Association of histologic subtypes with genetic alteration and PD-L1 expression in pulmonary adenocarcinoma

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Abstract. Genetic alteration and programmed death-ligand 1 (PD-L1) expression have been revealed to be associated with various subtypes of pulmonary adenocarcinoma (ADC). The present study aimed to explore the association between histological subtypes and genetic alterations and PD-L1 expression. A total of 375 cases of pulmonary ADC were included. Genetic alterations were determined using next generation sequencing (NGS) in 136 cases. PD-L1 expression was detected by immunohistochemistry (based on clone 22C3) in the remaining 239 cases. Mutations in the epidermal growth factor receptor gene (*EGFR*) were detected in 76 (55.8%) cases associated with the papillary subtype (P=0.038). Mutations in the Kirsten rat sarcoma viral oncogene homolog gene (*KRAS*) were present in 46 (33.8%) cases associated with the lepidic subtype (P<0.001) and mucinous ADC (P=0.037). PD-L1 expression was identified in 63 (26.4%) cases associated with the solid subtype (P<0.001). In conclusion, the present study demonstrated that *EGFR* and *KRAS* mutations, alongside PD-L1 protein expression are significantly associated with specific subtypes of pulmonary ADC. These results should aid our ability to accurately select appropriate areas of the heterogeneous tumor for molecular testing methods and to predict patient outcomes and prognosis.

Introduction

Lung cancer is the common carcinoma worldwide in both men and women, and is the leading cause of carcinoma-related death (1). Lung cancer is classified into two main subtypes: Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which are identified in 85 and 15% of diagnosed patients, respectively. NSCLC may be classified further into three subtypes: Adenocarcinoma (ADC) is the most common type (60%), followed by squamous cell carcinoma (SCC) and large cell carcinoma (LCC). The incidence of ADC has increased greatly over the course of the last few decades (2). Several previous studies have identified genetically driven mutations associated with the tyrosine kinase receptor pathway in patients with NSCLC, especially in the case of ADC, including mutations of the genes for epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), in addition to translocation of the anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*) and rearranged during transfection (*RET*) genes (3-8), which have led to novel treatment outcomes involving specific targeted tyrosine kinase inhibitors (TKIs). In terms of the histological evaluation of ADC, the recently formed International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classified invasive ADC according to its predominant growth pattern, including lepidic, acinar, papillary, micropapillary and solid subtypes, as well as ADC variants, including mucinous, colloid, enteric and fetal ADC (9). Recently published studies have demonstrated that the histological subtypes of pulmonary ADC are associated with 5-year survival, overall survival and disease-free survival rates across all tumor stages during treatment with adjuvant therapy (4,10-11). Histological subtypes and molecular factor correlations have also been reported on. Previous studies revealed that the *EGFR* mutation is associated with the lepidic and papillary subtypes (4,12), whereas solid subtypes are predominantly associated with the *ALK* mutation (13). Another study suggested that the predominance of papillary features in ADC is predictive of the response to gefitinib, a finding that implied that the *EGFR* mutation could be more common among this subtype (14).

In advanced stage lung cancer, a large body of evidence derived from clinical trials has led to an understanding that immunotherapy is more effective than systemic chemotherapy in terms of treating unresectable or advanced NSCLC (15-19). In the field of cancer immunotherapy, an understanding of the complex interplay between the tumor and the immune systems has been proposed. Programmed death 1 (PD-1) protein, a T-cell co-inhibitory receptor that binds to its ligand, programmed death-ligand 1 (PD-L1), serves a pivotal role

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in regulating T-cell activation and proliferation, functioning as an immune checkpoint. The mechanism of PD-1/PD-L1 interaction provides one of the major pathways used by certain tumors to escape immune surveillance (20,21). Studies using various PD-L1 detection antibodies and immunohistochemistry (IHC) assays have identified that a high level of PD-L1 expression in tumor cells is associated with poor prognosis in NSCLC (22,23), whereas in other studies, PD-L1 expression was associated with longer survival rates (24,25). The association between PD-L1 expression and clinicopathological characteristics, including histopathological parameters, revealed no significant correlations in certain studies (23,26), whereas another study identified that PD-L1 expression was associated with more advanced tumor status, node involvement status and the pathological stage. A further study suggested that PD-L1 expression was likely to be predominantly associated with the solid subtype (27).

This aim of the present study was to identify the association between histological subtypes of pulmonary ADC in the Thai population and genetic alterations identified by next-generation sequencing (NGS), as well as PD-L1 expression identified by PD-L1 (clone 22C3) IHC. The association among clinicopathological parameters and PDL1 expression was also explored.

