis was performed to assess the genetic spectrum of the EGFR in patients with resectable

NSCLC from July 2016 to November 2018 in our hospital. Enrollment criteria in our clinical cohort were: 1) age over 18 years; 2) pathological NSCLC at stage 0-III A; and 3) EGFR genetic testing performed. The Wuxi People's Hospital affiliated to Nanjing Medical University approved the study (no. HS2019013) and consent was obtained from all patients.

For The Cancer Genome Atlas (TCGA) cohort, the enrollment criteria were: 1) pathologic diagnosis confirmed as NSCLC at stage I-III A; and 2) exon 18–21 mutations in EGFR. A total of 21 hotspot mutations in EGFR in exons 18-21 in the clinical cohort were subject to mutation-based amplification (CFDA #. 3401228, AmoyDx, Xiamen, China). Based on the pre-design protocol, EGFR mutation information and corresponding patient demographic data (i.e., gender, age, stage, differentiation grade, pathological type, and smoking status) were recorded. Whole exome sequencing data (21 mutation sites in EGFR exons 18–21) and clinicopathological information were obtained from TCGA cohort (www.cbioportal.org) [17].

UCSC Xena functional genomics explorer analysis

In patients with lung squamous carcinoma (LUSC) or lung adenocarcinoma (LUAD), heat maps of EGFR copy number segments, mRNA expression, exon expression, and pan-cancer gene expression were examined by data mining in TCGA database using the UCSC Xena browser (<http://xena.ucsc.edu/>) [18]. Subsequently, Kaplan-Meier curves were plotted for overall survival (OS) and recurrence-free survival (RFS) in resectable NSCLC patients (stages I–III A). EGFR pan-cancer expression in each pathologic stage of the same patient cohort was verified.

cBioPortal for cancer genomics analyses

EGFR genetic and copy number alterations in TCGA-LUAD and TCGA-LUSC patients were examined using cBioPortal for Cancer Genomics [17]. Kaplan-Meier survival curves were used to assess the association between EGFR gene alterations and OS and progression-free survival (PFS) in LUAD and LUSC patients. The relationships between EGFR and PD1 (PD1) and CD274 (PDL1) in the same NSCLC cohort were also analyzed using cBioPortal.

Tumor Immune Estimation Resource (TIMER) analysis

Tumor Immune Estimation Resource (TIMER) quantifies the abundance of B cells, CD4 T cells, CD8 T cells, macrophages, neutrophils, and dendritic cells that infiltrate the tumor microenvironment (<https://cistrome.shinyapps.io/timer/>) [19]. EGFR expression in TCGA tumors versus healthy tissue was compared using the Exp module. The Gene module was used to correlate EGFR expression with immune cell infiltration. Changes in the rates of survival according to the levels of EGFR and immune

Table 1. Subgroup analysis for EGFR mutations in the clinical cohort.

Subgroup	Positive	Negative	P-value*	P-value**	HR (95% CI)**
Chinese cohort					
Total	331	180	151		
Age				0.505	
<64	204	108	96		
≥65	127	72	55		
Gender				0.013	0.717 (0.448–1.736)
Male	138	64	74		
Female	193	116	77		
Smoking				<0.001	0.346 (0.156–0.771)
No	259	152	107		
Yes	62	18	44		
Pathology				0.002	0.017 (12.819–104.892)
Adenocarcinoma	317	179	138		
Squamous carcinoma	12	1	11		
Differentiation				0.001	
Low differentiation	73	32	41		
Median differentiation	166	111	55		0.092 (0.413–1.154)
High differentiation	27	20	7		0.509 (0.284–1.866)
Stage				0.305	
0–I	260	140	120		
II	30	20	10		
IIIA	41	20	21		

* Univariate analysis; ** multivariate analysis. EGFR – epidermal growth factor receptor; HR – hazard ratio; CI – confidence interval.

cell infiltrates were obtained from Kaplan-Meier curves. TIMER was used to generate multivariate Cox proportional hazard models to identify the factors regulating survival. Mutation models were used to quantify the levels of immune cell infiltration according to somatic copy number alterations in EGFR according to the SCNA module.

Statistical analyses

All data were compared using SPSS 25.0 software. EGFR mutations and clinical outcomes were compared using Fisher's or χ^2 tests. A multivariate logistic model was performed for specific factors. EGFR mRNA expression was compared between LUSC and LUAD patients using Welch's t-test. Kaplan-Meier curves were used for survival analysis and compared between groups via the log-rank test. Spearman's rank correlation, Pearson's correlation, or a partial correlation were used for estimating correlations. Multivariate Cox proportional

hazard models were used to assess and analyze patient prognosis. *P*-values <0.05 were considered statistically significant (confidence intervals [CI]: 95%).

Results

Clinical and TCGA cohort analyses

Within the NSCLC patient cohort, 331 resectable cases were deemed eligible. The presence of EGFR mutations was assessed by ARMS. Patient demographics are summarized in Table 1. Male patients accounted for 41.69% (138 of 331 cases), whereas 38.37% (127 of 331 cases) were 65 years of age or older, and 18.73% (62 of 331 cases) were smokers. LUAD and LUSC accounted for 95.77% (317 of 331 cases) and 3.63% (12 of 331 cases) of the lung cancers, respectively. The percentages of patients with low, medium, and high tumor differentiation were

Table 2. Subgroup analysis for EGFR mutations in TCGA cohort.

Subgroup	Positive	Negative	P-value*	P-value**	HR (95% CI)**
TCGA cohort					
Total	83	957			
Gender			<0.001	0.001	0.386 (0.221–0.674)
Male	20	586			
Female	434	371			
Smoking			<0.001	<0.001	0.160 (0.094–0.272)
No	36	66			
Yes	909	864			
Pathology			<0.001	<0.001	8.153 (3.200–20.771)
Adenocarcinoma	586	78			
Squamous carcinoma	454	5			
Stage			0.587		
I	575	47			
II	307	21			
IIIA	158	15			

* Univariate analysis; ** multivariate analysis. EGFR – epidermal growth factor receptor; TCGA – The Cancer Genome Atlas; HR – hazard ratio; CI – confidence interval.

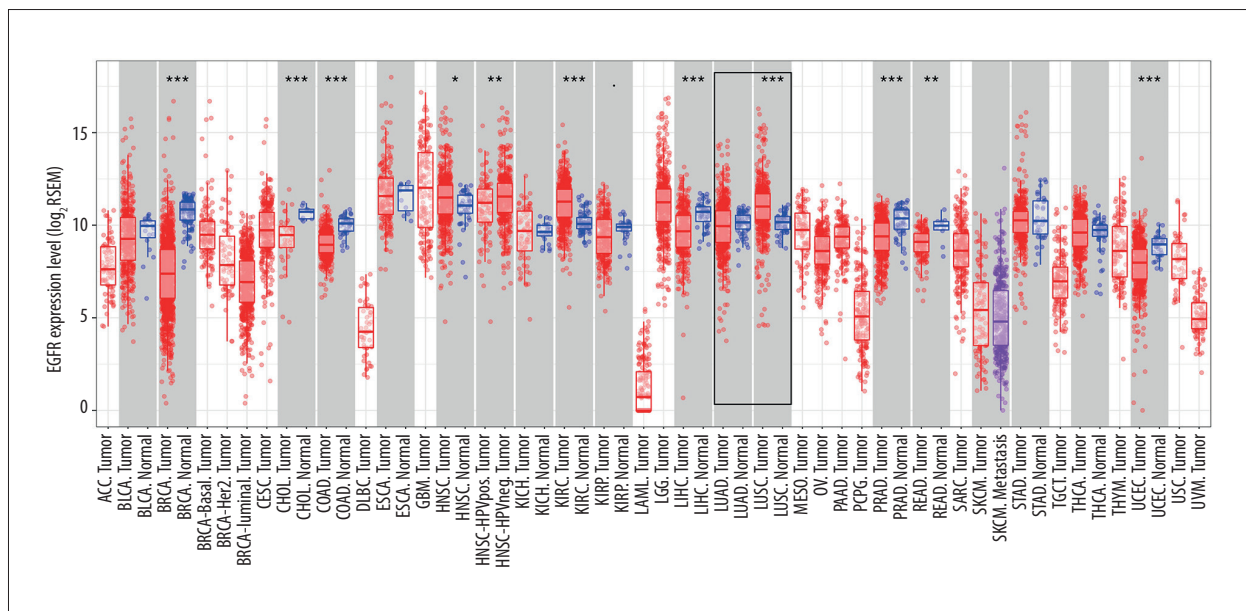


Figure 1. Epidermal growth factor receptor expression in tumors versus adjacent normal tissue. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

