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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Prognostic Effect of *TP53* and *PKD* Co-Mutations in Patients with Resected Epidermal Growth Factor Receptor-Mutated Lung Adenocarcinoma

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ABSTRACT

Background. The impact of specific co-mutations in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma is unclear.

Methods. Tissues from 147 consecutive patients with resected EGFR-mutated lung adenocarcinomas treated at Sun Yat-Sen University Cancer Center were analyzed by next-generation sequencing (NGS). Associations between mutation status, patient baseline characteristics, and survival outcomes (disease-free survival [DFS] and overall survival [OS]) after surgical resection were analyzed.

Results. TP53 and protein kinase D (PKD) mutations were the two most frequently observed co-mutations in this cohort. Dual PKD/EGFR and TP53/EGFR mutations were found in 39 (27%) and 72 patients (49%), respectively, with dual TP53/EGFR mutations more commonly observed in male patients ($P = 0.021$). Both TP53 (hazard ratio [HR] 2.08, 95% confidence interval [CI] 1.23–3.54, $P = 0.007$) and PKD co-mutations (HR 1.72, 95% CI 1.01–2.93, $P = 0.044$) were associated with shorter DFS, but not OS, in univariate analysis. In multivariate analysis, patients harboring PKD/TP53 co-mutations had shorter DFS compared with $PKD^{-}/TP53^{-}$ cases (HR 2.49, 95% CI 1.15–5.37, $P = 0.02$). In a subgroup of never-smokers, TP53 co-mutations were associated with significantly worse OS (HR 50.11, 95% CI 2.39–1049.83, $P = 0.012$).

Conclusion. TP53 and PKD mutations were the two most frequently observed co-mutations in resected EGFR-mutated lung adenocarcinoma. Both mutations were associated with poorer prognoses in affected patients.

Lung cancer is one of the most complex forms of cancer, particularly with regard to the underlying molecular and genetic aspects of the disease.[1] Although many of the genetic differences may not activate downstream pathways at the protein level, these mutations are still associated with driver genes that have prognostic importance in lung cancer. Since 2004, epidermal growth factor receptor (EGFR) mutations have received considerable attention for their role in disease pathogenesis,[2] and are now recognized as the most important genetic marker when determining therapeutic options for advanced lung adenocarcinoma. However, the response to EGFR–tyrosine kinase inhibitors (TKIs) varies considerably, even among patients with drug-sensitive EGFR mutations.[3]

One potential explanation for the diversity in treatment response is the possibility of additional mutations in patients harboring EGFR mutations, which may lead to alternate pathway activation and eventually cause drug insufficiency. For example, TP53 is reported to be the most frequently co-mutated gene in adenocarcinoma.[1] When compared with tumors harboring a single mutation, multiple somatic mutations have been shown to be associated with poorer overall survival (OS) and/or disease-free survival (DFS) in resected stage I–III NSCLC.[4] Present

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studies suggest that concurrent EGFR and TP53 mutation may be associated with resistance to EGFR TKIs and chemotherapy, resulting in poorer prognosis.[5–8]

To identify correlations and prognostic value of EGFR, TP53, and other co-mutations in surgically resected stage I–III adenocarcinomas, we studied the clinical outcomes of patients with EGFR-mutated adenocarcinoma, based on assessment for co-mutational status by next-generation sequencing (NGS).

MATERIALS AND METHODS

Patients and Tissues

From 2011 to 2015, 147 consecutive stage I–III EGFR-mutated lung adenocarcinoma patients from the Sun Yat-Sen University Cancer Center, Guangzhou, China, who underwent anatomic resection with systematic lymph node dissection were retrieved from the hospital database. Seven patients underwent mediastinoscopy, two of whom confirmed cN2 after lymph node biopsy. Five patients were proved to be cN2 by endobronchial ultrasound-guided transbronchial needle aspiration preoperatively. In sum, these seven patients underwent two to four courses of cisplatin-based neoadjuvant chemotherapy prior to operation. Additionally, 17/117 patients (14.5%) with N2 disease were considered clinical N0 preoperatively. In total, 78 patients underwent adjuvant therapy after surgery, 22 of whom received first-generation EGFR TKIs. The remaining 56 patients were treated with adjuvant chemotherapy, 9 of whom received adjuvant radiotherapy.

