Original Article

Aberrant status and clinicopathologic characteristic associations of 11 target genes in 1,321 Chinese patients with lung adenocarcinoma

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Background: The aberrant status of target genes and their associations with clinicopathologic characteristics are still unclear in primary lung adenocarcinoma.

Methods: The common mutations and translocations of nine target genes were evaluated in 1,247 specimens of surgically-resected primary lung adenocarcinoma. Immunohistochemistry was used to analyze the expressions of programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) in 731 specimens. The frequency of the aberrations and their associations with clinicopathologic characteristics were analyzed.

Results: Overall, 952 (76.3%) of 1,247 patients harbored at least one target mutation or translocation: epidermal growth factor receptor (*EGFR*) (729, 58.5%), v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (83, 6.7%), human epidermal growth factor receptor 2 (*HER2*) (82, 6.6%), anaplastic lymphoma kinase (*ALK)* (23, 1.8%), phosphoinositide-3-kinase catalytic alpha polypeptide (*PIK3CA*) (20, 1.6%), Ret proto-oncogene *RET* (15, 1.2%), ROS proto-oncogene 1 receptor tyrosine kinase (*ROS1*) (12, 1.0%), B-raf proto-oncogene (*BRAF*) (9, 0.7%), neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*) (3, 0.2%). Fourteen (1.9%) of 731 patients were PD-1 positive and 95 (13.0%) were PD-L1 positive in tumor cells. In men and smokers, there were more frequent *KRAS* mutations (both P<0.001) and PD-L1 positive tumors (P<0.001, P=0.005, respectively), and less frequent *EGFR* mutations (P=0.049, <0.001, respectively). In ground-glass opacity (GGO) or ground-glass nodules (GGN), there were more *HER2* (P=0.033) but less *EGFR* (P=0.025) and *PIK3CA* mutations (P=0.012), and *ALK* translocations (P=0.014). *EGFR* (P<0.001), *KRAS* mutations (P=0.004) and PD-L1 positive tumors (P=0.046) were more frequent in older patients, while *HER2* (P<0.001), *ALK* (P=0.005) and *ROS1* aberrations (P=0.044) were less frequent. Invasive mucinous adenocarcinoma was significantly associated with *KRAS* and *ALK* aberrations (both P<0.001), while solid predominant adenocarcinoma was associated with *ROS1* translocations (P=0.036) and PD-L1 expression (P<0.001). *KRAS*, *HER2*, and *ALK* aberrations were scarce in patients with *EGFR* mutations (all P<0.001), while PD-L1 positive tumors positively correlated with *ALK* translocations (P=0.031) and negatively correlated with *HER2* mutations (P=0.019).

Conclusions: Most patients with primary lung adenocarcinoma harbored target gene aberrations. The frequency of each alteration differed in patients depending on clinicopathologic characteristics.

Keywords: Lung adenocarcinoma; epidermal growth factor receptor (*EGFR*); v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS); human epidermal growth factor receptor 2 (*HER2*); programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1)

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Introduction

Lung cancer is the most common malignancy and the leading cause of cancer-related deaths worldwide (1) whereas lung adenocarcinoma is the most frequent histological subtype of lung cancer (2). Oncogene aberrations have been extensively studied in the adenocarcinoma subtype (3), and show great molecular heterogeneity. With the approval of gefitinib, a first-generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), in the early 2000s, the prognosis of the subgroup of patients with lung adenocarcinoma that harbor *EGFR* activating mutations has been dramatically improved. Subsequently, in addition to *EGFR* mutations, several target gene aberrations in lung adenocarcinoma have been discovered, including v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (4), human epidermal growth factor receptor 2 (*HER2*) (5), B-raf proto-oncogene (*BRAF*) mutations (6), and anaplastic lymphoma kinase (*ALK*) translocations (7). The critical immunoregulator role of programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) has been extensively studied in lung cancers as well as many other cancer types. By using molecularly targeted agents for specific alterations, corresponding patients with lung adenocarcinoma have exhibited a significant and durable response (8).

The identifications of the prevalence of target gene alterations and their associations with clinicopathologic characteristics can make a significant difference in the selection of the therapeutic modality and the improvement of clinical outcome in lung adenocarcinoma patients. To date, though the target gene characterizations of lung adenocarcinoma have been reported in several separate studies; no large-scale research has been carried out systematically, either in China or worldwide, to analyze the status of target genes and its association with clinicopathologic characteristics. In the last year, we began to routinely examine the aberrant status of *EGFR*, *KRAS*, *ALK*, *HER2*, *BRAF*, Ret proto-oncogene (*RET*), ROS proto-oncogene 1 receptor tyrosine kinase (*ROS1*), phosphoinositide-3-kinase catalytic alpha polypeptide (*PIK3CA*), and neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*), and the expressions of PD-1/PD-L1 in patients with primary lung adenocarcinoma who underwent surgical resection at our institution. Based on the clinical data that we have collected, we investigated the prevalence of the 11 target gene alterations and analyze their associations with clinicopathologic characteristics.

Methods

Ethics statement

This study was conducted with approval from the Ethics Committee of Zhongshan Hospital, Fudan University, Shanghai, China (Approval No. B2017-042). Written informed consent was obtained from all patients participating in this study at the time of hospitalization.

Patients

In this study, we screened all primary lung adenocarcinoma patients who underwent surgical resection of their tumors from April 2016 to March 2017, in the Department of Thoracic Surgery, Zhongshan Hospital, Fudan University. All the cases were clearly confirmed by pathologic evaluation. Of the 1,470 cases, both the expressions of PD-1/PD-L1 and the status of the nine target genes: *EGFR*, *KRAS*, *HER2*, *ALK*, *BRAF*, *RET*, *ROS1*, *PIK3CA*, and *NRAS* were detected in 657 patients. While the status of the nine target genes was analyzed in 590 different patients and the expressions of PD-1/PD-L1 in 74 different patients, respectively. A total of 1,321 patients were enrolled.