22.05% (73 of 331 cases), 50.15% (166 of 331 cases), and 8.16% (27 of 331 cases), respectively. The percentages of patients in stages 0–I, II, and IIIA were 78.55% (260 of 331 cases), 9.06% (30 of 331 cases), and 12.39% (41 of 331 cases), respectively. Gender, smoking, pathology, and differentiation were significantly associated with EGFR mutation frequency in the univariate analysis (Table 1, $P=0.013$, $P < 0.001$, $P < 0.002$, $P < 0.001$,

respectively). The multivariate logistic regression showed that smoking ($P=0.009$) and pathology ($P=0.017$) were 2 independent factors associated with the frequency of EGFR mutations.

From TCGA, 1040 resectable NSCLC patients were deemed eligible. Baseline demographic data are summarized in Table 2. Female patients accounted for 41.73% (434 of 1040 cases) and

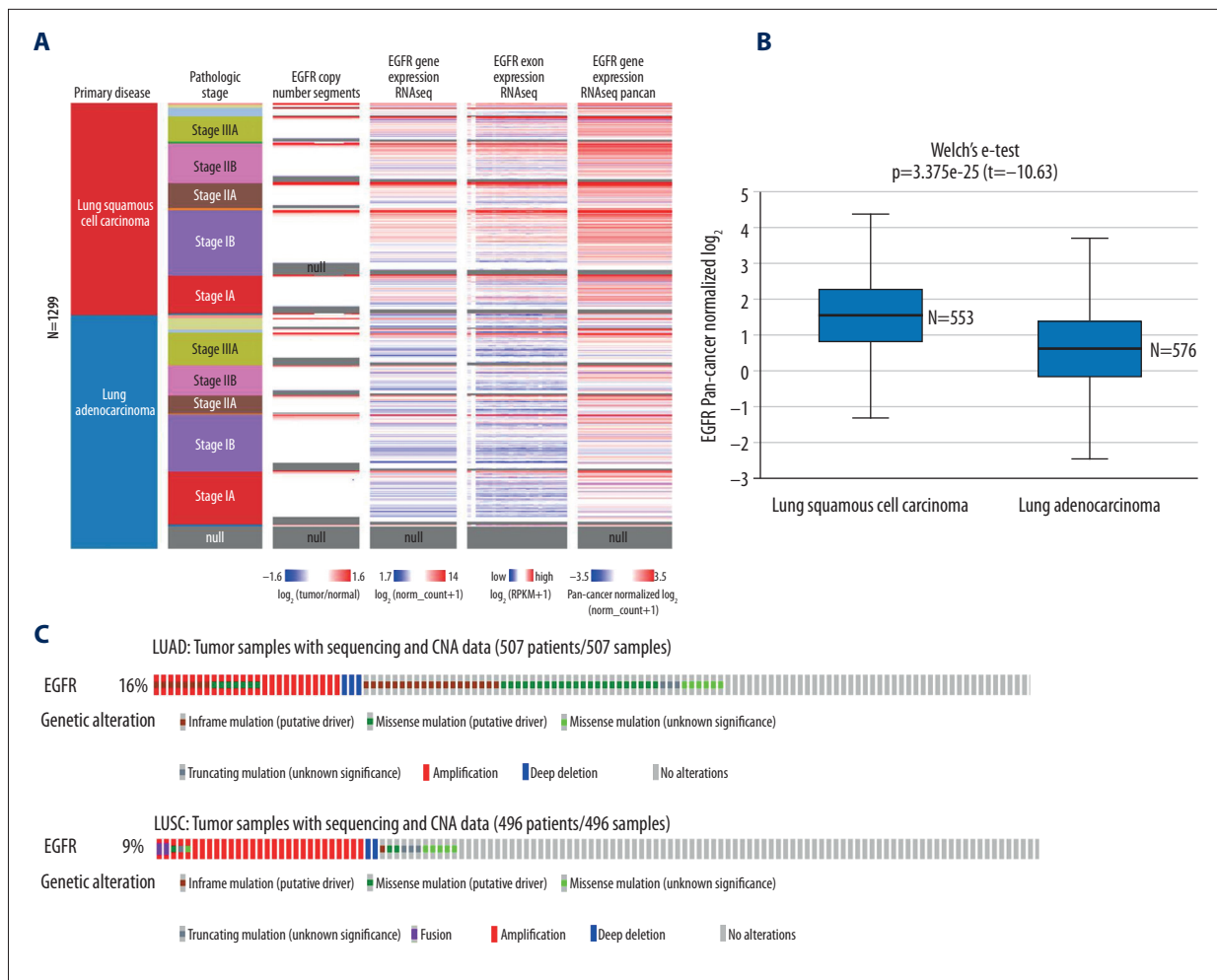


Figure 2. Epidermal growth factor receptor (EGFR) expression and copy number alterations in lung squamous carcinoma (LUSC) and lung adenocarcinoma (LUAD). **(A)** Heat maps of EGFR expression, pan-cancer expression, exon expression, and copy number segments in different tumor stages for both LUAD and LUSC. **(B)** Box plots of EGFR expression in LUAD and LUSC. **(C)** Genetic alterations of EGFR in 507 LUAD and 496 LUSC patients.

87.40% (909 of 1040 cases) were smokers. LUAD and LUSC accounted for 56.35% (586 of 1040 cases) and 43.65% (454 of 1040 cases) of the lung cancers, respectively. The percentages of patients in stages I, II, and IIIA were 55.29% (575 of 1040 cases), 29.52% (307 of 1040 cases), and 15.19% (158 of 1040 cases), respectively. The EGFR mutation frequency was 7.98% (83 of 1040 cases) in TCGA cohort. In the univariate analysis, gender, smoking, and pathology showed significant associations with the frequency of EGFR mutations (Table 2, $P < 0.001$ for all). The multivariate logistic regression showed that gender, smoking, and pathology were 3 independent factors associated with EGFR mutation frequency (Table 2, $P = 0.001$, $P < 0.001$, and $P < 0.001$, respectively).

EGFR amplification occurs in LUSC but not LUAD

EGFR expression was compared between healthy and tumor tissue in LUAD and LUSC using the DiffExp module of TIMER. In LUSC, the expression of EGFR was ~1.8-fold higher than healthy tissue and ~0.869-fold higher than LUAD (Figure 1). We extracted data for the EGFR copy number segments, gene expression, exon expression, and pan-cancer gene expression in TCGA-LUSC and TCGA-LUAD. Significantly higher EGFR expression was evident in LUSC versus LUAD (Figure 2A, 2B).

