

ORIGINAL ARTICLE



Tumor necrosis correlates with PD-L1 and PD-1 expression in lung adenocarcinoma

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ABSTRACT

Background: Predictive biomarkers for immunotherapy in lung cancer are intensively investigated; however, correlations between PD-L1/PD-1 expressions and clinical features or histopathological tumor characteristics determined on hematoxylin and eosin stained sections have not extensively been studied.

Material and methods: We determined PD-L1 expression of tumor cells (TC) and immune cells (IC), and PD-1 expression of IC by immunohistochemistry in 268 lung adenocarcinoma (LADC) patients, and correlated the data with smoking, COPD, tumor grade, necrosis, lepidic growth pattern, vascular invasion, density of stromal IC, and *EGFR/KRAS* status of the tumors.

Results: There was a positive correlation between PD-L1 expression of TC and IC, as well as PD-L1 and PD-1 expression of IC. Tumor necrosis was associated with higher PD-L1 expression of TC and PD-1 expression of IC. A negative correlation was observed between lepidic growth pattern and PD-L1 expression of TC and PD-L1/PD-1 expression of IC. *EGFR* mutation seemed to negatively correlate with PD-1 expression of IC, but this tendency could not be verified when applying corrections for multiple comparisons. No significant effect of the *KRAS* mutation on any of the studied variables could be established.

Conclusion: Here we first demonstrate that the presence of necrosis correlates with higher PD-L1 expression of TC and PD-1 expression of IC in LADC. Further studies are required to determine the predictive value of this observation in LADC patients receiving immunotherapy.

Abbreviations: COPD: chronic obstructive pulmonary disease; FFPE: formalin-fixed paraffin-embedded; H&E: hematoxylin and eosin; IC: immune cells; LADC: lung adenocarcinoma; NSCLC: non-small cell lung cancer; PD-L1: programmed cell death ligand-1; PD-1: programmed cell death-1; SCC: squamous cell carcinoma; TC: tumor cells

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Introduction

Lung cancer is the leading cause of cancer death worldwide; therefore, new therapeutic strategies are of great importance. During recent years, immunotherapy has become a promising treatment modality for advanced non-small cell lung cancer (NSCLC). Since immunotherapeutic agents are rather costly, particular emphasis should be placed on appropriate patient selection.

Up to now, programmed cell death ligand-1 (PD-L1) protein expression has been the only biomarker available in clinical

practice. PD-L1 staining, however, is not an ideal marker for predicting response to immunotherapy, as only half of the patients with a high PD-L1 level are responders, by contrast, certain patients with a PD-L1 negative tumor can also respond to immunotherapy [1].

Correlations between PD-L1/PD-1 (programmed cell death-1) expression and *EGFR/KRAS* status have already been investigated; however, the results of these studies are controversial [2].

Although some publications investigated the relationship between the PD-L1/PD-1 axis and Chronic Obstructive

Pulmonary Disease (COPD), to the best of our knowledge, a thorough exploration of the correlation between PD-L1/PD-1 expression and COPD in lung cancer patients has not yet been reported [3].

There are certain histological features described in routine diagnostics during the examination of hematoxylin and eosin (H&E) stained sections, such as tumor grade, lepidic growth pattern, presence of necrosis, vascular invasion, and density of stromal immune cells (IC), which have no direct therapeutic consequence [4,5]. The correlation between these parameters and PD-L1/PD-1 expression has never been investigated in lung adenocarcinoma (LADC), except for the correlation between lepidic growth pattern and PD-L1 expression of tumor cells (TC), which was found to be negative [6–8]. These studies, however, did not analyze the PD-L1/PD-1 expression of IC.

The aim of the present study was to examine the correlations between PD-L1 expression of both TC and IC, PD-1 expression of IC and certain clinical characteristics and histological parameters determined during routine histological examination. We also aimed to study correlations between PD-L1/PD-1 expression and *EGFR/KRAS* status in a large cohort of LADC.

Material and methods

We determined PD-L1 expression of TC and IC, and PD-1 expression of IC by immunohistochemistry in a large cohort of LADC patients, and correlated the data with specific clinicopathological characteristics.