Patients and methods

Patients. The study composed of 375 cases of pulmonary ADC diagnosed at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (Bangkok, Thailand) during 2013-2017 divided into two separated groups. A total of 136 cases in the first group had known genetic alteration detected by NGS and 239 cases in the second group had PD-L1 expression testing by IHC. Clinical features and patient characteristics including age, sex, smoking history, specimen size, specimen site, and staging were obtained from the patient's medical record.

Histologic evaluation. The pathologic report was retrospectively reviewed in all cases. The glass slides from transbronchial biopsy (TBBX), transthoracic needle biopsy (TTNB), Video-assisted thoracoscopic surgery (VATS) for wedge resection, lobectomy, pleural biopsy, and tumor removal specimen were retrieved and reviewed. The histologic subtype from all cases was recorded as predominant lepidic, acinar, papillary, micropapillary or solid subtype and mucinous ADC variant. The histologic subtypes are examined by the several first senior pathologists who assigned the final pathological reports. The pathology trainee and the second senior pathologist are re-analyzed the histologic subtype independently.

Genetic mutation analysis. All patients in genetic mutation group are performed by next generation sequencing (NGS). DNA extracted from NSCLC FFPE tumor using QIAsymphony DSP DNA Mini kit in the QIAsymphony SP system (Qiagen Inc.) with the protocol provided by the manufacturer. A total of 20 ng of extracted DNA has been measured by Qubit fluorometer using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Inc.) before proceeded to the DNA library step. Genomic DNA was fragmented then selected target regions of 45 genes associated with lung cancer, using Human Lung Cancer Panel (NGHS-005X) from GeneRead DNAseq Targeted Panels v2 (Qiagen Inc.) according to the manufacturer's instructions. The target genes of the Human Lung Cancer Panel (NGHS-005X) are as follow; *AKT1*, *ALK*, *APC*, *ATM*, *BAI3*, *BAP1*, *BRAF*, *CDKN2A*, *EGFR*, *EPHA5*, *ERBB2*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR2*, *GRM8*, *KDR*, *KEAP1*, *KIT*, *KMT2D*, *KRAS*, *LRP1B*, *MDM2*, *MET*, *MLH1*, *MUC16*, *MYC*, *NF1*, *NFE2L2*, *NOTCH1*, *PDGFRA*, *PIK3CA*, *PIK3CG*, *PKHD1*, *PTEN*, *RARB*, *RB1*, *RET*, *ROS1*, *RUNX1T1*, *SMAD4*, *SMARCA4*, *SOX2*, *STK11* and *TP53*. Variant discovery analysis was performed with VarSeq v2.1.1 (Golden Helix, Inc.). Only variants that have read depths (DP)≥10 x coverage, genotype quality (GQ)≥20 and presented more than 3% were selected.

PD-L1 IHC. In this study used Dako PD-L1 IHC 22c3 pharmDx SK006 IHC assay by using monoclonal mouse anti-PD-L1, clone 22c3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue and using Envision FLEX visualization system on Autostainer link 48 platform. The tonsil tissues were used as the internal control.

A minimum 0f 100 viable tumor cells are considered adequate for PD-L1 evaluation. Any perceptible membrane staining of tumor cells are included in the scoring. PD-L1 protein expression is determined by using tumor proportion score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The immune cells and normal cells are not included in the scoring. The TPS are recorded in negative/no PD-L1 expression (TPS<1%), weak positive PD-L1 expression (TPS1-49%), and strong positive/high PD-L1 expression (TPS≥50%) (28).

Statistical analysis. The relationship between genetic mutations and histologic subtypes was evaluated using Fisher exact/χ^2 test. Odds ratio (OR) was calculated in predominant histologic subtypes correlate with *EGFR* and *KRAS* mutation.

The association between PD-L1 expression and clinicopathologic variables were using Fisher's exact/ Chi-square test. OR was calculated in predominant histologic subtypes associate with PD-L1 expression.

SPSS (v25.0.0.0) was used for data analysis. P<0.05 was considered to indicate a statistically significant difference and OR>1.0 indicates an increase risk among the compared histologic subtype, whereas OR<1.0 indicates a decrease in risk.