Target gene analysis

The status of the nine target genes and expressions of PD-1/PD-L1 were obtained from pathologists' reports. In pathological examination, our institute routinely detects

the status of the nine target genes in patients with lung adenocarcinoma using a detection kit for the mutation of human *EGFR* and another eight genes based on fluorescence real-time polymerase chain reaction (Amoy Diagnostics Co., Ltd., Xiamen, China) (*Tables S1* and *S2*), including *EGFR*, *KRAS*, *HER2*, *ALK*, *BRAF*, *RET*, *ROS1*, *PIK3CA* and *NRAS*.

Immunohistochemical analysis

Immunohistochemical (IHC) staining of PD-L1 expression was performed on 4–6 μm thick formalin-fixed, paraffin-embedded tissue according to the manufacturer's guidelines. Briefly, the primary antibodies specific for PD-L1 were applied to detect expression. Stained specimens were then viewed at 100× by the investigators. Gene expression was determined by assessing the percentage of marked cells as previously reported (9).

More than 5% of PD-1/PD-L1 positive cells in each specimen were considered positive because this percentage was reported to be related to the clinical response of anti-PD-1 therapy in a number of previous studies (10-13). Four antibodies were used to detect the expression of PD-L1, including 28–8, SP142, E1L3N, and BP6001. To simplify calculations, the ultimate number of PD-L1 positive cells were the average detected by more than one kind of antibody.

Clinicopathologic characteristics

Clinical data were obtained from patients' electronic medical record database, including gender, age, smoking status, tumor location, tumour, node and metastasis (TNM) stage, histological subtype, and chest CT features. Histologic subtypes of lung adenocarcinoma were classified according to the new International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) multidisciplinary classification of lung adenocarcinoma (14). TNM stages were classified according to the revision of the 8th edition IASLC classification of TNM staging of lung cancer (15).

Statistical analysis

Correlations between target alterations and different subgroups stratified by sex, smoking status, tumor location, CT features, the classification of invasive adenocarcinomas, and the expressions of PD-1/PD-L1 were analyzed with chi-square and Fisher's exact tests when appropriate. Independent two-sample *t*-tests were used to analyze the associations of age with the 11 target genes. Wilcoxon rank-sum tests were performed to analyze the associations of the 11 target genes with T stage, N stage, M stage, TNM stage, and histological subtypes. Associations of PD-1/PD-L1 positive tumor cells with positive stromal lymphocytes were analyzed with McNemar's test. Spearman's rank correlation tests were used to analyze the correlations among the nine target gene aberrations and the expressions of PD-1/PD-L1. All tests were two-sided, and P values <0.05 were considered significant. All statistical analyses were performed using SPSS, version 24 (IBM, Armonk, NY, USA).

Results

Patient characteristics

A total of 1,321 patients with primary lung adenocarcinoma who had not received preoperative chemotherapy and targeted therapy were included in the present study. The demographics of all 1,321 patients with adenocarcinoma are listed in *Table S3*. Overall, 821 (62.1%) patients were women, 1,164 (88.1%) patients were never smokers, and the median age of all patients was 60 years (range, 24–85 years). Several patients had acinar predominant adenocarcinoma (n=748, 56.6%) and were at stage I (n=1,057, 80.0%). Additionally, 571 (43.2%) patients exhibited ground-glass opacity (GGO) or ground-glass nodule (GGN) in the CT images.

Prevalence of oncogene aberrations

Of the 1,247 patients who had been examined to identify the mutations or translocations of the nine target genes, 952 (76.3%) patients harbored at least one gene aberration. Among these 1,247 patients, *EGFR* mutations were detected in 729 (58.5%) patients, *KRAS* mutations in 83 (6.7%) patients, *HER2* mutations in 82 (6.6%) patients, while patients with alterations in the other six target genes were less than 2% (*Tables 1* and *2*). In addition, 24 (1.9%) patients harbored aberrations in two genes but none were found with aberrations in three or more genes.

EGFR mutations status and associations with clinicopathologic characteristics

The most frequent subtype of *EGFR* mutation was a leucine

to arginine mutation at amino acid position 858 (L858R) in exon 21, which occurred in 380 (30.5%) patients, followed by exon 19 deletions (19-del) in 323 patients (25.9%). The other four subtypes of *EGFR* mutations (G719A/C, S768I, L861Q) were all less than 1.2%, while eight (0.6%) patients harbored the substitution of threonine with methionine at amino acid position 790 (T790M) in exon 20 that relates to resistance to EGFR-TKIs. Additionally, there were 10 (0.8%) cases with two coexisting subtypes of *EGFR* mutations. No patient harbored three or more coexisting subtypes.

As shown in *Table 1* and *Table S4*, *EGFR* mutations were significantly more frequent in women (P=0.049), patients who had never smoked (P<0.001), with the absence of GGO/GGN (P=0.025) and acinar predominant adenocarcinoma (P<0.001). Furthermore, patients with *EGFR* mutations were older than those without *EGFR* mutations (P<0.001). Stage T2, M1 were significantly associated with *EGFR* mutations (P=0.043, 0.029, respectively). No significant association was observed with tumor location.

EGFR L858R mutations were significantly associated with patients who had never smoked (P=0.021), while EGFR 19-del mutations were significantly associated with the absence of GGO/GGN (P=0.009). Furthermore, older age was significantly associated with EGFR L858R mutations (P<0.001) and EGFR L861Q mutations (P=0.009).

Associations of alterations in KRAS, HER2, ALK, ROS1, RET, BRAF, PIK3CA and NRAS with clinicopathologic characteristics

As *Table 2* shows, *KRAS* mutations were significantly more frequent in men (P<0.001), and current or former smokers (P<0.001); while no significant correlation of gender was observed with other target gene alterations. In patients with GGO/GGN, there were significantly more frequent *HER2* mutations (P=0.033), while less frequent *ALK* translocations (P=0.014) and *PIK3CA* mutations (P=0.012). In older patients, there were significantly more frequent *KRAS* mutations (P=0.004), while less frequent *HER2* mutations (P<0.001), *ALK* translocations (P=0.005), and *ROS1* translocations (P=0.044).