Figure 2C shows that EGFR mutations occurred in ~16% and ~9% of LUAD and LUSC cases, respectively. EGFR amplification was predominant in both cancer types as revealed from the EGFR copy numbers (Figure 3A, 3B).

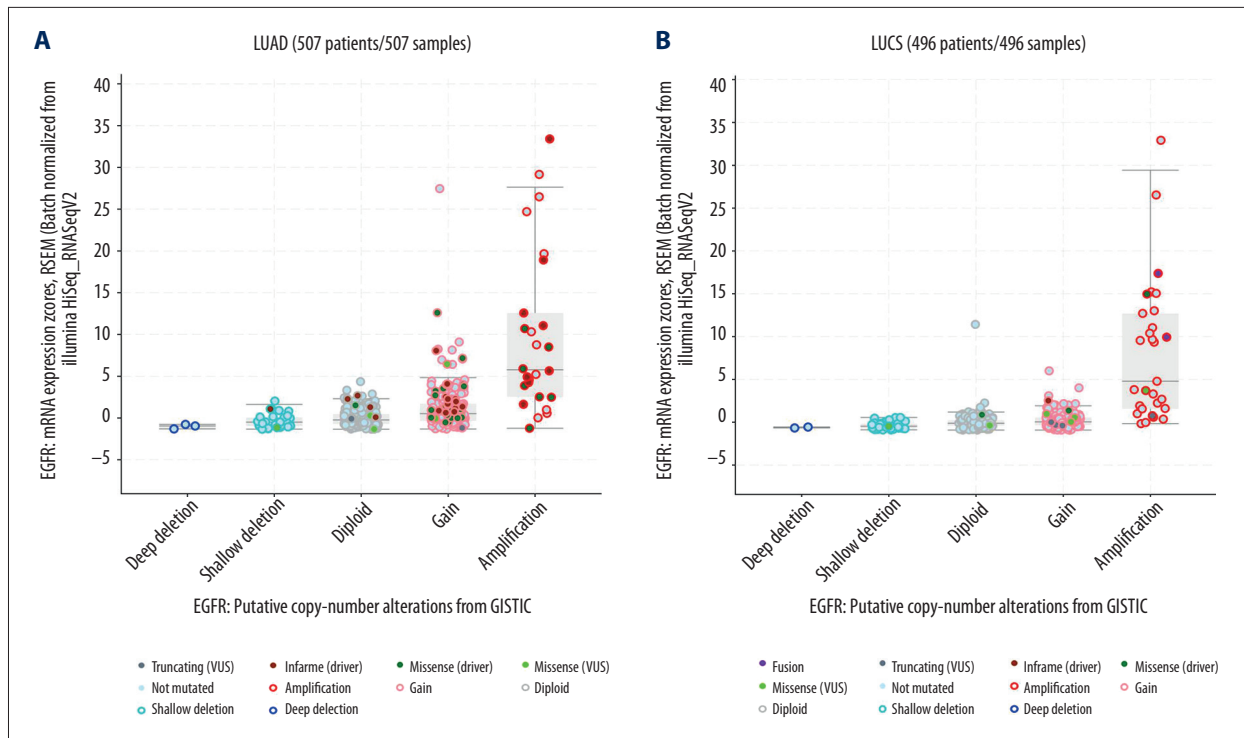


Figure 3. Box plots of epidermal growth factor receptor expression in (A) lung adenocarcinoma (LUAD) and (B) lung squamous cell carcinoma (LUSC) based on the genetic status for LUAD and LUSC according to The Cancer Genome Atlas.

Elevated EGFR levels do not correlate with poor OS or RFS in resectable LUAD or LUSC patients

Figure 4A shows that EGFR mRNA expression was unaffected by the pathological stage in resectable NSCLC (stages I–IIIA). Further analyses showed the same trends in both resectable LUAD and LUSC (Figure 4D, 4G). EGFR expression was not related to OS or RFS (Figure 4B, 4C). Subgroup analyses of LUAD (Figure 4E, 4F) and LUSC (Figure 4H, 4I) revealed no evidence that EGFR influenced OS or RFS.

EGFR gene alterations are associated with worse OS in LUAD

LUAD patients with EGFR gene alterations had significantly worse OS (Figure 5A; $P < 0.01$). However, there was no association of EGFR alterations with PFS in LUAD patients (Figure 5B). By contrast, EGFR gene alterations did not prominently affect OS or PFS in LUSC patients (Figure 5C, 5D).

EGFR expression and its relationship to immune signatures

Kaplan-Meier assessments of EGFR expression and immune cell infiltrates were compared to determine their contribution to the survival outcomes. In LUAD patients, infiltrates of B and dendritic cells were significantly associated with prognosis. However, in LUSC patients, no survival difference related to the

6 immune cell infiltrates was observed (Figure 6). Pathologic stage II (hazard ratio [HR]: 2.309, 95% CI: 1.493–3.573, $P < 0.001$) and stage III (HR: 2.562, 95% CI: 1.627–4.036, $P < 0.001$) were independent prognostic factors for poor survival in LUAD as assessed through multivariate Cox proportional hazard models. However, LUAD patients who exhibited higher B cell infiltrates had better outcomes (HR: 0.040, 95% CI: 0.002–0.668, $P = 0.025$). In LUSC patients, the multivariate analysis indicated that being male (HR: 1.725, 95% CI: 1.149–2.591, $P = 0.009$) and pathologic stage III (HR: 1.884, 95% CI: 1.214–2.923, $P = 0.005$) significantly affected survival.

We confirmed the positive correlations by analyzing lung cancer patient data in the TIMER database. The levels of EGFR expression in LUAD patients correlated with the infiltration of all immune cells assessed (Figure 7). However, EGFR expression was negatively associated with the infiltration of B and CD8+ T cells in LUSC patients. These findings suggested that EGFR is closely related to immune cells in lung cancer.

In LUAD patients, those with mutated EGFR had significantly higher infiltration of B and dendritic cells (Figure 8). This implied that the immune response to LUAD in mutated and wild-type patients was disparate. Data were not available for LUSC patients in TIMER.