The collected formalin-fixed paraffin-embedded (FFPE) samples were sent to the core facility of Nanjing Geneseeq Technology Inc. (Nanjing, China) for genetic testing using a custom xGen lockdown probe panel (Integrated DNA Technologies, Coralville, IA, USA) consisting of 416 predefined genes. Library construction was performed according to the manufacturer's instructions, and sequenced on a HiSeq 4000 (Illumina, San Diego, CA, USA) to coverage depths of at least 300 × for FFPE samples after removing polymerase chain reaction (PCR) duplicates. The study protocol was approved by the Institutional Review Board and written consent for tissue analysis was obtained from each patient preoperatively.

Statistical Analysis

OS was defined as the time of surgery to death, while DFS was defined as the interval between resection and first recurrence. Patients without an event were censored at the end of follow-up. Categorical variables were analyzed using the Chi square test or Fisher's exact test, as appropriate, and continuous data were analyzed using Student's *t* test. Completeness of follow-up was measured by modification of Clark's method, called C*.[9] The survival curves were estimated and compared using the Kaplan–Meier method and log-rank tests. After adjusting for clinical and pathological factors, univariable and multivariable Cox proportional hazards models were employed to investigate the association between prognosis and mutation status. All reported *P*-values were two-tailed, with *P*-values < 0.05 considered statistically significant. Statistical analyses were performed using SPSS software version 22.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Baseline Demographics

The five most frequently observed co-mutations in this cohort were TP53 (49%), protein kinase D (PKD) (27%), NOTCH (22%), BRCA (16%), and PIK3 (12.9%). From this dataset, we selected the two most frequently observed co-mutations for further analysis. Dual PKD/EGFR mutations were observed in 39 patients, while dual TP53/EGFR mutations were found in 72 patients, with TP53/EGFR being significantly more common in male patients ($P = 0.021$). Simultaneous PKD/TP53 mutations were found in 10 patients (7%). The baseline characteristics of patients are summarized in Table 1.

Effect of Co-Mutational Status on Survival in Stage I–III Surgically Resected Epidermal Growth Factor Receptor (EGFR) + Adenocarcinoma Patients

The median follow-up time was 31.3 months (range 5.0–65.6). Seventeen patients died during follow-up. The follow-up statistic was 83.8% complete by the C*. The 3-year OS and DFS rates in the entire cohort were 86.9% and 52.7%, respectively, and the median DFS was 37 months (range 2.0–53.0).

Patients harboring classic drug-sensitive EGFR mutations (Exon 19 and 21) had better OS (log-rank $P = 0.018$) and DFS (log-rank $P = 0.009$) compared with those with rare EGFR mutations (Fig. 1a, b), while patients with TP53 or PKD mutations had significantly worse DFS (log-rank $P = 0.006$ and 0.042, respectively) compared with patients without these co-mutations (Fig. 1f, d); however, these differences did not affect OS (Fig. 1c, e). Furthermore, statistically shorter OS (log-rank $P = 0.018$) and DFS (log-rank $P = 0.011$) were also observed in patients harboring triple TP53, PKD, and EGFR co-mutations, compared with those with EGFR mutations only (Fig. 1g, h). Kaplan–Meier curves for DFS (as more events occurred) with and without PKD and TP53 co-mutations in different stages were drawn to evaluate the correlation between the co-mutations and pathologic stage. No upstaging across