Invasive mucinous adenocarcinoma was significantly associated with *KRAS* mutations (P<0.001) and *ALK* translocations (P<0.001). Solid predominant adenocarcinoma was significantly associated with *ROS1* translocations (P=0.036). No significant association was observed with pulmonary lobe harboring tumor, except *ROS1* translocations (P=0.030). *HER2* mutations and *ALK* translocations were significantly more frequent in stage 0 (P<0.001) and stage III (P<0.001), respectively (*Tables 2*,*S5*).

Associations of PD-1/PD-L1 expression with clinicopathologic characteristics

Of the 731 patients who had been examined to identify the expressions of PD-1/PD-L1, only 14 (1.9%) patients were PD-1 positive in tumor cells, yet 369 (50.5%) were PD-1 positive in stromal lymphocytes (P<0.001). Ninety-five (13.0%) patients were PD-L1 positive in tumor cells, yet 492 (67.3%) were PD-1 positive in stromal lymphocytes (P<0.001) (*Table 3*).

No significant associations of PD-1 positive tumors were observed with clinical features, which may be the result of the small number of patients with PD-1 positive tumors. PD-L1 positive tumors were more frequent in men (P<0.001), current or former smokers (P=0.005), patients without GGO/GGN (P<0.001), older patients (P=0.046), and those with solid predominant adenocarcinoma (P<0.001) (*Tables 3*,*S6*).

Correlations among the status of nine target genes and expression of PD-1/PD-L1

As *Table 4* shows, of the 1,247 patients who had been examined to identify the status of the nine target genes, we observed that *KRAS* mutations, *HER2* mutations, and *ALK* translocations were scarce in patients with *EGFR* mutations (P<0.001, respectively), particularly in patients with EGFR 19-del mutations and EGFR L858R mutations. Owing to the small number of *ROS1* and *RET* translocations, *PIK3CA*, *BRAF*, and *NRAS* mutations, no obvious correlation was observed among them and other target aberrations.

PD-1 positive tumors positively correlated with PD-L1 positive tumors (P<0.001). PD-L1 positive tumors positively correlated with *ALK* translocations (P=0.031) and negatively correlated with *HER2* mutations (P=0.019). There was no significant correlation of PD-L1 positive tumors with total *EGFR* mutation and its subtypes. (*Tables 4*,*S7*).

Discussion

In this study, we investigated the status of 11 common target genes in more than 1,000 lung adenocarcinoma

Table 1 Associations of *EGFR* mutations with clinicopathologic characteristics

Clinicopathologic characteristics	No. of patients							
	No.	Total *EGFR*	G719A/C	19-del	S768I	T790M	L858R	L861Q
No.		729	11	323	2	8	380	14
Sex (P)		0.049*	0.308	0.406	0.531	0.713	0.476	0.071
Male	468	257	2	115	0	2	137	2
Female	779	472	9	208	2	6	243	12
Smoking history (P)		<0.001*	1.000	0.205	1.000	0.244	0.021*	0.334
Never	1,099	662	10	291	2	6	347	14
Former/current	148	67	1	32	0	2	33	0
GGO/GGN (P)		0.025*	0.609	0.009*	0.511	0.521	0.980	0.564
Present	529	290	6	117	0	2	161	7
Absent	718	439	5	206	2	6	219	7
Age (mean ± SD) (P)		<0.001*	0.956	0.811	0.471	0.482	<0.001*	0.009*
With aberrations		60.0±10.4	58.5±9.7	58.5±11.3	53.0±17.0	61.4±7.6	61.2±9.5	65.6±8.6
Without aberrations		56.7±11.6	58.6±11.1	58.7±11.0	58.7±11.0	58.6±11.1	57.6±11.5	58.6±11.1
Histological subtype (P)		<0.001*	0.779	0.008*	0.873	0.055	0.174	0.161
AAH/AIS	16	5	0	3	0	0	1	1
MIA	276	93	2	38	0	0	50	3
Acinar	735	536	7	233	2	5	285	10
Lepidic	41	25	0	9	0	0	16	0
Papillary	60	37	1	20	0	1	17	0
Micropapillary	6	2	1	1	0	0	0	0
Solid	53	20	0	10	0	2	9	0
Unknown predominance	14	5	0	5	0	0	0	0
IMA	46	6	0	4	0	0	2	0
TNM stage (P)		0.075	0.369	0.049*	0.316	0.513	0.678	0.496
0	16	5	0	3	0	1	1	1
I	1,013	589	8	253	1	9	319	9
II	86	49	1	24	1	1	22	1
III	104	64	2	32	0	2	28	2
IV	28	22	0	11	0	1	10	1

*, indicates P value <0.05. G719A/C, glycine to alanine or cysteine mutation at amino acid position 719; 19-del, exon 19 deletions; T790M, substitution of threonine with methionine at amino acid position 790; S768I, serine to isoleucine mutation at amino acid position 768; L858R, leucine to arginine mutation at amino acid position 858; L861Q, leucine to glutamine mutation at amino acid position 861; GGO, ground-glass opacity; GGN, ground-glass nodule; AAH/AIS, atypical adenomatous hyperplasia or adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IMA, invasive mucinous adenocarcinoma; EGFR, epidermal growth factor receptor; TNM, tumour, node and metastasis; SD, standard deviation.