Results

Genetic alterations determined from the NGS study. Patient characteristics. From a total of 136 patients with known genetic alterations identified via the NGS analysis, 82 (60.2%) cases were women and 54 (39.7%) cases were men. The range of ages, and median age were found to be 28-65 and 63 years, respectively. Specimens were collected from the primary site in 109 (80.1%) of the cases, whereas they were collected from the metastatic site, including brain, bone, lymph node, liver, pleura and pericardium, in the other 27 (19.9%) cases. The size of tissue sample taken in 101 (74.3%) cases was ≥0.5 cm in its greatest dimension based on lobectomy, wedge resection, excision, and biopsy, whereas 35 (25.7%) cases were of biopsy specimen size <0.5 cm in diameter. A total of

78 (57.3%) cases were non-smokers, whereas 58 (42.7%) cases were ex- and current smokers. The majority of the patients [103 (75.7%) cases] were at stage IV of the disease. The patient characteristics are highlighted in Table I.

Association between histological features and genetic mutation. Based on the 136 samples, the tumors were classified into acinar, papillary, lepidic, micropapillary and solid predominant subtypes in 57 (41.2%), 24 (17.6%), 20 (14.7%), 6 (4.4%) and 3 (2.2%) cases, respectively. The mucinous ADC variant was identified in 26 (19.1%) cases. The *EGFR* mutation was detected in 76 (55.8%) cases, followed by the identification of the *KRAS* mutation in 46 (33.8%) cases. *BRAF*, *MET*, *AKT1*, *ROS* and *PTEN* mutations were identified in 12 (8.8%), 11 8.0%), 8 (5.9%), 3 (2.2%) and 1 (0.7%) cases, respectively. *EGFR-KRAS*, *EGFR-BRAF* and *EGFR-KRAS-BRAF* co-mutations were present in 20, 8 and 3 cases, respectively. The associations identified between the genetic mutation and the predominant histological subtype are shown in Table II. Only the *EGFR* and *KRAS* mutations were shown to have a significant level of association with the histological subtype (P=0.050 and P<0.001, respectively). Among the cases with *EGFR* and *KRAS* mutations, the papillary predominant subtype was significantly associated with the *EGFR* mutation (P=0.038; Table III). Lepidic subtype and mucinous ADC variant were the parameters found to be significantly associated with the *KRAS* mutation (P<0.001 and P=0.037, respectively; Table III).

PD-L1 expression Patient characteristics. Out of the 239 patients, 135 (56.5%) cases were women and 104 (43.5%) cases were men. The mean age was 63 years. The range of ages, and median age were 28-89 and 63 years, respectively. Ninety-one (58.7%) patients were non-smokers, and 64 (41.3%) were ex- and current smokers. The majority of the patients were at stage IV of the disease [117 (85.4%) cases]. The specimens were collected from the primary site in 182 (76.2%) cases, and collected from the metastatic sites, including the brain, bone, lymph node, liver, pleura and skin, in the remaining 57 (23.8%) cases. A total of 170 (71.1%) specimens taken via lobectomy, wedge resection, excision, and biopsy measured ≥0.5 cm in their greatest dimension, whereas 69 (28.9%) specimens were of biopsy size <0.5 cm in diameter (Table IV).

Association between clinicopathological parameters and PD-L1 expression. A pattern of PD-L1 expression was identified in 63 (26.4%) of the 239 patients, including 12 (5%) cases of strong positive (TPS≥50%) and 51 (21.4%) cases of weak positive (TPS≥1%) expression. A total of 176 (73.6%) cases were negative for PD-L1 expression (TPS<1%). PD-L1 expression was found to be significantly associated with specimen size ≥0.5 cm (P=0.037), whereas no significant associations were identified comparing PD-L1 expression with the parameters of age, gender, staging or site of the tumor (primary vs. metastasis; Table IV).

Association between histological features and PD-L1 expression. The most common histological subtype was the acinar subtype in 107 (44.8%) cases, followed by solid subtype in 55 (23.0%) cases, papillary subtype in 36 (15.1%) cases, lepidic subtype in 25 (10.4%) cases, micropapillary subtype in 10 (4.2%) cases, and mucinous ADC variant in 6 (2.5%) cases. The solid subtype was significantly associated with PD-L1 expression (P<0.001; OR, 3.96) in terms of positive prediction. On the other hand, the lepidic subtype was significantly associated with PD-L1 expression in terms of the negative predictive value (P=0.007; OR, 0.102; Table V).

Table I. Patient characteristics of 136 cases of the genetic mutation group.