TABLE 1 Baseline characteristics of patients

Characteristic	PKD mutation			TP53 mutation		
	No [*n* = 108]	Yes [*n* = 39]	*P*-value	No [*n* = 75]	Yes [*n* = 72]	*P*-value[a]
Age, years						
≤ 60	62 (57.4)	22 (56.4)	1.000	45 (60.0)	39 (54.2)	0.486
> 60	46 (42.6)	17 (43.6)		30 (40.0)	33 (45.8)	
Sex						
Female	59 (54.6)	21 (53.8)	1.000	48 (64.0)	32 (44.4)	**0.021**
Male	49 (45.4)	18 (46.2)		27 (36.0)	40 (55.6)	
EGFR mutation type						
Exon 19 (+)	42 (38.9)	15 (38.5)	1.000	27 (36.0)	30 (41.7)	0.239
Exon 21 (+)	59 (54.6)	21 (53.8)		45 (60.0)	35 (48.6)	
Rare mutation[b]	7 (6.5)	3 (7.7)		3 (4.0)	7 (9.7)	
Smoking						
No	78 (72.2)	26 (66.7)	0.542	57 (76.0)	47 (65.3)	0.204
Yes	30 (27.8)	13 (33.3)		18 (24.0)	25 (34.7)	
T stage[b]						
T1-2	99 (91.7)	37 (94.9)	0.728	70 (93.3)	66 (91.7)	0.762
T3-4	9 (8.3)	2 (5.1)		5 (6.7)	6 (8.3)	
N stage[b]						
N0	63 (58.3)	20 (51.3)	0.755	49 (65.3)	34 (47.2)	0.063
N1	15 (13.9)	6 (15.4)		10 (13.3)	11 (15.3)	
N2	30 (27.8)	13 (33.3)		16 (21.3)	27 (37.5)	
Pathological stage						
I	57 (52.8)	20 (51.3)	0.967	46 (61.3)	31 (43.1)	0.082
II	17 (15.7)	6 (15.4)		10 (13.3)	13 (18.1)	
III	34 (31.5)	13 (33.3)		19 (25.3)	28 (38.9)	
Type of surgery						
Lobectomy	98 (90.7)	34 (87.2)	0.544	64 (85.3)	68 (94.4)	0.101
Bilobectomy	10 (9.3)	5 (12.8)		11 (14.7)	4 (5.6)	
Adjuvant treatment						
No	50 (46.3)	19 (48.7)	0.853	37 (49.3)	32 (44.4)	0.621
Yes	58 (53.7)	20 (51.3)		38 (50.7)	40 (55.6)	

Data are expressed as *n* (%)

Exon 18 (*n* = 2), exon 20 (*n* = 3), exons 21 and 18 (*n* = 1), exons 21 and 19 (*n* = 1), exons 21 and 20 (*n* = 3)

EGFR epidermal growth factor receptor, *PKD* protein kinase D, *UICC* Union for International Cancer Control, *AJCC* American Joint Committee on Cancer

P-values < 0.05 are given in bold

[a]*P*-values were calculated using the Chi square test

[b]According to the 8th edition of the UICC/AJCC staging system

different *p*-stages was observed, as shown in electronic supplementary Fig. 2.

Univariate and Multivariate Analysis of Prognostic Factors

Analysis of prognostic factors performed using the Cox proportional hazards regression model is shown in Table 2. Both TP53 (hazard ratio [HR] 2.08, 95% confidence interval [CI] 1.23–3.54, $P = 0.007$) and PKD mutations (HR 1.72, 95% CI 1.01–2.93, $P = 0.044$) were associated with shorter DFS in univariate analysis; however, neither of these mutations was statistically significant in multivariate analysis (Table 3). Patients with simultaneous PKD/TP53 mutations had worse DFS than those negative for dual PKD/TP53 mutations (HR 2.49, 95% CI 1.15–5.37, $P = 0.02$), although there was no significant difference in OS (HR 2.06, 95% CI 0.58–7.33, $P = 0.265$)