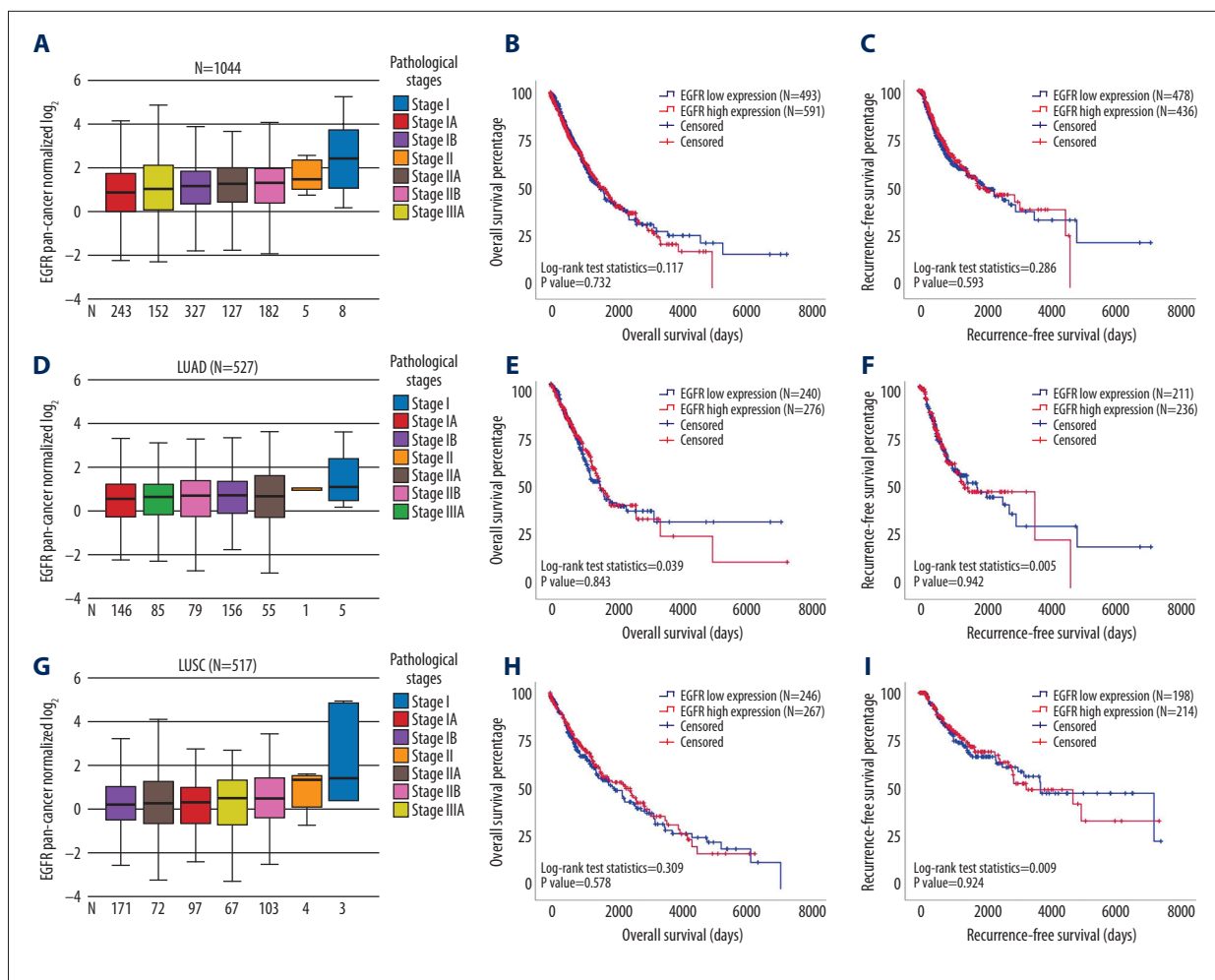


Figure 4. Epidermal growth factor receptor (EGFR) expression and survival in stage I-IIIa resectable non-small cell lung cancer (NSCLC). EGFR expression in the different pathological stages of (A) NSCLC, (D) lung adenocarcinoma (LUAD), and (G) lung squamous cell carcinoma (LUSC). EGFR expression and its association to overall survival (OS) and recurrence-free survival (RFS) in total NSCLC (B, C), LUAD (E, F), and LUSC (H, I) patients.

In different copy number subsets of EGFR mutations, the immune cell infiltration level decreased in deep deletion, arm-level deletion, arm-level gain, and high amplification mutations, as compared to diploid/normal, in both LUAD and LUSC patients (Figure 9). Further correlation analyses showed that EGFR expression was significantly positively correlated with CD274 (PDL1) in LUAD (Figure 10A; $r=0.27$, $P<0.001$), but was not related with PDCD1 (PD1) (Figure 10B). In LUSC patients, there was a significant negative correlation between EGFR and PDCD1 (PD1) ($r=-0.19$, $P=0.002$, Figure 10D). An inverse correlation of EGFR and CD274 (PDL1) was not observed (Figure 10C). These findings indicate that EGFR might be closely involved in the effect of immunotherapies targeting PD-1 and PD-L1 in lung cancer.

Discussion

Currently, the usage of EGFR TKIs in resectable NSCLC for neo-adjuvant and adjuvant therapy is considered of great clinical value [20–22]. In this study, clinical and TCGA cohorts were used to assess EGFR mutation profiles and their clinicopathological significance. A larger number of EGFR mutations were observed in the clinical compared to TCGA cohort. Tumor immune cell infiltrates in LUAD and LUSC were different, and EGFR mutations may cause the decline or lack of immune infiltration in the NSCLC microenvironment.

Compared with TCGA cohort, a larger number of EGFR mutations occurred in clinical samples. Both cohorts highlighted the relationship between smoking and pathology for EGFR mutations. In a previous study, Liu et al. also found the occurrence rate was higher in the smoking and pathology subgroups,

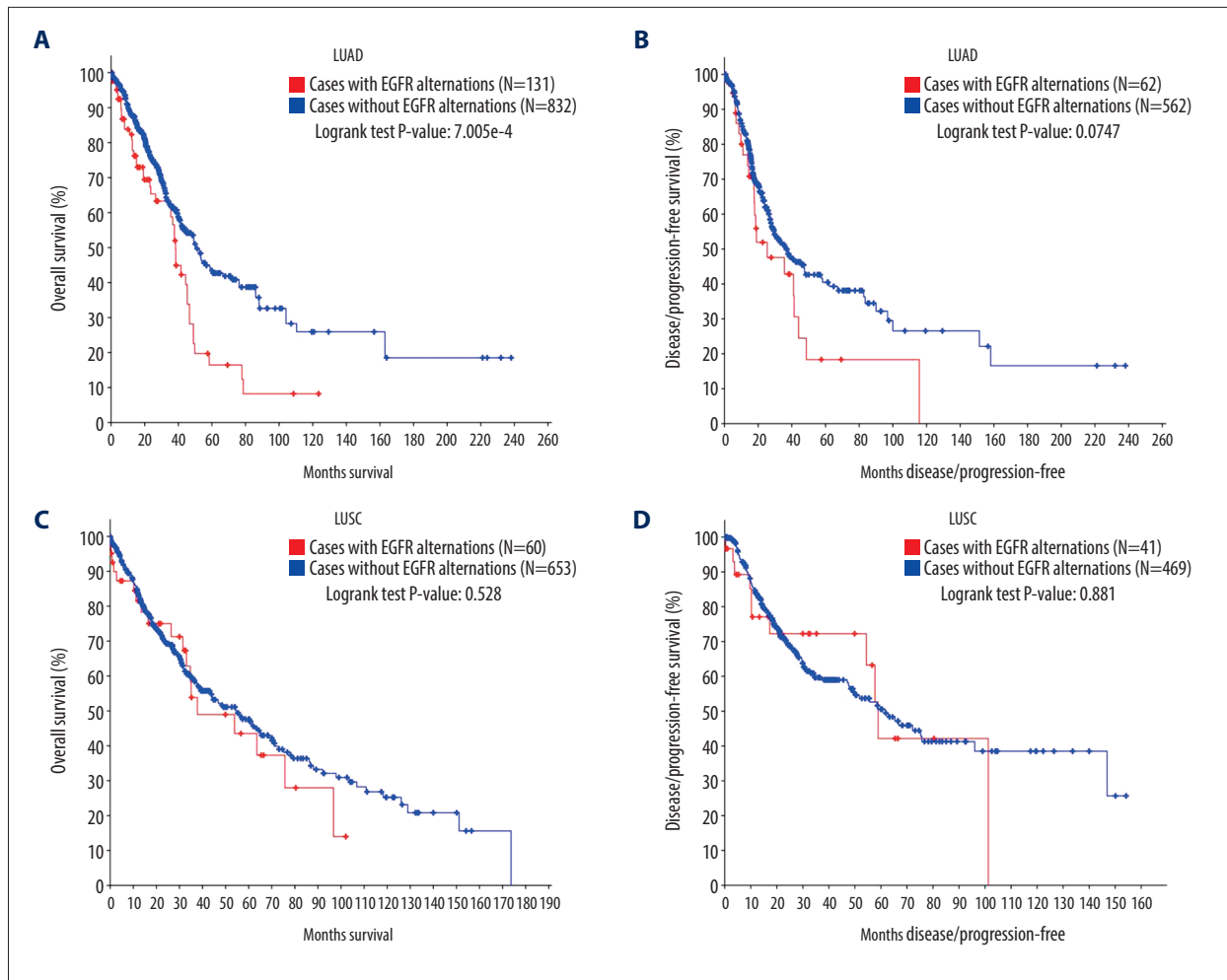


Figure 5. Association of epidermal growth factor receptor (EGFR) mutations and overall survival (OS) and progression-free survival (PFS) in lung adenocarcinoma (LUAD) (A, B) and lung squamous cell carcinoma (LUSC) (C, D) patients.