Variables	N (%)
Age, years range (median)	28-65 (63)
Mean	62.5
<60 years	45 (33.1)
≥60 years	91 (66.9)
Sex	
Male	54 (39.7)
Female	82 (60.3)
Specimen type	
Primary	109 (80.1)
Metastatic[a]	27 (19.9)
Specimen size	
<0.5 cm	37 (27.2)
≥0.5 cm	99 (72.8)
Smoking status	
Non-smokers	78 (57.3)
Ex- and current smokers	58 (42.7)
Tumor staging, n (%)	
IA	4 (3.0)
IB	6 (4.4)
IIA	12 (8.8)
IIIA	7 (5.1)
IIIB	4 (3.0)
IV	103 (75.7)

[a]Brain, bone, lymph node, liver, pleura and pericardium.

Discussion

The present study has revealed that the most common histological subtype identified in lung ADC regarding the IASLC/ATS/ERS classification system was the acinar subtype (43.0%). The *EGFR* mutation was the most common mutation, which was detected in 76 out of 136 (55.8%) cases. The histological subtype with the highest level of significance in terms of its association with the *EGFR* mutation, based on the present study, was the papillary subtype (P=0.038; OR 2.793). This finding concurred with those of previous studies: A significant association between EGFR and micropapillary, lepidic, acinar or papillary subtypes were identified in studies by Wang *et al* (29) who analyzed 153 cases, Li *et al* (30) who investigated 230 cases, Yoshizawa *et al* (31) who analyzed 440 cases, and Dong *et al* (32) who analyzed 330 cases. In investigating *KRAS*-mutated NSCLC, several

Table II. Association between histological subtypes and genetic mutation in the 136 cases.

	Histologic subtypes						
Genetic mutation (n)	Lepidic	Acinar	Papillary	Micropapillary	Solid	Mucinous	P-value
EGFR (76)	12	33	18	3	10	0	0.050
KRAS (46)	14	11	4	2	12	3	<0.001
BRAF (12)	3	6	3	0	0	0	0.393
MET (11)	3	3	3	0	2	0	0.639
AKT1 (8)	3	1	2	0	1	1	0.063
ROS (3)	1	0	1	0	1	0	0.347
PTEN (1)	0	1	0	0	0	0	NA

NA, not available.

Table III. Association between histological subtypes and *EGFR* (n=76) and *KRAS* mutations (n=46) in genetic mutation group.

	EGFR mutation (n=76)				*KRAS* mutation (n=46)			
Histologic subtypes	Positive, n (%)	Negative, n (%)	P-value	OR	Positive, n (%)	Negative, n (%)	P-value	OR
Lepidic	12 (15.7)	64 (84.2)	0.688	1.219	14 (30.5)	32 (69.5)	<0.001	6.125
Acinar	33 (43.4)	43 (56.6)	0.688	1.151	11 (23.9)	35 (76.1)	0.002	0.301
Papillary	18 (23.7)	58 (76.3)	0.038	2.793	4 (8.7)	42 (91.3)	0.050	0.333
Micropapillary	3 (3.9)	73 (96.1)	1.000	0.718	2 (4.3)	44 (95.7)	1.000	0.977
Solid	10 (13.2)	66 (86.8)	0.047	0.417	12 (26.1)	34 (73.9)	0.140	1.916
Mucinous	0 (0.0)	76 (100.0)	NA	NA	3 (6.5)	43 (93.5)	0.037	NA

NA, not available; OR, odds ratio; *EGFR*, epidermal growth factor receptor gene; KRAS, Kirsten rat sarcoma viral oncogene homolog gene.

studies have demonstrated that the *KRAS* mutation is associated with mucinous ADC, including studies by Dong *et al* (32) (330 cases) and Marchetti *et al* (33) (58 cases). A study by Kadota *et al* (34), that included 864 patients with ADC revealed that mucinous ADC, as well as extracellular mucin production, were also significantly associated with the *KRAS* mutation. The *KRAS* mutation in the present study was significantly associated with mucinous ADC variant (P=0.037), a finding that was in accordance with all mentioned studies (32-34). On the other hand, in the present study, the subtype for which the predominantly significant association was identified was the lepidic (P<0.001) subtype, as opposed to the solid subtype. There was a possibility of contrast outcome, which might have been influenced by the *KRAS* mutation identified in our study: In 23 of 46 cases, *KRAS* was mainly co-mutated with *BRAF* and/or *EGFR* mutations, resulting in predominant patterns that differed with respect to other co-mutations, especially *EGFR*. Moreover, the acinar predominant subtype was also identified to be statistically significant as a protective prediction factor, and it was unlikely to harbour the *KRAS* mutation (P=0.002; OR, 0.301). The acinar pattern was predominantly found in 12 cases that harboured the *BRAF* mutation; however, no statistically significant association was identified in these cases (P=0.393).