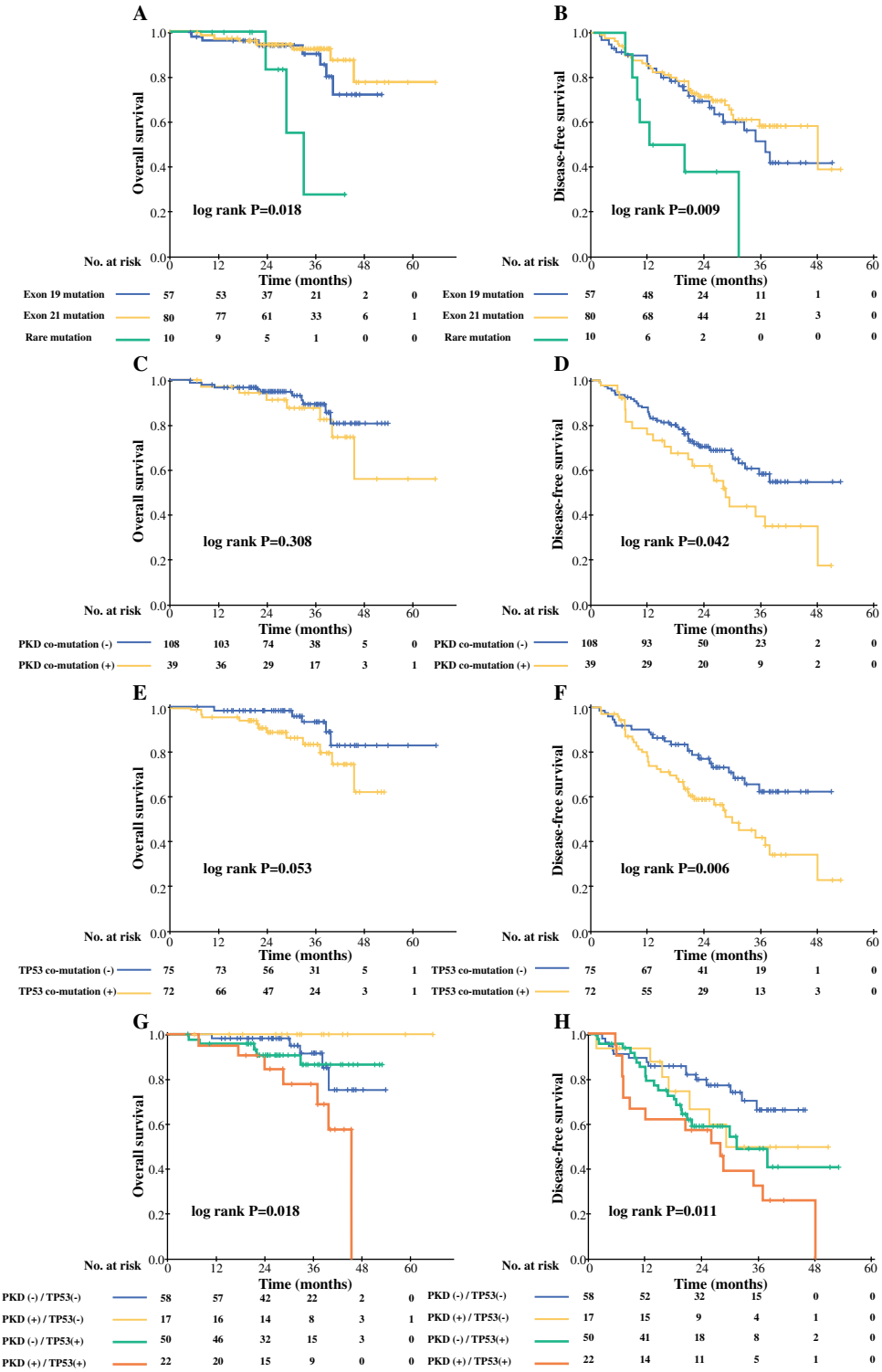

FIG. 1 Kaplan–Meier curves in 147 adenocarcinoma patients with different EGFR mutation types for (**a**) overall survival and (**b**) disease-free survival; with and without PKD mutation for (**c**) overall survival and (**d**) disease-free survival; with and without TP53 mutation for (**e**) overall survival and (**f**) disease-free survival; with different mutation status of PKD and TP53 for (**g**) overall survival and (**h**) disease-free survival. *EGFR* epidermal growth factor receptor, *PKD* protein kinase D

[Table 4]. TP53 mutational status was not associated with any differences in OS or DFS; however, in a subgroup of never-smokers, TP53 mutations were observed to have a significantly worse OS (HR 60.25, 95% CI 3.02–1200.45, $P = 0.007$) [electronic supplementary Table 1, Fig. 1].

DISCUSSION

Among the 147 EGFR + adenocarcinoma patients included in this study, 49% also tested positive for TP53 mutations, while an additional 27% had PKD mutations.