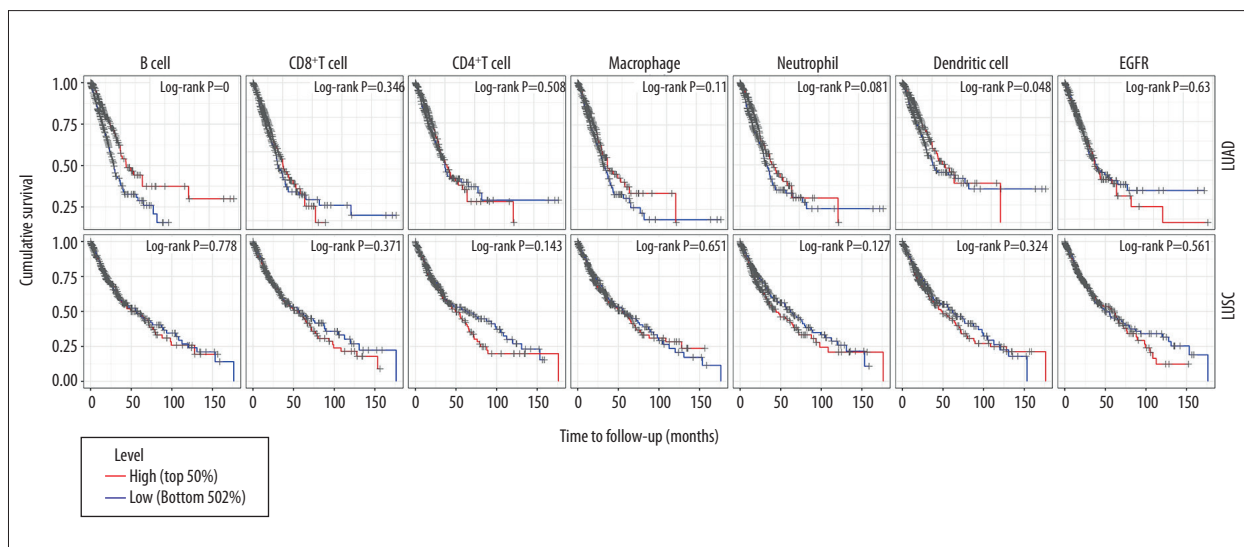


Figure 6. Kaplan-Meier curves of 6 subsets of immune cell infiltrates and epidermal growth factor receptor (EGFR) expression in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) patients.

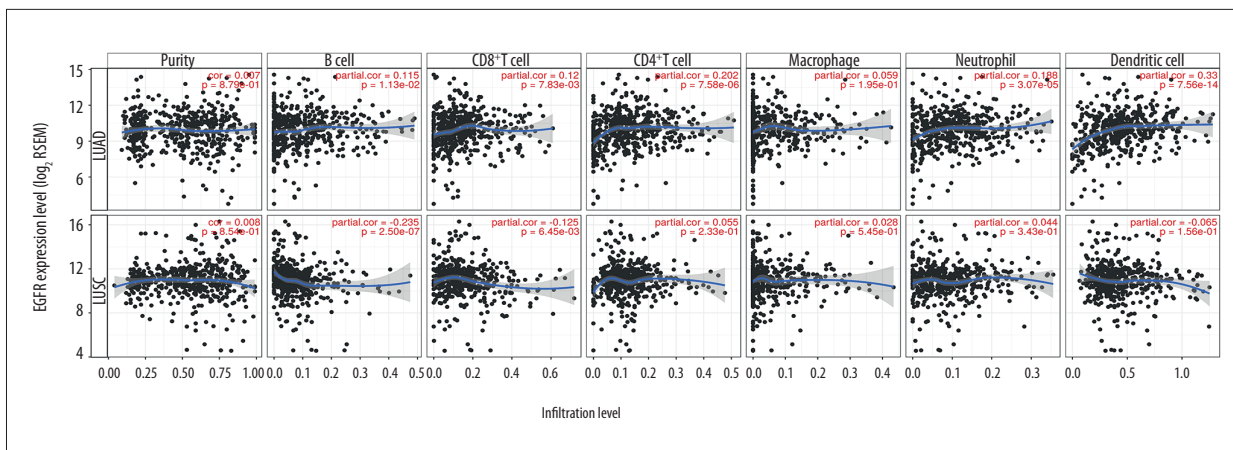


Figure 7. Relationship between epidermal growth factor receptor (EGFR) expression and the different subsets of immune cell infiltrates in lung squamous carcinoma (LUSC) and lung adenocarcinoma (LUAD) patients using the TIMER database.

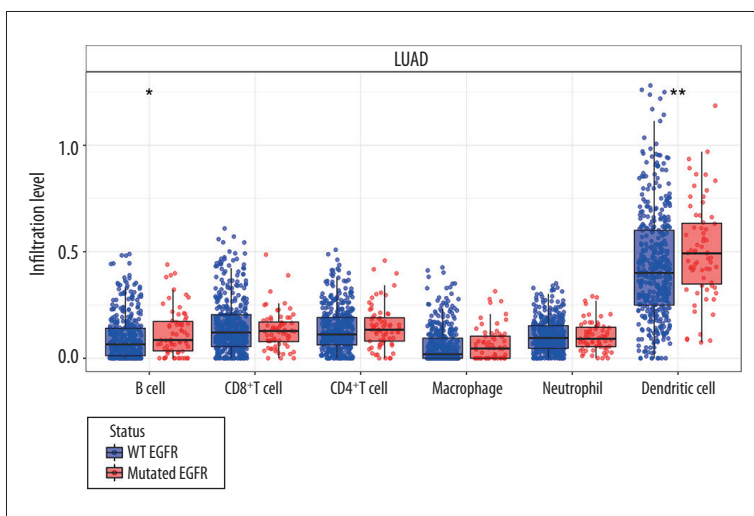


Figure 8. Infiltration levels of 6 subsets of immune cells in mutant versus wild-type epidermal growth factor receptor (EGFR) patients. * $P < 0.05$, ** $P < 0.01$.

confirming our findings [23]. In our study, the EGFR mutation frequency in the clinical and TCGA cohorts had no relation with disease stage, indicating that EGFR mutations are likely to occur during the early stages of NSCLC development.

In the current study, we found that EGFR expression was remarkably increased in LUSC compared with adjacent normal tissues. However, EGFR expression had no prognostic ability on OS or PFS in resectable LUAD and LUSC patients based on a Kaplan-Meier survival analysis. Additionally, EGFR alterations in LUAD had an unfavorable influence on OS, but were not associated with changes in PFS. Meanwhile, no significant relationship in LUSC was found between EGFR alterations and OS or PFS. These results suggested that EGFR alterations might be a vital mechanism involved in long-term survival of LUAD patients.