Table 2 Associations of the other eight target gene aberrations with clinicopathologic characteristics

Clinicopathologic characteristics	No. of patients								
	No.	*ALK*	*ROS-1*	*RET*	*KRAS*	*NRAS*	*BRAF*	*PIK3CA*	*HER2*
No.		23	12	15	83	3	9	20	82
Sex (P)		0.784	0.998	0.462	<0.001*	0.655	0.195	0.483	0.173
Male	468	8	4	7	51	2	1	6	25
Female	779	15	8	8	32	1	8	14	57
Smoking history (P)		1.000	1.000	0.029*	<0.001*	0.316	0.557	0.930	0.187
Never	1,099	20	11	10	60	2	9	17	76
Former/current	148	3	1	5	23	1	0	3	6
GGO/GGN (P)		0.014*	0.220	0.849	0.153	1.000	0.644	0.012*	0.033*
Present	529	4	3	6	29	1	5	3	44
Absent	718	19	9	9	54	2	4	17	38
Age (mean ± SD) (P)		0.005*	0.044*	0.822	0.004*	0.996	0.656	0.748	<0.001*
With aberrations		52.3±9.9	52.3±11.5	58.0±10.5	62.0±10.3	58.7±9.0	57.0±8.9	57.8±12.4	47.4±12.0
Without aberrations		58.8±11.0	58.7±11.0	58.7±11.1	58.4±11.1	58.6±11.1	58.7±11.1	58.7±11.0	59.4±10.6
Histological subtype (P)		<0.001*	0.036*	0.333	<0.001*	0.005*	0.426	0.584	<0.001*
AAH/AIS	16	0	0	0	0	0	0	0	2
MIA	276	0	1	3	18	0	4	2	51
Acinar	735	14	6	8	32	0	3	16	18
Lepidic	41	0	0	0	1	1	0	0	0
Papillary	60	1	2	0	7	0	1	0	2
Micropapillary	6	0	0	0	0	0	0	0	0
Solid	53	2	3	1	6	1	1	1	2
Unknown predominance	14	0	0	1	1	0	0	0	4
IMA	46	6	0	2	18	1	0	1	3
TNM stage (P)		<0.001*	0.412	0.734	0.177	0.475	0.718	0.083	<0.001*
0	16	0	0	0	0	0	0	0	2
I	1,013	12	9	13	64	3	8	14	77
II	86	4	1	1	10	0	0	1	0
III	104	6	1	1	9	0	1	3	3
IV	28	1	1	0	0	0	0	2	0

*, indicates P value <0.05. *ALK*, anaplastic lymphoma kinase; *ROS-1*, ROS proto-oncogene 1 receptor tyrosine kinase; *RET*, Ret proto-oncogene; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; *BRAF*, B-raf proto-oncogene; *PIK3CA*, phosphoinositide-3-kinase catalytic alpha polypeptide; *HER2*, human epidermal growth factor receptor 2; GGO, ground-glass opacity; GGN, ground-glass nodule; AAH/AIS, atypical adenomatous hyperplasia or adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IMA, invasive mucinous adenocarcinoma; TNM, tumour, node and metastasis; SD, standard deviation.

Table 3 Associations of PD-1/PD-L1 expression with clinicopathologic characteristics

Clinicopathologic characteristics	No. of patients		
	No.	PD-1 positive	PD-L1 positive
No.		14	95
Sex (P)		0.357	<0.001*
Male	279	7	53
Female	452	7	42
Smoking history (P)		1.000	0.005*
Never	641	12	75
Former/current	90	2	20
GGO/GGN (P)		0.878	<0.001*
Present	328	6	21
Absent	403	8	74
Age (mean ± SD) (P)		0.854	0.046*
Positive		57.2±12.4	59.7±9.9
Negative		57.8±11.3	57.5±11.5
Histological subtype (P)		0.849	<0.001*
AAH/AIS	35	0	2
MIA	191	6	8
Acinar	381	4	56
Lepidic	23	0	1
Papillary	35	0	7
Micropapillary	4	0	1
Solid	28	2	16
Unknown predominance	10	0	1
Enteric	1	0	0
IMA	23	2	3
TNM stage (P)		0.391	<0.001*
0	35	0	2
I	581	11	59
II	37	1	12
III	63	2	19
IV	15	0	3

*, indicates P value <0.05. PD-1, programmed death-1; PD-L1, programmed death-ligand 1; GGO, ground-glass opacity; GGN, ground-glass nodule; AAH/AIS, atypical adenomatous hyperplasia or adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IMA, invasive mucinous adenocarcinoma; TNM, tumour, node and metastasis; SD, standard deviation.

patients. We found that over half of the patients harbored *EGFR* mutations, followed by *KRAS* mutations and *HER2* mutations, which both occurred in approximately one in 15 patients. *ALK*, *RET*, and *ROS1* translocations, *PIK3CA*, *BRAF*, and *NRAS* mutations were rare and all were identified in less than 2% of patients. PD-1 positive tumors were identified in 14 (1.9%) patients, and PD-L1 positive tumors in 95 (13.0%) patients. To the best of our knowledge, the present study is the first report elaborating such a comprehensive prevalence of nine target gene aberrations and the expressions of PD-1/PD-L1, as well as their associations with clinicopathologic features.

As reported previously, *EGFR* mutations, the most common target gene of lung adenocarcinoma, represented 75% of all the gene alterations and occurred more frequently in females, patients who had never smoked, the elderly, and acinar predominant adenocarcinoma (16-19). Interestingly, Hong *et al.* (20) found that *EGFR* mutations were significantly more frequent in tumors with GGO than in solid tumors, which was contrary to our findings. This may be the result of different sample sizes, areas where the participants live, and reagents used to detect the status of the *EGFR* gene. However, in our study, there were more women and fewer smokers (20). Additionally, the detected subtypes of *EGFR* mutations in our study were partly different (20). In the 729 *EGFR*-mutant patients, consistent with a previous study (21), EGFR L858R mutations and 19-del mutations were the most common subtypes, followed by EGFR L861Q mutations and EGFR G719A/C mutations. Chiu *et al.* (22) revealed that patients with EGFR G719X/L861Q/S768I mutations exhibited a significantly inferior tumor response rate and progression-free survival than patients with EGFR 19-del and L858R mutations after receiving gefitinib and erlotinib treatment, suggesting the limited efficacy of first-generation EGFR-TKIs in those patients. EGFR T790M mutations are the most common and well-characterized resistance mechanism of acquired resistance to gefitinib and erlotinib. Currently, osimertinib is the cornerstone for patients with EGFR-T790M mutations in second-line therapy (23). EGFR T790M mutations were identified in 1.1% of patients with mutant *EGFR*, indicating that this resistance may only occur in few patients with lung adenocarcinoma who have not received a preoperative targeted therapy.