Tissue samples

Formalin-fixed paraffin-embedded (FFPE) tissue samples of 268 patients with surgically resected primary LADC were selected for further analyses from the archive of the National Koranyi Institute of Pulmonology, Budapest, Hungary. Tumors were classified according to the latest WHO Classification [9]. Clinicopathological data including age, gender, smoking history (never-, ex- and current smoker), presence of COPD, and *EGFR/KRAS* molecular status of the tumor were recorded. Permissions to use the archived tissue have been obtained from the Regional Ethical Committee (ETT-TUKEB nos. 510/2013, 86/2015, 77-6/2016), and from the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (SE-TUKEB 241/2016). The clinicopathological characteristics of all patients are summarized in Table 1.

Histological characteristics of H&E-stained sections

The tumor grade, lepidic growth pattern, presence of necrosis, vascular invasion, and density of stromal IC were determined on H&E stained section of each sample by two independent pathologists. Lepidic growth pattern was defined as a binary variable: samples were deemed to have lepidic growth pattern, whenever it was present in at least 20% of the tumor area.

Table 1. Clinical and pathological data of lung adenocarcinoma patients.

Characteristics	No	%
Patients	268	
Age (years)		
Mean ($\pm$ SD)		68.76 ($\pm$ 8.64)
Range		36–85
Gender		
Male	125	46.6
Female	143	53.4
Smoking status	231	
Current-smoker	114	49.4
Ex-smoker	66	28.6
Non-smoker	51	22.0
COPD	244	
Yes	88	36.1
No	156	63.9
Tumor grade	267	
1	22	8.3
2	207	77.5
3	38	14.2
Lepidic growth pattern	268	
$\geq$ 10%	92	34.3
<10%	176	65.7
Presence of necrosis	268	
Present	154	57.5
Not present	114	42.5
Vascular involvement	268	
Present	94	35.1
Not present	174	64.9
<i>EGFR/KRAS</i> status	154 ^b	
<i>EGFR</i> mutant ^a	23	14.9
<i>KRAS</i> mutant	45	29.2
Double wild type	86	55.9

^aOnly classical sensitizing mutations included.

^bSeven overlaps between *EGFR* & *KRAS* mutations.

Tumor necrosis was defined as present if any amount of necrotic tissue was visible in the tumor mass. Necrotic tumor tissue was specified as an area showing increased eosinophilia without tumor cell nuclei or with nuclear shrinkage or fragmentation. In some necrotic areas shadows of tumor cells were also visible (Supplementary Figure 1).

The amount of stromal mononuclear IC, including lymphocytes, histiocytes and plasma cells was determined on H&E stained sections, and was recorded by a semi-quantitative method as follows: (1) no or minimal (2) moderate or (3) intense infiltration of IC.

Immunohistochemistry

Immunohistochemistry for PD-L1 (clone SP142, dilution 1:100; Spring Bioscience, Ventana; Oro Valley, AZ) and PD-1 (ab52587, dilution 1:100; Abcam, Cambridge, UK) was performed on 3- μ m thick sections of tissue microarray blocks; each case was represented by 3 cores. Antigen retrieval was carried out using Leica Bond-Max automated immunostaining system (Leica Biosystems, Wetzlar, Germany) according to standard laboratory practice. The amount of positive TC and IC was determined by a semi-quantitative method as percentage of positive cells. For TC 1%, 5%, 10%, and 50%, while for IC 1%, 5%, and 10% cutoff levels were recorded (Supplementary Figure 2), which are the most commonly used thresholds [10,11].

EGFR and KRAS mutation status analysis

EGFR and KRAS mutation analyses were performed as described previously [12–16]. Briefly, regions of tumor samples embedded in paraffin blocks containing the highest concentrations of TC were macrodissected. DNA was extracted using the MasterPure DNA Purification Kit according to the manufacturer's instructions. KRAS mutations were identified by microcapillary-based restriction fragment length analysis followed by Sanger sequencing. For EGFR mutations, polymerase chain reaction amplification of the EGFR exons 18, 19, 20, and 21 was performed as the first step, followed by bidirectional Sanger sequencing.

Statistical analysis

Spearman correlation between different variables was calculated using python version 3.5.3 with the help of the scipy-stats statistical package. Corrections for multiple comparisons were obtained using the multiple tests function of the statsmodels.sandbox.stats.multicomp package with the 'holm' method selected for p -value (p^*) adjustment and .05 set as the significance level.