PD-1/PD-L1 expression in NSCLC has been widely studied in terms of exploring significant associations with clinicopathological parameters and prognostic indicators. A meta-analysis of 11,444 cases from 47 studies by Zhang *et al* (35) revealed that the expression of PD-L1 was increased in males, smokers, patients with SCC, and patients with a higher histological grade, larger tumor size, nodal metastasis, and later clinical stage. Another meta-analysis of 1,157 patients from 6 studies by Wang *et al* (23) concluded that PD-L1 expression was significantly associated with poorly differentiated tumors, resulting in poor overall survival of the patients with NSCLC, whereas a meta-analysis of 1,550 patients from 9 studies by Pan *et al* (36) demonstrated that the majority of the clinicopathological features, including histological type, smoking status, tumor depth, lymph node status and tumor-lymph node-metastasis (TNM) stage, were not significantly associated with PD-L1 expression, with the exception of tumor differentiation in NSCLC. A meta-analysis of 3,128 patients from 11 studies by Yang *et al* (37), also failed to reveal any significant associations among clinicopathological characteristics and PD-L1 expression in patients with NSCLC. These data may be compared with the present study, in which specimen size was the only clinicopathological parameter that exerted a significant impact on PD-L1 expression. The larger tumor size (≥0.5 cm in the greatest dimension) was more significantly associated with PD-L1 expression compared with the smaller tumor size (<0.5 cm; P=0.037).

Table IV. Association between clinicopathological characteristics and PD-L1 expression in 239 cases of PD-L1.

		PD-L1 expression		
Parameters	N (%)	Negative[a] (n)	Positive[b] (n)	P-value
Age, years, n (%)				0.266
Range, median	29-89, 63			
Mean	63.7			
<60 year	74 (31.0)	58	16	
≥60 year	165 (69.0)	118	47	
Sex, n (%)				0.177
Male	104 (43.5)	73	31	
Female	135 (56.5)	103	32	
Smoking status				0.266
Non-smokers	91 (58.7)	67	24	
Ex- and current smokers	64 (41.3)	41	23	
Tumor staging				0.392
IA	3 (2.2)	3	0	
IB	9 (6.6)	9	1	
IIA	2 (1.5)	2	0	
IIIA	4 (2.9)	4	0	
IIIB	2 (1.5)	1	1	
IV	117 (85.4)	80	37	
Specimen type				0.087
Primary	182 (76.2)	139	43	
Metastatic[c]	57 (23.8)	37	20	
Specimen size				0.037
<0.5 cm	69 (28.9)	51	18	
≥0.5 cm	170 (71.1)	125	145	

[a]PD-L1 negative, TPS<1%. [b]PD-L1 positive, TPS≥1%. [c]brain, bone, lymph node, liver, pleura and skin. PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

Table V. Association between histological subtypes and PD-L1 expression in 239 cases of PD-L1.

		PD-L1 expression			
Histologic subtypes	n (%)	Negative[a] (n)	Positive[b] (n)	P-value	OR
Lepidic	25 (10.4)	24	1	0.007	0.102
Acinar	107 (44.8)	80	27	0.722	0.900
Papillary	36 (15.1)	30	6	0.152	0.152
Micropapillary	10 (4.2)	9	1	0.462	0.299
Solid	55 (23.0)	28	27	<0.001	3.964
Mucinous	6 (2.5)	5	1	>0.999	0.952

[a]PD-L1 negative, TPS<1%. [b]PD-L1 positive, TPS≥1%; PD-L1, programmed death-ligand 1; OR, odds ratio; TPS, tumor proportion score.

This could be accounted for by the fact that, the larger the specimen size, the more likely it is that there will be enhanced PD-L1 expression due to tumor heterogeneity. The remaining clinical parameters, including smoking status, age, sex, stage, and specimen type, were not found to be significantly associated with PD-L1 expression, and these results were in accordance with those of Pan *et al* (36) and Yang *et al* (37). The discrepancies identified in the results obtained from these meta-analyses might have been due to the differing sample sizes, differences in subtype among the patients with NSCLC,

variable levels of PD-L1 expression detected using different assays, and other interpretation criteria.