TABLE 2 Univariate analysis of prognostic factors

Characteristic	Overall survival		Disease-free survival	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age, years [> 60 vs. ≤ 60]	0.480 (0.17–1.37)	0.169	0.76 (0.45–1.28)	0.295
Sex [male vs. female]	2.23 (0.82–6.03)	0.115	1.70 (1.02–2.84)	**0.044**
EGFR mutation type				
Exon 21 (+) vs. Exon 19 (+)	0.64 (0.22–1.81)	0.396	0.79 (0.45–1.36)	0.387
Rare mutation vs. Exon 19 (+)	3.83 (0.97–15.11)	0.055	2.74 (1.16–6.46)	**0.021**
T stage [T3-4 vs. T1-2]	2.49 (0.71–8.70)	0.152	1.85 (0.84–4.10)	0.129
N stage				
N1 vs. N0	14.66 (2.81–76.64)	**0.001**	2.54 (1.18–5.47)	**0.017**
N2 vs. N0	11.46 (2.50–52.44)	**0.002**	4.87 (2.73–8.67)	< **0.001**
Smoking [yes vs. no]	2.22 (0.85–5.80)	0.102	1.78 (1.05–3.02)	**0.031**
Type of surgery [bilobectomy vs. lobectomy]	1.68 (0.48–5.87)	0.414	0.76 (0.30–1.89)	0.548
Adjuvant treatment [yes vs. no]	1.05 (0.40–2.78)	0.919	1.55 (0.91–2.64)	0.105
PKD mutation [yes vs. no]	1.65 (0.62–4.36)	0.313	1.72 (1.01–2.93)	**0.044**
TP53 mutation [yes vs. no]	2.70 (0.95–7.67)	0.063	2.08 (1.23–3.54)	**0.007**
Triple mutational status				
PKD(+)/TP53(−) vs. PKD(−)/TP53(−)	NC	0.980	1.69 (0.69–4.14)	0.255
PKD(−)/TP53(+) vs. PKD(−)/TP53(−)	1.21 (0.35–4.21)	0.758	2.05 (1.06–3.96)	**0.033**
PKD(+)/TP53(+) vs. PKD(−)/TP53(−)	3.51 (1.11–11.14)	**0.033**	3.18 (1.55–6.52)	**0.002**

A Cox proportional hazards regression model was used to detect variables one by one without adjustment

P-values < 0.05 are given in bold

HR hazard ratio, *CI* confidence interval, *EGFR* epidermal growth factor receptor, *PKD* protein kinase D, *NC* HR could not be calculated as no incident occurred in the PKD(+)/TP53(−) subgroup

TABLE 3 Multivariate analysis of PKD and TP53 mutation and other prognostic factors

Characteristic	Overall survival		Disease-free survival	
	HR[a] (95% CI)	*P*-value	HR[a] (95% CI)	*P*-value
Age, years [> 60 vs. ≤ 60]	0.44 (0.13–1.43)	0.171	0.51 (0.28–0.93)	**0.028**
Sex [male vs. female]	0.99 (0.21–4.72)	0.990	1.13 (0.52–2.46)	0.755
EGFR mutation type				
Exon 21 (+) vs. Exon 19 (+)	0.67 (0.19–2.35)	0.527	1.19 (0.65–2.17)	0.581
Rare mutation vs. Exon 19 (+)	4.12 (0.75–22.74)	0.104	1.90 (0.74–4.90)	0.182
T stage [T3-4 vs. T1-2]	2.17 (0.49–9.57)	0.305	1.05 (0.40–2.77)	0.916
N stage				
N1 vs. N0	17.00 (2.77–104.32)	**0.002**	2.18 (0.97–4.93)	0.061
N2 vs. N0	7.80 (1.36–44.66)	**0.021**	4.89 (2.49–9.62)	< **0.001**
Smoking [yes vs. no]	0.75 (0.15–3.69)	0.723	1.35 (0.61–2.95)	0.460
Type of surgery [bilobectomy vs. lobectomy]	2.08 (0.42–10.47)	0.373	0.68 (0.25–1.82)	0.439
Adjuvant treatment [yes vs. no]	0.57 (0.18–1.76)	0.327	0.87 (0.48–1.57)	0.634
PKD mutation [yes vs. no]	0.99 (0.31–3.13)	0.986	1.69 (0.95–3.00)	0.074
TP53 mutation [yes vs. no]	2.25 (0.62–8.21)	0.220	1.48 (0.83–2.64)	0.182

P-values < 0.05 are given in bold

PKD protein kinase D, *HR* hazard ratio, *CI* confidence interval, *EGFR* epidermal growth factor receptor

[a]HRs estimated by Cox proportional hazards regression

TABLE 4 Multivariate analysis of triple mutational status and other prognostic factors