KRAS mutations were identified in 83 (6.7%) patients, consistent with a previous report (24). Tanaka *et al.* and Xu *et al.* (24) demonstrated that *KRAS* mutations were significantly more frequent in males, current or former

Table 4 Correlations among 11 target gene aberrations

Aberrations	*ALK*	*ROS-1*	*RET*	*EGFR*	*KRAS*	*NRAS*	*BRAF*	*PIK3CA*	*HER2*	PD-1
ALK (P)										
ROS-1 (P)	0.634									
RET (P)	0.594	0.701								
EGFR (P)	<0.001**	<0.001**	<0.001**							
KRAS (P)	0.196	0.353	0.298	<0.001**						
NRAS (P)	0.812	0.864	0.848	0.377	0.644					
BRAF (P)	0.680	0.767	0.740	<0.001*	0.422	0.883				
PIK3CA (P)	0.537	0.657	0.619	0.550	0.131	0.825	0.701			
HER2 (P)	0.199	0.356	0.288	<0.001**	0.041*	0.646	0.425	0.232		
PD-1 (P)	0.649	0.749	0.770	0.725	0.616	0.896	0.729	0.694	0.343	
PD-L1 (P)	0.031*	0.130	0.628	0.332	0.251	0.702	0.905	0.063	0.019*	<0.001**

*, indicates P value <0.05; **, indicates P value <0.001. The underlined P values indicate a positive correlation. *ALK*, anaplastic lymphoma kinase; *ROS-1*, ROS proto-oncogene 1 receptor tyrosine kinase; *RET,* Ret proto-oncogene; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; *BRAF*, B-raf proto-oncogene; *PIK3CA*, phosphoinositide-3-kinase catalytic alpha polypeptide; *HER2*, human epidermal growth factor receptor 2; PD-1, programmed death-1; PD-L1, programmed death-ligand 1.

smokers, and the elderly, which was also found in the present study. No significant association between *KRAS* mutations and the presence of GGO/GGN was found, as previously reported (25). In agreement with a previous report, there was no significant association of *HER2* mutations with gender and smoking status (26), whereas a significant association with younger patients was found (27).

Because of the small number of *ALK* translocations (n=23, 1.8%), *PIK3CA* mutations (n=20, 1.6%), *BRAF* mutations (n=9, 0.7%), *NRAS* mutations (n=3, 0.2%), *ROS1* translocations (n=12, 1.0%), and *RET* translocations (n=15, 1.2%), agents targeting these target genes may be applied in the minority of patients with lung adenocarcinoma harboring these alterations.

Previous clinical trials found that the blockade of the PD-L1 and PD-1 interaction with specific antibodies had promising antitumor efficacy in patients with NSCLC (10,28). However, in our study, only a few patients were PD-1/PD-L1 positive. Azuma *et al.* (29) and Akbay *et al.* (30) found that *EGFR* mutations could up-regulate PD-L1 expression and that EGFR-TKIs could down-regulate PD-L1 expression. D'Incecco *et al.* (31) demonstrated that PD-1 positivity was significantly associated with current smoking status and with *KRAS* mutations, which was not observed in our study and needs to be further validated. Azuma *et al.* (29) found that expression of PD-L1 was significantly associated with females, patients who had never smoked, and *EGFR* mutations. Song *et al.* (32) also found that expression of PD-L1 was significantly associated with *EGFR* mutations, while no significant association was observed with other clinicopathologic parameters. Whereas, Jiang *et al.* (33) observed that PD-L1 expression in tumor cells was significantly higher in males, smokers, and patients with a higher histologic grade and that none of the *EGFR*, *KRAS*, *MET*, *ALK* and *ROS1* gene abnormalities showed any statistical association with PD-L1 positivity. We also observed that PD-L1 positive tumors were significantly associated with males and current or former smokers, yet positively correlated with *ALK* translocations and negatively correlated with *HER2* mutations. Various antibodies and the thresholds for PD-L1 positivity might lead to different results. Thus, further investigations to standardize the assay for PD-L1 expression are warranted.

There are some limitations in our study. Notably, the enrolled patients in the present study were mostly a Chinese Han population, so our results may not be applicable to people from other areas. Moreover, there was insufficient time to follow up the prognostic information. Therefore, we did not analyze the association of these target genes and corresponding targeted therapies with patients' prognosis

because the follow-up time was too short.

Conclusions

We demonstrated the prevalence of the 11 target genes and their comprehensive associations with clinicopathologic parameters in more than 1,000 Chinese lung adenocarcinoma patients. We hope our results will provide a reference for personalized medicine in patients with lung adenocarcinoma and improve their final prognosis.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was conducted with approval from the Ethics Committee of Zhongshan Hospital, Fudan University, Shanghai, China (Approval No. B2017-042). Written informed consent was obtained from all patients participating in this study at the time of hospitalization.