Tumor-infiltrating immune cells are a key point of the anti-cancer effect in the immune system, and are involved in the

response to therapy and cancer escape mechanisms [24,25]. Nevertheless, it remains challenging to confirm the association of immune cell infiltrates with tumor cells owing to the complexity of cancer genomics and the indeterminate immune system of patients. Cytolytic activity regulates the immune response to cancer and its treatment. This represents immunogenicity and acts as a predictor of the response to checkpoint blockade [26].

We speculated that immune evasion or dysfunction of tumor-infiltrating immune cells may be factors for inducing tumor progression and predicting outcomes. In untreated early-stage NSCLC, the immune microenvironment imposes a strong selection pressure on tumor evolution, producing complex mechanisms of immune evasion, namely neoantigen-directed immune escape, which might forecast decreased disease-free survival times [27]. Immune evasion before tumor invasion is also evident in early LUSC, and is associated with the dynamic evolution of pre-invasive bronchial cells and the relevant immune

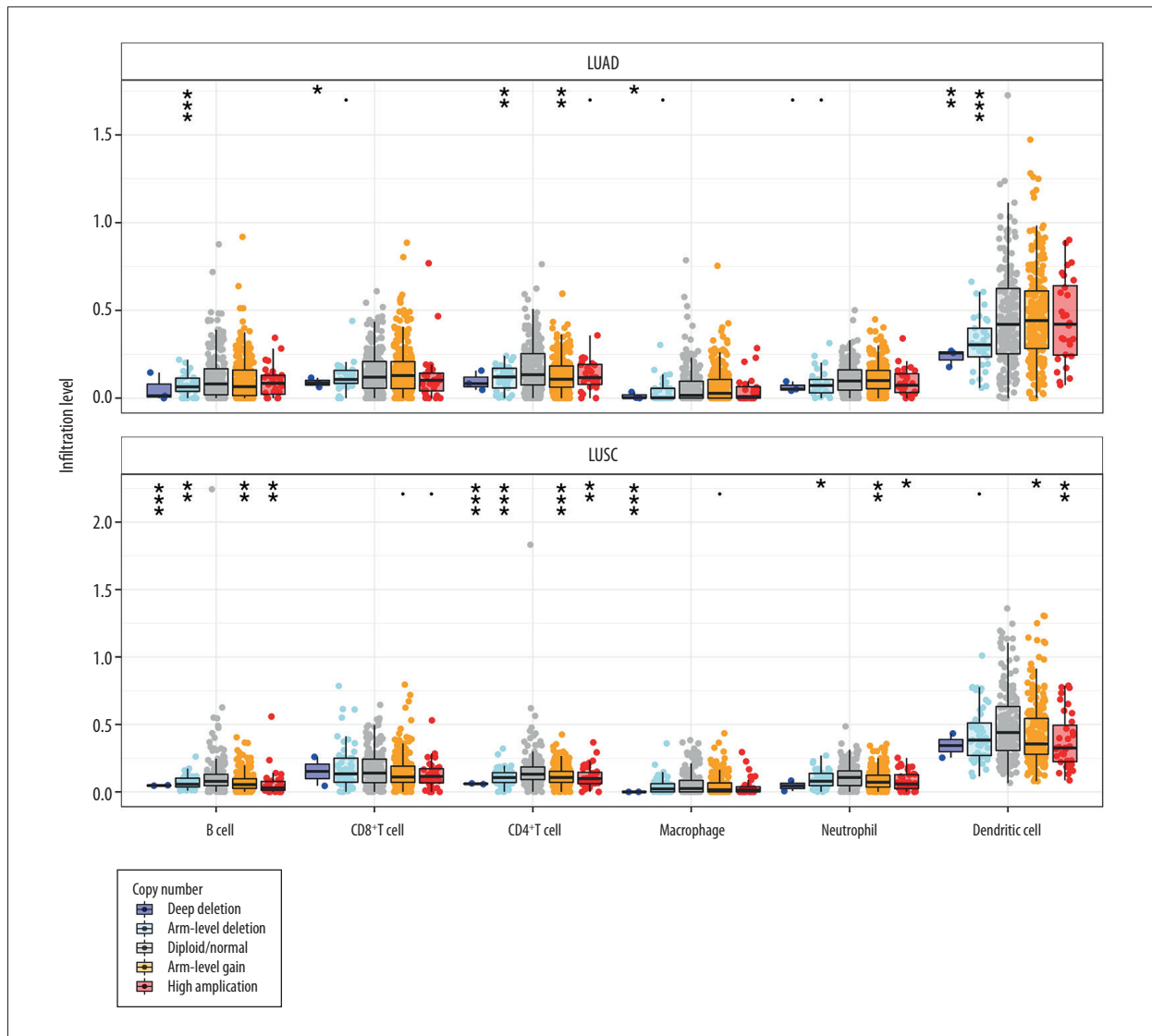


Figure 9. Tumor immune cell infiltrates in those with disparate epidermal growth factor receptor (EGFR) copy numbers. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, • $P<0.1$.

response [28]. A lack of infiltrating immune cells in premalignant lesions could produce tumor progression to LUSC owing to corresponding transcriptomic alterations [29]. Meanwhile, we identified different immune cell infiltrates in LUAD and LUSC and found that lower levels of infiltrating B and dendritic cells predicted poor prognosis in LUAD. Conversely, the level of immune cell infiltrates was not related to a significant survival disparity in LUSC.

The EGFR provides immuno-competence in lung cancer. In our study, positive associations in LUAD with EGFR expression were observed for the infiltration level of B cells, CD8+ T cells, CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells. However, we found a negative relationship between the infiltration of B and CD8+ T cells with EGFR expression in LUSC, in accordance

with a previous study [30]. This trend was also demonstrated in B and dendritic cells of patients with mutated compared to wild-type EGFR (Figure 9), although the relationship did not reach statistical significance for CD4 T cells, CD8 T cells, macrophages, and neutrophils. In addition, to determine the role of copy number alterations of EGFR in the immune cell infiltration level in LUAD and LUSC patients, further analyses revealed that the levels in deep deletion, arm-level deletion, arm-level gain, and high amplification mutation subtypes were decreased compared to diploid/normal. Overall, these findings indicate that mutations of EGFR might induce a decline or lack of immune cell infiltration in the tumor microenvironment, reducing the anticancer effect of immune cells.