Characteristic	Overall survival		Disease-free survival	
	HR[a] (95% CI)	*P*-value	HR[a] (95% CI)	*P*-value
Age, years [> 60 vs. ≤ 60]	0.53 (0.15–1.87)	0.322	0.50 (0.27–0.92)	**0.026**
Sex [male vs. female]	0.94 (0.20–4.52)	0.938	1.11 (0.51–2.44)	0.790
EGFR mutation type				
Exon 21 (+) vs. Exon 19 (+)	0.68 (0.19–2.42)	0.550	1.20 (0.65–2.20)	0.558
Rare mutation vs. Exon 19 (+)	4.15 (0.73–23.41)	0.107	1.89 (0.73–4.87)	0.187
T stage [T3-4 vs. T1-2]	1.72 (0.38–7.69)	0.478	1.10 (0.41–2.97)	0.849
N stage				
N1 vs. N0	17.59 (2.76–112.08)	**0.002**	2.22 (0.98–5.04)	0.057
N2 vs. N0	7.43 (1.28–43.29)	**0.026**	4.91 (2.50–9.66)	< **0.001**
Smoking [yes vs. no]	0.79 (0.16–3.92)	0.776	1.37 (0.62–3.03)	0.438
Type of surgery [bilobectomy vs. lobectomy]	1.63 (0.33–8.09)	0.547	0.69 (0.25–1.86)	0.462
Adjuvant treatment [yes vs. no]	0.59 (0.18–1.90)	0.371	0.85 (0.47–1.56)	0.603
Triple mutational status				
PKD(+)/TP53(−) vs. PKD(−)/TP53(−)	NC	0.983	1.94 (0.76–4.97)	0.166
PKD(−)/TP53(+) vs. PKD(−)/TP53(−)	1.28 (0.28–5.81)	0.752	1.61 (0.77–3.36)	0.202
PKD(+)/TP53(+) vs. PKD(−)/TP53(−)	2.06 (0.58–7.33)	0.265	2.49 (1.15–5.37)	**0.020**

P-values < 0.05 are given in bold

HR hazard ratio, *CI* confidence interval, *EGFR* epidermal growth factor receptor, *NC* HR cannot be calculated as no incident occurred in the PKD(+)/TP53(−) subgroup

[a]HRs estimated by Cox proportional hazards regression

TP53 and PKD mutations were the most frequently observed co-mutations in the cohort, with mutations found to be associated with poorer prognoses.

EGFR mutational status is the most important predictor of EGFR-TKI efficacy; however, patients undergoing targeted therapy exhibit significant heterogeneity in terms of TKI response. It remains unclear as to what additional mutations, in combination with EGFR mutational status, affect tumor aggressiveness, and whether they contribute to drug insufficiency.

TP53, one of the most well-known tumor suppressor gene mutations, with a mutation rate of approximately 39–46% in adenocarcinomas,[10] encodes the p53 protein, a major stress-induced transcription factor involved in cell cycle arrest, apoptosis, and senescence. p53 also regulates metabolic pathways and cytokine responses that are required for embryo implantation.[11] Multiple studies have suggested a negative prognostic effect of TP53 mutations in non-small cell lung cancer (NSCLC), although the true impact of such mutations remains inconclusive.[12,13] Furthermore, no drugs capable of targeting TP53 mutations in cancer have been approved to date.

The prevalence of dual TP53/EGFR mutations in our study was 49%, which is consistent with previous studies showing a prevalence of 30–60% TP53 mutations in EGFR-mutated tumors.[14] In this study, the co-existence of TP53 mutations in patients with EGFR-mutated adenocarcinoma had little effect on DFS or OS after surgical resection, consistent with that seen in a study by the LACE-Bio Collaborative Group.[10] However, in a subgroup of non-smokers, we found the co-existence of the TP53 mutation had a significantly worse OS. Similar observations were reported by Halvorsen et al.[15] in which 36% of never-smokers harboring a TP53 co-mutation exhibited significantly reduced progression-free survival (PFS). Additionally, TP53 co-mutation was considered an independent negative prognostic factor in this subgroup. This could be partly explained by the hypothesis that lung cancer in never-smokers is a separate entity, with fewer overall mutations relative to smokers who generally harbor large numbers of mutations. The LACE-Bio Collaborative Group also found that patients with EGFR and TP53 co-mutations gained fewer benefits from adjuvant chemotherapy,[10] which may be associated with reduced sensitivity to EGFR TKIs in advanced NSCLC.[14] While the data presented here are suggestive, the sample sizes used in our subgroup analyses were too small to evaluate the effect of adjuvant therapy based on the mutational categories. Although the current study showed that the presence of PKD and TP53 co-mutations are associated with worse outcomes, it is unclear which specific genetic mechanism

might be the underlying force driving the negative prognostic impact as certain non-disruptive TP53 mutations are not considered functionally relevant.[16]