In the results section, those comparisons are described that have a p -value of lower than .05 and an absolute Spearman correlation coefficient (R -value) of at least 0.20 in the investigated cohort. Results obtained by correcting for multiple comparisons are discussed as well.

As true associations between quantitative variables are best detected without applying artificial cutoff levels, we aimed at establishing significant correlations using the original semi-quantitative scale of the histology parameters described above. However, as all the values of this scale are commonly used cutoff levels to separate patients into two distinct groups in both previous studies and clinical practice [17,18], additional investigations were carried out to determine significant correlations when patients are stratified based on a given threshold level.

Results

Patients' characteristics

Clinical data including age, gender, smoking status, and presence of COPD, together with pathological data including tumor grade, lepidic growth pattern, presence of necrosis, vascular involvement and EGFR/KRAS status are summarized in Table 1.

Correlation between tumor grade and necrosis or lepidic growth pattern

Tumor grade showed a positive correlation with the presence of necrosis ($R = 0.325$, $p^* = .014$) and a negative correlation with lepidic growth pattern ($R = 0.339$, $p^* < .001$).

Correlation between the PD-L1 and PD-1 protein expression

There was a strong positive correlation between PD-L1 expression of TC and IC without cutoff levels ($p < .001$, $R = 0.430$), which result remained significant when applying correction for multiple comparisons as well ($p^* < .001$).

A positive correlation could be observed between PD-L1 expression of TC and PD-1 expression of IC without cutoff levels ($p < .001$, $R = 0.250$), as well as between PD-L1 and PD-1 expression of IC ($p < .001$, $R = 0.289$). However, only the correlation between PD-L1 and PD-1 expression of IC remained significant when applying correction for multiple comparisons ($p^* = .003$). By using different cutoff levels, significant results could be achieved for the PD-L1 TC and PD-1 IC correlation as well, even when corrected for multiple comparisons (see Table 2).

Correlation of PD-L1/PD-1 expression and clinical parameters

Smoking status showed a relatively strong correlation with PD-1 expression of IC using no cutoff levels ($p < .001$, $R = 0.275$), which tendency remained significant with the use of multiple comparison correction as well ($p^* = .037$) (Table 3).

There were no correlations between the PD-L1/PD-1 expression and the other clinical parameters including age, gender, and COPD (data not shown).

Correlation of PD-L1/PD-1 expression and different histological parameters

Tumor grade

There was a positive correlation between the tumor grade and the PD-1 expression of IC ($p < .001$, $R = 0.231$) using no cutoff levels, which did not remain significant with corrections for multiple comparisons. However, using different cutoff levels, the obtained results are significant even with multiple comparisons corrections (Table 3). Very weak positive correlations were seen with PD-L1 expressions of IC and TC and, none of which remained significant with corrections (data not shown).

Tumor necrosis

The presence of tumor necrosis was associated with higher PD-L1 and PD-1 expression of IC and PD-L1 expression of TC with no cutoff levels ($p < .001$, $R = 0.215$; $p < .001$, $R = 0.290$ and $p < .001$, $R = 0.283$, respectively) (Table 3), the latter two remaining also significant with the use of correction for multiple comparisons ($p^* = .002$ for PD-1 IC and $p^* = 0.004$ for PD-L1 TC).

Lepidic growth pattern

Lepidic growth pattern was associated with lower PD-L1 expression of TC and IC, and PD-1 expression of IC ($p < .001$, $R = -0.329$; $p < .001$, $R = -0.306$ and $p < .001$, $R = -0.302$) with no cutoff levels, all of which results remained significant

Table 2. Correlations between PD-L1/PD-1 expression of tumor cells and immune cells in LADC.