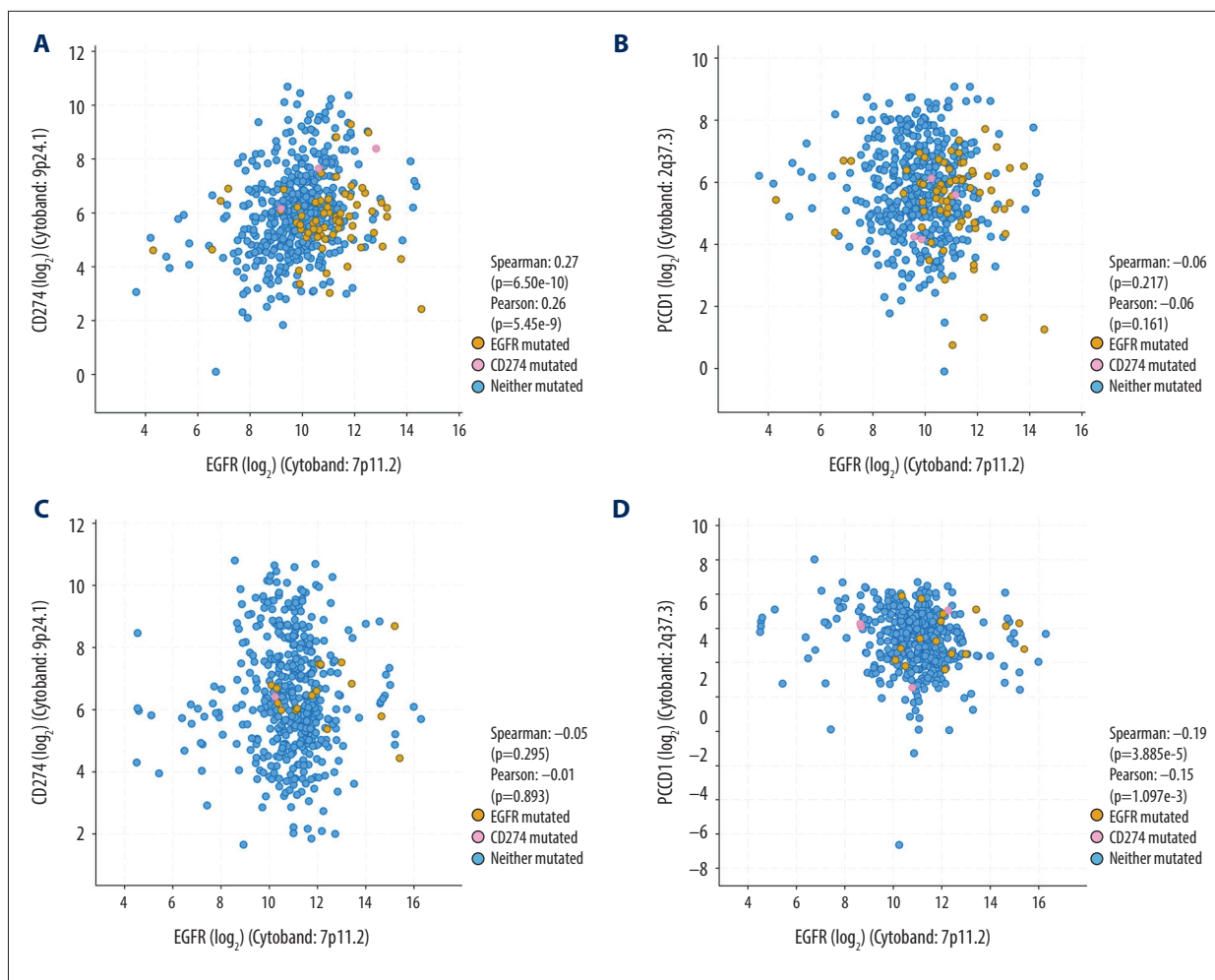


Figure 10. Correlation between epidermal growth factor receptor (EGFR) and programmed cell death 1 ligand (PDL1) or its receptor PD1. **(A)** EGFR with CD274 (PDL1) in lung adenocarcinoma (LUAD) patients. **(B)** EGFR with PDCD1 (PD1) in LUAD patients. **(C)** Correlation of EGFR and CD274 (PDL1) in lung squamous cell carcinoma (LUSC) patients. **(D)** Correlation of EGFR and PDCD1 (PD1) in LUSC patients. Analyses were performed using cBioPortal for Cancer Genomics using The Cancer Genome Atlas LUAD and LUSC data.

The PDL1/PD1 pathway plays essential roles in the immune evasion of tumor cells, and is a key immune co-inhibitory pathway for NSCLC immunotherapy [31]. Through correlation analyses, we observed that EGFR expression was positively correlated with PDL1 expression in LUAD, and negatively associated with PD1 in LUSC. However, the correlation between PDL1/PD1 expression and EGFR expression/mutation remains controversial. Azuma et al. highlighted the association of elevated PDL1 levels with EGFR mutations by immunohistochemistry in 164 surgically resected NSCLC specimens [32]. Similarly, Gatalica et al. showed that PDL1 positivity was related to the occurrence of EGFR mutations [33]. Ji et al. highlighted a negative correlation between PDL1 and EGFR mutational status in 100 resected patients with primary LUAD, with higher rates of mutations occurring in those with low levels of PDL1 expression [34]. This was confirmed by a meta-analysis showing that wild-type EGFR

NSCLC was more likely to be PDL1-positive compared to mutant EGFR NSCLC [35].

TKIs inhibiting EGFR activity can reduce PDL1 expression by inhibiting NF- κ B in EGFR mutant NSCLC [36]. Furthermore, NSCLC tumors can evade the immune response by upregulating PDL1 via EGFR activation [37]. Conversely, Mu et al. reported no significant correlation between PD-L1 and EGFR expression in stage I NSCLC patients [38]. Additionally, Zhang et al. found no significant relationship between PDL1 and EGFR expression in LUAD [39]. The expression of PDL1 was associated with the levels of wild-type EGFR, but no significant changes in PDL1 expression were observed according to EGFR mutational status [40].

First-line treatment with pembrolizumab (which blocks PD1) was ineffective in 10 patients with advanced NSCLC with mutated EGFR and positive PDL1 who did not receive TKI therapy in a phase II clinical trial [41]. Second-line therapy with immune checkpoint inhibitors did not improve survival over chemotherapy in EGFR-mutant advanced NSCLC [42]. High PDL1 expression correlated with poor responses to TKI therapy in those harboring EGFR mutations [43], whilst higher PDL1 expression was associated with TKI resistance in advanced LUAD patients harboring EGFR mutations [44]. Despite the poor efficacy of TKI therapy in those with high PDL1 expression upon first-line treatment, the efficacy improved in third generation treatment and appeared unaffected by PDL1 expression. Given these findings, EGFR mutant and PDL1-positive patients should receive TKIs targeting the EGFR as a first-line therapy.

There are several limitations in our study. Firstly, as a retrospective study, the interpretation of the data and the conclusions may be limited to a certain extent. PDL1 immunohistochemical analysis was not performed in the clinical cohort, and the correlation of PDL1 and EGFR mutations in resectable NSCLC must be performed. Secondly, this was a single center retrospective study. A multiple center study would provide more compelling clinical judgements. In addition, experiments were not conducted in our patients to detect the tumor immune cell infiltration level and immunity signature. Thus, the results of

our study should be evaluated carefully, and additional research on the current topic is recommended.

Conclusions

This study demonstrated that EGFR mutations are frequent in resectable lung cancer and contribute to the long-term survival outcomes of LUAD patients. The levels of tumor immune cell infiltrates in LUAD and LUSC differed and, in part, were significantly related to EGFR expression. EGFR mutations decrease immune cell infiltration in the tumor microenvironment, decreasing the anticancer effect of immune cells. The correlation between PDL1/PD1 expression and EGFR expression/mutation must now be investigated to elucidate whether combination therapy with EGFR TKIs and immune checkpoint blockers is beneficial for subsets of NSCLC patients.

Acknowledgements

We thank EdiTar Bio-Tech Ltd. (Nanking, China) for language editing.

Conflict of interests

None.