Protein kinase D (PKD) is a serine/threonine kinase first categorized as a member of the protein kinase C (PKC) family (as PKCμ), although more recent analyses have demonstrated its homology to calcium/calmodulin-dependent protein kinases.[17] With structure, enzymology, and regulatory properties different from that of PKC family members, PKD is now classified as a member of a new family of PKD enzymes, of which there are three members: PKD1, PKD2 and PKD3. Studies have shown that PKD participates in a range of biological processes, including signal transduction, cell adhesion, migration, survival, proliferation, differentiation, and apoptosis.[18,19] It is reasonable to postulate that PKD plays an important role in cancer as altered PKD expression has been identified as a prognostic indicator in multiple epithelial tumors, including pancreatic,[20] gastric,[20] colorectal,[20,21] and prostate[22] cancer. A decreased expression level of PKD1 is also observed in NSCLC compared with normal tissue, suggesting a loss of PKD1 expression increases the proliferation of NSCLC cells.[23]

We found that PKD/TP53 co-mutations had a significantly worse DFS and were identified as independent negative prognostic factors in stage I–III resected EGFR + adenocarcinomas. Most studies examining PKD emphasize its molecular aspects and signaling-related mechanisms. It has been reported that HSP90 pathways are interconnected, converging on PKD2 to promote tumor angiogenesis as well as tumor growth.[24] Similarly, Nurr1 expression mediated by PKD plays an important role in vascular endothelial growth factor (VEGF)-induced tumor angiogenesis.[25] As part of our routine clinical screening of cancer patients, we test for mutations in several genes, including EGFR, ROS1, HER2, BRAF, KRAS, RET, and MET amplification, along with ALK rearrangement in NSCLC patients; however, PKD and TP53 are not routinely included. Our study highlights that consideration should be given to the future potential of PKD and TP53 testing.

To our knowledge, this is the first study to focus on the impact of PKD mutations and PKD/TP53 co-mutations in surgically resected stage I–III EGFR-mutated lung adenocarcinoma. However, our study has several limitations. First, the size of the cohort is small, and, second, our data are retrospective in nature, and are taken from a single institution. The median study follow-up time was 31.3 months, which is also a relatively short follow-up interval. Although our results indicate that PKD and TP53 mutations, along with PKD/TP53 co-mutations, have a negative prognostic role in patients with resected EGFR-driven adenocarcinoma, further research into the predictive effects of multiple co-mutations in larger data sets is warranted.

CONCLUSION

The present results showed TP53 and PKD mutations were the two most frequently observed co-mutations in resected EGFR-mutated lung adenocarcinoma. According to our results, both TP53 and PKD mutations were associated with poorer prognoses. These findings may have clinical relevance to predict the outcomes of affected patients.

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AUTHOR'S CONTRIBUTIONS Conception and design: Di-Han Liu, Ze-Rui Zhao, Hao Long. Provided the study materials or patients: Di-Han Liu, Ze-Rui Zhao, Yao-Bin Lin, Wen-Jie Zhou, Jing-Yu Hou, Zheng-Hao Ye, Hao Long. Collected and assembled the data: Di-Han Liu, Yao-Bin Lin. Data analysis and interpretation: Di-Han Liu, Ze-Rui Zhao. Writing of the manuscript and approving the final version: Di-Han Liu, Ze-Rui Zhao, Yao-Bin Lin, Wen-Jie Zhou, Jing-Yu Hou, Zheng-Hao Ye, and Hao Long.

DISCLOSURES Di-Han Liu, Ze-Rui Zhao, Yao-Bin Lin, Wen-Jie Zhou, Jing-Yu Hou, Zheng-Hao Ye, and Hao Long have no conflicts of interest to declare.

REFERENCES

1. The Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature.* 2014;511(7511):543–50.
2. Lynch T, Bell D, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med.* 2004;350(21):2129–39.
3. Hirsh V. Turning mutation-positive non-small-cell lung cancer into a chronic disease: optimal sequential therapy with EGFR tyrosine kinase inhibitors. *Ther Adv Med Oncol.* 2018;10:1758834017753338.
4. Jao K, Tomasini P, Kamel-Reid S, et al. The prognostic effect of single and multiple cancer-related somatic mutations in resected non-small-cell lung cancer. *Lung Cancer.* 2018;123:22–9.
5. Molina-Vila M, Bertran-Alamillo J, Gascó A, et al. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. *Clin Cancer Res.* 2014;20(17):4647–59.
6. Ulivi P, Delmonte A, Chiadini E, et al. Gene mutation analysis in EGFR wild type NSCLC responsive to erlotinib: are there features to guide patient selection? *Int J Mol Sci.* 2014;16(1):747–57.
7. Huang S, Benavente S, Armstrong E, Li C, Wheeler D, Harari P. p53 modulates acquired resistance to EGFR inhibitors and radiation. *Cancer Res.* 2011;71(22):7071–9.
8. Lim S, Kim H, Cho E, et al. Targeted sequencing identifies genetic alterations that confer primary resistance to EGFR