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Supplementary

Table S1 Detected mutation subtypes of target genes

Mutation	Exon	Base change	Cosmic ID
G719A	*EGFR exon* 18	2156 G > C	6239
G719C		2155 G > T	6253
E746_A750 del (1)	*EGFR exon* 19	2235_2249 del 15	6223
E746_A750 del (2)		2236_2250 del 15	6225
L747_P753 > S		2240_2257 del 18	12370
E746_T751 > I		2235_2252 > AAT (complex)	13551
E746_T751 del		2236_2253 del 18	12728
E746_S752 > V		2237_2255 > T (complex)	12384
L747_T751 > Q		2238_2252 > GCA (complex)	12419
L747_E749 del		2239_2247 del 19	6218
L747_S752 del		2239_2256 del 18	6255
L747_A750 > P		2239_2248 TTAAGAGAAG > C (complex)	12382
L747_P753 > Q		2239_2258 > CA (complex)	12387
L747_T751 del		2240_2254 del 15	12369
L747_T751 > P		2239_2251 > C (complex)	12383
S768I	*EGFR exon* 20	2303 G > T	6241
T790M		2369 C > T	6240
L858R	*EGFR exon* 21	2573 T > G	6224
L861Q		2582 T > A	6213
G12D	*KRAS exon* 2	35 G > A	521
G12S		34 G > A	517
G12A		35 G > C	522
G12V		35 G >T	520
G12R		34 G > C	518
G12C		34 G > T	516
G13C		37 G > T	527
V600E	*BRAF exon* 15	1799 T > A	476
A775_G776 ins YVMA	*HER2 exon* 20	2325_2326 ins 12 (TACGTGATGGCT)	12558
A775_G776 ins YVMA		2324_2325 ins 12 (ATACGTGATGGC)	20959
M774_A775 ins AYVM		2322_2323 ins 12 (GCATACGTGATG)	682
G776 > VC		2326_2327 ins 3 (TGT)	12553
P780_Y781 ins GSP		2339_2340 ins 9 (TGGCTCCCC)	303948
G13R	*NRAS exon* 2	37 G > C	569
G12C		34 G > T	562
G12V		35 G > T	566
G12A		35 G > C	565
G13V		38 G > T	574
Q61R	*NRAS exon* 3	182 A > G	584
Q61K		181 C > A	580
Q61L		182 A > T	583
Q61H		183 A > C	586
E545K	*PIK3CA exon* 9	1633 G > A	763
H1047R	*PIK3CA exon* 20	3140 A > G	775

EGFR, epidermal growth factor receptor; *BRAF*, B-raf proto-oncogene; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; *HER2*, human epidermal growth factor receptor 2; *PIK3CA*, phosphoinositide-3-kinase catalytic alpha polypeptide, AAT, adenine adenine thymine; GCA, guanine cytosine adenine.

Table S2 Detected fusion subtypes of target genes

Driver gene	Fusion	Cosmic ID
ALK	*EML4* exon 13; *ALK* exon 20	463
	EML4 exon 6 ins 33; *ALK* exon 20	493
	EML4 exon 20; *ALK* exon 20	465
	EML4 exon 18; *ALK* exon 20	488
	EML4 exon 2; *ALK* exon 20	480
ROS1	*SLC34A2* exon 4; *ROS1* exon 32	1197
	SLC34A2 exon 14 del; *ROS1* exon 32	1260
	CD74 exon 6; *ROS1* exon 32	1203
	SDC4 exon 2; *ROS1* exon 32	1266
	SDC4 exon 4; *ROS1* exon 32	1279
	SLC34A2 exon 4; *ROS1* exon 34	–
	SLC34A2 exon 14 del; *ROS1* exon 34	–
	CD74 exon 6; *ROS1* exon 34	1201
	SDC4 exon 4; *ROS1* exon 34	–
	EZR exon 10; *ROS1* exon 34	1268
	TPM3 exon 8; *ROS1* exon 35	1274
	LRIG3 exon 16; *ROS1* exon 35	1270
	GOPC exon 8; *ROS1* exon 35	1251
RET	*CCDC6* exon 1; *RET* exon 12	5918
	NCOA4 exon 9; *RET* exon 12	–
	KIF5B exon 15; *RET* exon 12	60431
	KIF5B exon 16; *RET* exon 12	60431
	KIF5B exon 23; *RET* exon 12	60431
	KIF5B exon 22; *RET* exon 12	60431

ALK, anaplastic lymphoma kinase; *EML4*, echinoderm microtubule-associated protein-like 4; *SLC34A2*, solute carrier family 34 member 2; *ROS1*, ROS proto-oncogene 1 receptor tyrosine kinase; *EZR*, ezrin; *TPM3*, tropomyosin 3; *LRIG3*, leucine-rich repeats and immunoglobulin-like domains 3; *GOPC*, golgi-associated PDZ and coiled-coil motif-containing; *CCDC6*, coiled-coil domain containing 6; *NCOA4*, nuclear receptor coactivator 4; *KIF5B*, kinesin family member 5B; *RET*, ret proto-oncogene.

Table S3 Characteristics of 1,321 patients with primary lung adenocarcinoma

Characteristic	No. of patients (%)
Age: median [range], y	60 [24-85]
Sex	
Male	500 (37.9)
Female	821 (62.1)
Smoking history	
Never	1,164 (88.1)
Former/current	157 (11.9)
GGO/GGN	
Present	571 (43.2)
Absent	750 (56.8)
Tumor location	
Left lung	519 (39.3)
Right lung	784 (59.3)
Bilateral lung	18 (1.4)
Pulmonary lobe with tumor	
Left upper lobe	326 (24.7)
Left lower lobe	167 (12.6)
Right upper lobe	411 (31.1)
Right middle lobe	92 (7.0)
Right lower lobe	205 (15.5)
Multiple lobes	120 (9.1)
Histological subtype	
AAH/AIS	38 (2.9)
MIA	304 (23.0)
Invasive adenocarcinoma	930 (70.4)
Acinar	748 (56.6)
Lepidic	42 (3.2)
Papillary	61 (4.6)
Micropapillary	6 (0.5)
Solid	58 (4.4)
Unknown predominance	15 (1.1)
IMA	48 (3.6)
Enteric	1 (0.1)
T stage	
Tis	38 (2.9)
T1	898 (68.0)
T2	327 (24.7)
T3	30 (2.3)
T4	28 (2.1)
N stage	
N0	1158 (87.6)
N1	67 (5.1)
N2	87 (6.6)
Nx	9 (0.7)
M stage	
M0	1,291 (97.7)
M1	30 (2.3)
TNM stage	
0	38 (2.9)
I	1,057 (80.0)
II	87 (6.6)
III	109 (8.2)
IV	30 (2.3)

GGO, ground-glass opacity; GGN, ground-glass nodule; AAH/AIS, atypical adenomatous hyperplasia or adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IMA, invasive mucinous adenocarcinoma.