References:

1. Chen W, Zheng R, Baade PD et al: Cancer statistics in China, 2015. *Cancer J Clin*, 2016; 66(2): 115–32
2. Zeng H, Zheng R, Guo Y et al: Cancer survival in China, 2003–2005: A population-based study. *Int J Cancer*, 2015; 136(8): 1921–30
3. Park K, Tan EH, O'Byrne K et al: Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): A phase 2B, open-label, randomised controlled trial. *Lancet Oncol*, 2016; 17(5): 577–89
4. Solomon BJ, Mok T, Kim DW et al., PROFILE 1014 Investigators: First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*, 2014; 371(23): 2167–77
5. Shaw AT, Ou SH, Bang YJ et al: Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med*, 2014; 371(21): 1963–71
6. Sun X, Xu Y, Wang Y et al: Synergistic inhibition of thalidomide and icotinib on human non-small cell lung carcinomas through ERK and AKT signaling. *Med Sci Monit*, 2018; 24: 3193–203
7. Hirsch FR, Scagliotti GV, Mulshine JL et al: Lung cancer: Current therapies and new targeted treatments. *Lancet*, 2017; 389(10066): 299–311
8. Wu YL, Cheng Y, Zhou X et al: Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHEER 1050): A randomised, open-label, phase 3 trial. *Lancet Oncol*, 2017; 18(11): 1454–66
9. Ning Y, Bao M, Yan X et al: Surgery for advanced non-small cell lung cancer patient after epidermal growth factor receptor tyrosine kinase inhibitor neoadjuvant therapy. *Ann Transl Med*, 2018; 6(20): 407
10. Zhong WZ, Wang Q, Mao WM et al., ADJUVANT investigators: Gefitinib versus vinorelbine plus cisplatin as adjuvant treatment for stage II-IIIa (N1-N2) EGFR-mutant NSCLC (ADJUVANT/CTONG1104): A randomised, open-label, phase 3 study. *Lancet Oncol*, 2018; 19(1): 139–48
11. Intlekofer AM, Thompson CB: At the bench: preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy. *J Leukoc Biol*, 2013; 94(1): 25–29
12. Brahmer JR, Tykodi SS, Chow LQ et al: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*, 2012; 366(26): 2455–65
13. Wang S, Lan F, Xia Y: LncRA ANCR Inhibits non-small cell lung cancer cell migration and invasion by inactivating TGF-beta pathway. *Med Sci Monit*, 2018; 24: 6002–9
14. Li P, Zhang Z, Zhang F et al: Effects of 3-tetrazolyl methyl-3-hydroxy-oxindole hybrid (THOH) on cell proliferation, apoptosis, and G2/M Cell cycle arrest occurs by targeting platelet-derived growth factor D (PDGF-D) and the MEK/ERK signaling pathway in human lung cell lines SK-LU-1, A549, and A-427. *Med Sci Monit*, 2018; 24: 4547–54
15. Sasada T, Azuma K, Ohtake J, Fujimoto Y: Immune responses to epidermal growth factor receptor (EGFR) and their application for cancer treatment. *Front Pharmacol*, 2016; 7: 405
16. Ji M, Liu Y, Li Q et al: PD-1/PD-L1 pathway in non-small-cell lung cancer and its relation with EGFR mutation. *J Transl Med*, 2015; 13: 5
17. Gao J, Aksoy BA, Dogrusoz U et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*, 2013; 6(269): pl1
18. Cline MS, Craft B, Swatloski T et al: Exploring TCGA pan-cancer data at the UCSC Cancer Genomics Browser. *Sci Rep*, 2013; 3: 2652
19. Li T, Fan J, Wang B et al: TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res*, 2017; 77(21): e108–10
20. Xiong L, Li R, Sun J et al: Erlotinib as neoadjuvant therapy in stage IIIa (N2) EGFR mutation-positive non-small cell lung cancer: A prospective, single-arm, phase II study. *The Oncologist*, 2019; 24(2): 157–64
21. Nagasaka M, Gadgeel SM: Role of chemotherapy and targeted therapy in early-stage non-small cell lung cancer. *Expert Rev Anticancer Ther*, 2018; 18(1): 63–70

22. Ahn HK, Choi YL, Han JH et al: Epidermal growth factor receptor mutation and treatment outcome of mediastinoscopic N2 positive non-small cell lung cancer patients treated with neoadjuvant chemoradiotherapy followed by surgery. *Lung Cancer*, 2013; 79(3): 300–6
23. Liu HP, Isaac Wu HD, Chang JW et al: Prognostic implications of epidermal growth factor receptor and KRAS gene mutations and epidermal growth factor receptor gene copy numbers in patients with surgically resectable non-small cell lung cancer in Taiwan. *J Thorac Oncol*, 2010; 5(8): 1175–84
24. Yu X, Wang X: Tumor immunity landscape in non-small cell lung cancer. *Peer J*, 2018; 6: e4546
25. Lim WA, June CH: The principles of engineering immune cells to treat cancer. *Cell*, 2017; 168(4): 724–40
26. Charoentong P, Finotello F, Angelova M et al: Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep*, 2017; 18(1): 248–62
27. Rosenthal R, Cadieux EL, Salgado R et al, TRACERx consortium: Neoantigen-directed immune escape in lung cancer evolution. *Nature*, 2019; 567(7749): 479–85
28. Mascaux C, Angelova M, Vasaturo A et al: Immune evasion before tumour invasion in early lung squamous carcinogenesis. *Nature*, 2019; 571(7766): 570–75
29. Beane JE, Mazzilli SA, Campbell JD et al: Molecular subtyping reveals immune alterations associated with progression of bronchial premalignant lesions. *Nat Commun*, 2019; 10(1): 1856
30. Zhang XC, Wang J, Shao GG et al: Comprehensive genomic and immunological characterization of Chinese non-small cell lung cancer patients. *Nat Commun*, 2019; 10(1): 1772
31. Butte MJ, Keir ME, Phamduy TB et al: Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity*, 2007; 27(1): 111–22
32. Azuma K, Ota K, Kawahara A et al: Association of PD-L1 overexpression with activating EGFR mutations in surgically resected non small-cell lung cancer. *Ann Oncol*, 2014; 25(10): 1935–40
33. Gatalica Z, Snyder C, Maney T et al: Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. *Cancer Epidemiol Biomarker Prev*, 2014; 23(12): 2965–70
34. Ji M, Liu Y, Li Q et al: PD-1/PD-L1 expression in non-small-cell lung cancer and its correlation with EGFR/KRAS mutations. *Cancer Biol Ther*, 2016; 17(4): 407–13
35. Soo RA, Lim SM, Syn NL et al: Immune checkpoint inhibitors in epidermal growth factor receptor mutant non-small cell lung cancer: Current controversies and future directions. *Lung Cancer*, 2018; 11: 12–20
36. Lin K, Cheng J, Yang T et al: EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-kappaB. *Biochem Biophys Res Commun*, 2015; 463(1–2): 95–101
37. Akbay EA, Koyama S, Carretero J et al: Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov*, 2013; 3(12): 1355–63
38. Mu CY, Huang JA, Chen Y et al: High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. *Med Oncol*, 2011; 28(3): 682–88
39. Zhang Y, Wang L, Li Y et al: Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. *Onco Targets Ther*, 2014; 7: 567–73
40. Takada K, Toyokawa G, Tagawa T et al: PD-L1 expression according to the EGFR status in primary lung adenocarcinoma. *Lung Cancer*, 2018; 116: 1–6
41. Lisberg A, Cummings A, Goldman JW et al: A phase II study of pembrolizumab in EGFR-Mutant, PD-L1+, tyrosine kinase inhibitor naive patients with advanced NSCLC. *J Thorac Oncol*, 2018; 13(8): 1138–45
42. Lee CK, Man J, Lord S et al: Checkpoint inhibitors in metastatic EGFR-mutated non-small cell lung cancer—a meta-analysis. *J Thorac Oncol*, 2017; 12(2): 403–7
43. Su S, Dong ZY, Xie Z et al: Strong programmed death ligand 1 expression predicts poor response and *de novo* resistance to EGFR tyrosine kinase inhibitors among NSCLC patients with EGFR mutation. *J Thorac Oncol*, 2018; 13(11): 1668–75
44. Hsu KH, Huang YH, Tseng JS et al: High PD-L1 expression correlates with primary resistance to EGFR-TKIs in treatment naive advanced EGFR-mutant lung adenocarcinoma patients. *Lung Cancer*, 2019; 127: 37–43