tyrosine kinase inhibitor (Korean Lung Cancer Consortium). *Oncotarget.* 2016;7(24):36311–20.
9. Wu Y, Takkenberg JJ, Grunkemeier GL. Measuring follow-up completeness. *Ann Thorac Surg.* 2008;85(4):1155–7.
10. Shepherd F, Lacas B, Le Teuff G, et al. Pooled analysis of the prognostic and predictive effects of TP53 comutation status combined with KRAS or EGFR mutation in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. *J Clin Oncol.* 2017;35(18):2018–27.
11. Levine A, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer.* 2009;9(10):749–58.
12. Ma X, Le Teuff G, Lacas B, et al. Prognostic and predictive effect of TP53 mutations in patients with non-small cell lung cancer from adjuvant cisplatin-based therapy randomized trials: a LACE-bio pooled analysis. *J Thorac Oncol.* 2016;11(6):850–61.
13. Scoccianti C, Vesin A, Martel G, et al. Prognostic value of TP53, KRAS and EGFR mutations in nonsmall cell lung cancer: the EUELC cohort. *Eur Respir J.* 2012;40(1):177–84.
14. Labbé C, Cabanero M, Korpanty G, et al. Prognostic and predictive effects of TP53 co-mutation in patients with EGFR-mutated non-small cell lung cancer (NSCLC). *Lung Cancer.* 2017;111:23–9.
15. Halvorsen A, Silwal-Pandit L, Meza-Zepeda L, et al. TP53 mutation spectrum in smokers and never smoking lung cancer patients. *Front Genet.* 2016;7:85.
16. Govindan R, Weber J. TP53 mutations and lung cancer: not all mutations are created equal. *Clin Cancer Res.* 2014;20(17):4419–21.
17. Bollag W, Dodd M, Shapiro B. Protein kinase D and keratinocyte proliferation. *Drug N Perspect.* 2004;17(2):117–26.
18. Liljedahl M, Maeda Y, Colanzi A, Ayala I, Van Lint J, Malhotra V. Protein kinase D regulates the fission of cell surface destined transport carriers from the trans-Golgi network. *Cell.* 2001;104(3):409–20.
19. Azoitei N, Cobbaut M, Becher A, Van Lint J, Seufferlein T. Protein kinase D2: a versatile player in cancer biology. *Oncogene.* 2018;37(10):1263–78.
20. Azoitei N, Pusapati G, Kleger A, et al. Protein kinase D2 is a crucial regulator of tumour cell-endothelial cell communication in gastrointestinal tumours. *Gut.* 2010;59(10):1316–30.
21. Wei N, Chu E, Wipf P, Schmitz J. Protein kinase d as a potential chemotherapeutic target for colorectal cancer. *Mol Cancer Ther.* 2014;13(5):1130–41.
22. Jaggi M, Rao P, Smith D, et al. E-cadherin phosphorylation by protein kinase D1/protein kinase C{mu} is associated with altered cellular aggregation and motility in prostate cancer. *Cancer Res.* 2005;65(2):483–92.
23. Ni Y, Wang L, Zhang J, Pang Z, Liu Q, Du J. PKD1 is down-regulated in non-small cell lung cancer and mediates the feedback inhibition of mTORC1-S6K1 axis in response to phorbol ester. *Int J Biochem Cell Biol.* 2015;60:34–42.
24. Azoitei N, Diepold K, Brunner C, et al. HSP90 supports tumor growth and angiogenesis through PRKD2 protein stabilization. *Cancer Res.* 2014;74(23):7125–36.
25. Zhao D, Desai S, Zeng H. VEGF stimulates PKD-mediated CREB-dependent orphan nuclear receptor Nurr1 expression: role in VEGF-induced angiogenesis. *Int J Cancer.* 2011;128(11):2602–12.

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