Table S4 Associations of *EGFR* mutations with tumor location, T, N, and M stage

Clinicopathologic characteristics	No. of patients							
	No.	Total *EGFR*	G719A/C	19-del	S768I	T790M	L858R	L861Q
No.		729	11	323	2	8	380	14
Side of tumor location (P)		0.129	0.326	0.946	1.000	0.541	0.156	0.658
Left lung	492	272	2	127	1	2	136	7
Right lung	738	445	9	191	1	6	237	7
Bilateral lung	17	12	0	5	0	0	7	0
Lobe of tumor location (P)		0.555	0.297	0.467	0.056	0.436	0.133	0.875
Left upper	312	176	1	81	0	2	91	3
Left lower	157	86	1	45	1	0	37	3
Right upper	394	240	4	95	0	2	137	5
Right middle	82	53	2	24	1	1	26	1
Right lower	193	113	1	56	0	3	53	1
Multiple lobes	109	61	2	22	0	0	36	1
T stage (P)		0.043*	0.409	0.033*	0.388	0.181	0.835	0.153
Tis	16	5	0	3	0	0	1	1
T1	859	492	7	208	2	4	266	11
T2	317	201	2	98	0	3	99	1
T3	30	16	0	9	0	1	7	0
T4	25	15	2	5	0	0	7	1
N stage (P)		0.036*	0.722	<0.001*	0.131	0.262	0.269	0.797
N0	1090	624	10	266	1	6	336	12
N1	65	42	1	22	1	1	17	1
N2	83	56	0	33	0	1	22	1
Nx	9	7	0	2	0	0	5	0
M stage (P)		0.029*	0.614	0.102	0.830	0.050	0.542	0.214
M0	1219	702	11	312	2	7	370	13
M1	28	22	1	11	0	1	10	1

*, indicates P value <0.05. *EGFR*, epidermal growth factor receptor; G719A/C, glycine to alanine or cysteine mutation at amino acid position 719; 19-del, exon 19 deletions; T790M, substitution of threonine with methionine at amino acid position 790; S768I, serine to isoleucine mutation at amino acid position 768; L858R, leucine to arginine mutation at amino acid position 858; L861Q, leucine to glutamine mutation at amino acid position 861.

Table S5 Associations of the other eight target gene aberrations with tumor location, T, N, and M stage

Clinicopathologic characteristics	No. of patients								
	No.	ALK	ROS-1	RET	KRAS	NRAS	BRAF	PIK3CA	HER2
No.		23	12	15	83	3	9	20	82
Side of tumor location (P)		0.877	0.355	0.432	0.365	0.585	0.067	0.022*	0.824
Left lung	492	10	7	8	37	2	7	9	35
Right lung	738	13	5	7	46	1	2	9	46
Bilateral lung	17	0	0	0	0	0	0	2	1
Lobe of tumor location (P)		0.906	0.030*	0.096	0.856	0.792	0.104	0.159	0.068
Left upper	312	6	1	6	22	2	3	3	19
Left lower	157	2	6	1	12	0	3	5	14
Right upper	394	6	3	1	21	1	1	4	15
Right middle	82	2	0	1	5	0	0	0	6
Right lower	193	5	1	5	15	0	0	5	17
Multiple lobes	109	2	1	1	8	0	2	3	11
T stage (P)		0.066	0.830	0.777	0.044*	0.915	0.245	0.054	<0.001*
Tis	16	0	0	0	0	0	0	0	2
T1	859	12	8	11	52	2	8	10	69
T2	317	10	4	4	22	1	1	9	10
T3	30	1	0	0	3	0	0	1	1
T4	25	0	0	0	6	0	0	0	0
N stage (P)		<0.001*	0.623	0.878	0.463	0.524	0.981	0.168	0.017*
N0	1090	13	10	13	75	3	8	15	79
N1	65	3	1	1	5	0	0	0	1
N2	83	6	1	1	3	0	1	4	2
Nx	9	1	0	0	0	0	0	1	0
M stage (P)		0.492	0.153	0.555	0.153	0.793	0.648	0.018*	0.156
M0	1219	22	11	15	83	3	9	18	82
M1	28	1	1	0	0	0	0	2	0

*, indicates P value <0.05. ALK, anaplastic lymphoma kinase; ROS-1, ROS proto-oncogene 1 receptor tyrosine kinase; RET, Ret proto-oncogene; EGFR, epidermal growth factor receptor; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; BRAF, B-raf proto-oncogene; PIK3CA, phosphoinositide-3-kinase catalytic alpha polypeptide; HER2, human epidermal growth factor receptor 2.

Table S6 Associations of PD-1/PD-L1 expression with tumor location, T, N, and M stage

Clinicopathologic characteristics	No. of patients		
	No.	PD-1 positive	PD-L1 positive
No.		14	95
Side of tumor location (P)		0.540	0.730
Left lung	272	7	38
Right lung	446	7	56
Bilateral lung	13	0	1
Lobe of tumor location (P)		0.436	0.256
Left upper	166	4	27
Left lower	91	1	9
Right upper	229	3	22
Right middle	48	0	8
Right lower	115	2	19
Multiple lobes	82	4	10
T stage (P)		0.326	<0.001*
Tis	35	0	2
T1	496	9	45
T2	177	4	42
T3	6	0	1
T4	17	1	5
N stage (P)		0.687	<0.001*
N0	648	12	69
N1	27	1	10
N2	51	1	15
Nx	5	0	1
M stage (P)		0.585	0.415
M0	716	14	92
M1	15	0	3

*, indicates P value <0.05. PD-1, programmed death-1; PD-L1, programmed death-ligand 1.