	Percentage of positive cells	No cutoff			<1% versus $\geq 1\%$			<5% versus $\geq 5\%$			<10% versus $\geq 10\%$		
		p Value	Pearson's R	p*	p Value	Pearson's R	p*	p Value	Pearson's R	p*	p Value	Pearson's R	p*
PD-L1 tumor cells	No cutoff	<.001	0.456	<.001	<.001	0.360	PD-L1 immune cells	<.001	0.368	<.001	<.001	0.230	ns.
	<1% versus $\geq 1\%$	<.001	0.410	<.001	<.001	0.348	<.001	<.001	0.326	<.001	<.001	0.216	ns.
	<5% versus $\geq 5\%$	<.001	0.382	<.001	<.001	0.305	<.001	<.001	0.382	<.001	<.001	0.241	ns.
	<50% versus $\geq 50\%$	<.001	0.279	.007	<.001	0.219	ns.	<.001	0.307	<.001	.002	0.188	ns.
PD-L1 tumor cells	No cutoff	<.001	0.250	ns.	<.001	0.215	PD-1 immune cells	<.001	0.149	ns.	ns.	0.084	ns.
	<1% versus $\geq 1\%$	.007	0.167	ns.	<.001	0.205	ns.	.016	0.053	ns.	ns.	0.019	ns.
	<5% versus $\geq 5\%$	.005	0.174	ns.	.002	0.192	ns.	ns.	0.041	ns.	ns.	0.055	ns.
	<50% versus $\geq 50\%$	ns.	0.070	ns.	ns.	0.028	ns.	ns.	0.068	ns.	ns.	−0.004	ns.
PD-L1 immune cells	No cutoff	<.001	0.289	.003	<.001	0.287	PD-1 immune cells	<.001	0.137	ns.	ns.	0.081	ns.
	<1% versus $\geq 1\%$	<.001	0.264	.020	<.001	0.261	.004	.027	0.139	ns.	ns.	0.078	ns.
	<5% versus $\geq 5\%$	.042	0.126	ns.	.006	0.170	.027	.024	−0.008	ns.	ns.	0.025	ns.
	<10% versus $\geq 10\%$	ns.	0.087	ns.	.035	0.131	ns.	ns.	−0.004	ns.	ns.	−0.015	ns.

ns.: not significant; p*: corrected p-value with multiple comparisons.

after correction for multiple comparisons ($p^* < .001$ for all three) (Table 3).

Stromal immune cells

A positive correlation could be observed between the amount of stromal IC and the PD-L1 expression of TC without cutoff levels ($p < .001$, $R = 0.226$), which did not remain significant with the use of corrections for multiple comparisons.

No correlation could be established between the amount of stromal IC and the PD-L1/PD-1 expression of IC (data not shown).

Vascular invasion

There was no correlation between vascular invasion of the tumor and the PD-L1/PD-1 protein expression of TC and IC (data not shown).

Correlation of EGFR/KRAS mutation status and PD-L1/PD-1 expression or other histological and clinical parameters

EGFR mutation seemed to negatively correlate with PD-1 expression of IC with no cutoff ($p = .019$, $R = -0.225$) and with PD-L1 expression of TC with a 5% cutoff level ($p = .029$, $R = -0.212$), but neither of these trends were validated after using multiple comparisons correction.

No correlation could be observed between KRAS mutational status and any of the PD-L1/PD-1 expressions of IC and TC (data not shown).

The presence of EGFR mutation negatively correlated with smoking status ($p = .011$, $R = -0.255$), but this tendency did not remain significant after correction. Additional correlations were observed between EGFR mutation and other investigated parameters initially (Ilepidic growth: $p = .020$, $R = 0.222$; COPD: $p = .017$, $R = -0.236$; tumor necrosis: $p = .016$, $R = -0.231$), but none of these remained significant when applying multiple comparisons correction.

Similarly, no correlations could be established between KRAS mutation and any of the investigated parameters (data not shown).

Discussion

In this study, we have investigated correlations between tumor characteristics observed on routine H&E and on PD-L1/PD-1 stained sections.

We described for the first time the association between tumor necrosis and higher PD-L1 expression of TC and IC and PD-1 expression of IC in LADC. It may reflect a correlation with higher tumor grade, as necrosis and grade are significantly correlated. Chang et al. found similar results in pulmonary pleomorphic carcinomas, since high PD-L1 expression of TC significantly correlated with tumor necrosis and hypoxia-inducible factor-1 α [19]. We observed no direct anatomic correlation between tumor necrosis and PD-L1 expression of TC, however, higher PD-L1 expression might

Table 3. Correlations between PD-L1/PD-1 expression of tumor cells and immune cells and different histological characteristics in LADC.