More recent studies on PD-L1 expression in pulmonary ADC have also been published. PD-L1 expression in these studies was detected according to standardized Food and Drug Administration (FDA) approved assays, including the 22C3 PharmDx assay (Agilent Technologies/Dako) (38,39), the Ventana PD-L1 SP142 assay (Ventana Medical Systems Inc.) (40), and the Ventana PD-L1 SP263 assay (Ventana Medical Systems Inc.) (41,42). Each IHC antibody clone is associated with a specific type of targeted therapy. In the present study, Dako PD-L1 IHC 22c3 pharmDx was used, which is the IHC study that makes use of pembrolizumab. Pembrolizumab has been evaluated in large-scale clinical trials; patients with TPS≥50% are eligible to receive pembrolizumab as first-line therapy, and these have been shown to have significantly improved progression-free survival and overall survival rates compared with those patients with TPS<1% or 1-49% (43). Patients with TPS≥1% subsequently treated in a trial with pembrolizumab were shown to have significantly improved overall survival rates and risk-benefit profiles that reached the level of clinical relevance compared with standard care chemotherapy (43). Among the PD-L1 expression studies in patients with pulmonary ADC using PD-L1 IHC clone 22C3, Pan *et al* (38) identified PD-L1 expression (TPS≥1%) in 4.1% of the total of 221 cases, including 1 (0.5%) case with TPS≥50%. Miyazawa *et al* (39) identified PD-L1 expression (TPS≥1%) in 21% of 78 cases, including TPS≥50% in 7 (9%) cases, percentages that were more comparable with those in our study (TPS≥1%, 21 vs. 23%; and TPS≥50%, 9 vs. 5%). They also reported that PD-L1 tends not to be expressed in early invasive ADC or well differentiated subtypes, similar to our finding that negative PD-L1 (TPS<1%) expression was found to be associated with the lepidic subtype (P=0.007). On the other hand, Driver *et al* (40) identified a larger number of cases of PD-L1 expression (44% of 125 cases) compared with our study, possibly due to their use of a different PD-L1 IHC clone (SP142), and the criteria for positive PD-L1 expression from their study also included positivity of infiltrating immune cells.

In histological terms, solid predominant ADC was found to be the cancer type that was consistently associated with high PD-L1 expression. The studies by Pan *et al* (38) and Driver *et al* (40) demonstrated significantly higher PD-L1 expression levels in solid vs. non-solid subtypes of ADC. Our results also revealed a significant association between the solid predominant histological subtype and PD-L1 expression, consistent with those two studies. Moreover, the large genetic and immune profiles of solid ADC published by Dong *et al* (42) from 194 patients with lung ADC also demonstrated that solid ADC is significantly associated with a higher smoking index, tumor mutation burden and adaptive immune resistance (highly PD-L1-expressing tumor cells and highly CD-8 positive tumor-infiltrating lymphocytes), findings which draw attention to the benefits associated with using PD-1/PD-L1 immune checkpoint inhibitors in patients with solid predominant ADC.

It should be noted that our results did reveal certain differences compared with those of previous studies, possibly resulting from several limitations. Firstly, in pattern recognition that exist in small biopsies, which may be distortions of the true predominant pattern one would identify from the whole resection specimen. Secondly, dynamic changes in expression occurring between the primary tumour and the metastatic sites may also affect the histological pattern and/or genetic alterations or PD-L1 expression, and these possibilities should be investigated in future studies.

In conclusion, in the present study patients from a Thai population with lung ADC were collected and analyzed according to the histological subtypes in terms of their genetic mutations and PD-L1 expression levels. The *EGFR* and *KRAS* mutations were the first and second most common genetic alterations identified in the present study. The papillary histological subtype was associated with a high incidence of *EGFR* mutations. The lepidic subtype and mucinous ADC variant were significantly associated with the *KRAS* mutation. PD-L1 expression (TPS≥1%) was identified in 26% of the patients, and the solid subtype was associated with PD-L1 protein expression. Genetic mutations and the PD-L1 expression level therefore may serve as representative predictive factors for lung ADC, and may be of use in terms of targeted immunotherapeutic strategies. The histological subtype associated with the lung tumor is worthy of investigation in terms of identifying the genotype-phenotype association. These results may be helpful in selecting appropriate areas of the heterogeneous tumor feature for the patients who require personalized therapy, and predicting their prognosis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

PI conceived the study, participated in its design, coordinated the study, analyzed the data and drafted the manuscript. BP participated in study design and coordination, and drafted the manuscript. TR participated in provided clinical data and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Committee on Human Rights Related to Research Involving Human Subjects (Faculty of Medicine, Ramathibodi Hospital, Mahidol University; protocol no. 11-61-45). Consent for tissue collection and use was collected prior to experimentation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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