Table S7 Details about the correlations among 11 target gene aberration

Aberrations	*ALK*		*ROS-1*		*RET*		G719A/C		19-del		T790M		L858R		L861Q		S768I		Total *EGFR*		*KRAS*		*NRAS*		*BRAF*		*PIK3CA*		*HER2*		PD-1	
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
ALK																																
−																																
+																																
P																																
ROS-1																																
−	1,212	23																														
+	12	0																														
P	0.634																															
RET																																
−	1,209	23	1220	12																												
+	15	0	15	0																												
P	0.594		0.701																													
EGFR; G719A/C																																
−	1,213	23	1,224	12	1,221	15																										
+	11	0	11	0	11	0																										
P	0.648		0.743		0.713																											
EGFR; 19-del																																
−	903	21	912	12	909	15	913	11																								
+	321	2	323	0	323	0	323	0																								
P	0.057		0.040*		0.021*		0.049*																									
EGFR; T790M																																
−	1,216	23	1,227	12	1,224	15	1,228	11	917	322																						
+	8	0	8	0	8	0	8	0	7	1																						
P	0.698		0.780		0.754		0.789		0.386																							
EGFR; L858R																																
−	844	23	855	12	852	15	856	11	545	322	865	2																				
+	380	0	380	0	380	0	380	0	379	1	374	6																				
P	0.001**		0.021*		0.010*		0.027*		<0.001***		0.006**																					
EGFR; L861Q																																
−	1,210	23	1,222	11	1,218	15	1,222	11	910	323	1,225	8	853	380																		
+	14	0	13	1	14	0	14	0	14	0	14	0	14	0																		
P	0.606		0.017*		0.678		0.723		0.026*		0.763		0.013*																			
EGFR; S768I																																
−	1,222	23	1,233	12	1230	15	1,234	11	924	321	1,237	8	865	380	1,231	14																
+	2	0	2	0	2	0	2	0	0	2	2	0	2	0	2	0																
P	0.846		0.889		0.876		0.894		0.017*		0.910		0.349		0.880																	
Total; *EGFR*																																
−	497	21	507	11	503	15	518	0	518	0	518	0	518	0	518	0	518	0														
+	727	2	728	1	729	0	718	11	406	323	721	8	349	380	715	14	727	2														
P	<0.001***		<0.001***		<0.001***		0.005**		<0.001***		0.017*		<0.001***		0.001**		0.233															
KRAS																																
−	1,141	23	1,152	12	1,149	15	1,153	11	841	323	1,156	8	784	380	1,150	14	1,162	2	435	729												
+	83	0	83	0	83	0	83	0	83	0	83	0	83	0	83	0	83	0	83	0												
P	0.196		0.353		0.298		0.374		<0.001***		0.449		<0.001***		0.315		0.706		<0.001***													
NRAS																																
−	1,221	23	1,232	12	1,229	15	1,233	11	921	323	1,236	8	865	379	1,230	14	1,242	2	516	728	1,161	83										
+	3	0	3	0	3	0	3	0	3	0	3	0	2	1	3	0	3	0	2	1	3	0										
P	0.812		0.864		0.848		0.870		0.306		0.889		0.914		0.854		0.945		0.377		0.644											
BRAF																																
−	1,215	23	1,226	12	1,223	15	1,227	11	915	323	1,230	8	858	380	1,224	14	1,236	2	509	729	1,155	83	1,235	3								
+	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0								
P	0.680		0.767		0.740		0.777		0.075		0.809		0.046*		0.749		0.904		<0.001***		0.422		0.883									

Table S7 *(continued)*

Table S7 *(continued)*

Aberrations	*ALK*		*ROS-1*		*RET*		G719A/C		19-del		T790M		L858R		L861Q		S768I		Total *EGFR*		*KRAS*		*NRAS*		*BRAF*		*PIK3CA*		*HER2*		PD-1	
	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+
PIK3CA																																
–	1,204	23	1,215	12	1,212	15	1,216	11	911	316	1,220	7	852	375	1,214	13	1,225	2	511	716	1,147	80	1,224	4	1,218	9						
+	20	0	20	0	20	0	20	0	13	7	19	1	15	5	19	1	20	0	7	13	17	3	20	0	20	0						
P	0.537		0.657		0.619		0.671		0.350		0.014*		0.592		0.097		0.857		0.550		0.131		0.825		0.701							
HER2																																
–	1,142	23	1,153	12	1,152	13	1,154	11	843	322	1,157	8	785	380	1,151	14	1,163	2	437	728	1,083	82	1,162	3	1,156	9	1,145	20				
+	82	0	82	0	80	2	82	0	81	1	82	0	82	0	82	0	82	0	81	1	81	1	82	0	82	0	82	0				
P	0.199		0.356		0.288		0.377		<0.001***		0.452		<0.001***		0.319		0.708		<0.001***		0.041*		0.646		0.425		0.232					
PD-1																																
–	634	12	640	6	641	5	639	7	480	166	642	4	446	200	624	4			269	377	610	36	645	1	639	7	637	9	597	49		
+	11	0	11	0	11	0	11	0	9	2	11	0	7	4	10	1			4	7	10	1	11	0	11	0	11	0	11	0		
P	0.649		0.749		0.770		0.729		0.572		0.794		0.701		0.001**				0.725		0.616		0.896		0.729		0.694		0.343			
PD-L1																																
–	565	8	569	4	569	4	566	7	429	144	569	4	390	183	568	5			234	339	543	30	572	1	567	6	567	6	525	48	629	7
+	80	4	82	2	83	1	84	0	60	24	84	0	63	21	84	0			39	45	77	7	84	0	83	1	81	3	83	1	88	7
P	0.031*		0.130		0.628		0.309		0.500		0.443		0.200		0.391				0.332		0.251		0.702		0.905		0.063		0.019*		0.001***	

*, indicates P value <0.05; **, indicates P value <0.01; ***, indicates P value <0.001. ALK, anaplastic lymphoma kinase; ROS-1, ROS proto-oncogene 1 receptor tyrosine kinase; RET, Ret proto-oncogene; EGFR, epidermal growth factor receptor; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; BRAF, B-raf proto-oncogene; PIK3CA, phosphoinositide-3-kinase catalytic alpha polypeptide; HER2, human epidermal growth factor receptor 2; PD-1, programmed death-1; PD-L1, programmed death-ligand 1. The underlined P values indicate a positive correlation. G719A/C, glycine to alanine or cysteine mutation at amino acid position 719; 19-del, EGFR 19 deletions; T790M, substitution of threonine with methionine at amino acid position 790; S768I, serine to isoleucine mutation at amino acid position 768; L858R, leucine to arginine mutation at amino acid position 858; L861Q, leucine to glutamine mutation at amino acid position 861.