		Percentage of positive cells	<i>p</i> value	Pearson's <i>R</i>	<i>p</i> *
Lepidic growth pattern	PD-L1 TC	No cutoff	<.001	−0.329	<.001
		<1% versus ≥1%	<.001	−0.280	.049
		<5% versus ≥5%	<.001	−0.283	.004
		<50% versus ≥50%	<.001	−0.225	ns.
	PD-L1 IC	No cutoff	<.001	−0.306	<.001
		<1% versus ≥1%	<.001	−0.252	.045
		<5% versus ≥5%	<.001	−0.216	ns.
		<10% versus ≥10%	ns.	−0.093	ns.
	PD-1 IC	No cutoff	<.001	−0.302	<.001
		<1% versus ≥1%	<.001	−0.231	ns.
		<5% versus ≥5%	<.001	−0.216	ns.
		<10% versus ≥10%	<.001	−0.208	ns.
Tumor necrosis	PD-L1 TC	No cutoff	<.001	0.283	.004
		<1% versus ≥1%	<.001	0.241	ns.
		<5% versus ≥5%	<.001	0.293	.002
		<50% versus ≥50%	<.001	0.254	.041
	PD-L1 IC	No cutoff	<.001	0.215	ns.
		<1% versus ≥1%	.016	0.149	ns.
		<5% versus ≥5%	.004	0.177	ns.
		<10% versus ≥10%	.043	0.125	ns.
	PD-1 IC	No cutoff	<.001	0.290	.002
		<1% versus ≥1%	<.001	0.270	.013
		<5% versus ≥5%	.003	0.184	ns.
		<10% versus ≥10%	.018	0.146	ns.

ns.: not significant; *p**: corrected *p*-value with multiple comparisons.

reflect to greater tendency to necrosis caused by more aggressive tumor phenotype and higher proliferation rate as it was described in a very recent study [20]. Similarly, a recent publication revealed that tumor necrosis factor- α upregulated PD-L1 expression in pancreatic ductal adenocarcinoma cells [21]. Our result might be in line with a recent animal study of Hartley et al., who demonstrated that monocyte PD-L1 expression can be upregulated by tumor necrosis- α [22]. Since newer targets of immunotherapy include members of the tumor necrosis factor receptor family, our result is likely to have clinical relevance [23]. In a very recent study, the correlation between hypoxia and PD-L1 upregulation was demonstrated by Samanta et al., who studied triple-negative breast cancer cells [24]. Treatment of mice with cytotoxic chemotherapy markedly increased the intratumoral ratio of regulatory/effector T cells. This effect was abrogated by HIF inhibition, so they concluded that combining chemotherapy with an HIF inhibitor may prevent counter therapeutic induction of proteins that mediate evasion of innate and adaptive antitumor immunity. In humans, the effect of chemotherapy on PD-L1 expression in primary lung cancer is somewhat controversial. Shin et al. reported increased tumor PD-L1 expression after platinum-based neoadjuvant chemotherapy in a significant proportion of non-small cell lung cancer patients, but others – including our group – demonstrated decrease in PD-L1 expression [25–28].

Until now only few studies and low number of cases have been published regarding the effect of chemoradiotherapy on PD-L1 expression in lung cancer tissue. Fujimoto et al. demonstrated increased, unchanged, or decreased PD-L1 expression after concurrent chemoradiation therapy in NSCLC [29]. They also investigated the changes in CD8+ lymphocytes in 34 patients, and found mainly increased and unchanged cases with one tumor with decreased cell count.

In preclinical model Deng et al. provided evidence for a close interaction between ionizing radiation, T cells, and the PD-L1/PD-1 axis, as administration of anti-PD-L1 enhanced the efficacy of ionizing radiation through a cytotoxic T cell-dependent mechanism [30]. Similarly, in mouse melanoma cells radiation led to a significant increase of surface PD-L1 [31]. In lung cancer, however, investigations regarding the effect of radiation on PD-L1/PD-1 expression using human tumor samples are still missing.

Until now, PD-L1 expression is the most commonly used biomarker for selecting NSCLC patients for lung cancer immunotherapy, however, tumor mutation burden (TMB) is another potential predictive marker. According to some authors, although TMB and PD-L1 expression did not co-associate in several clinical trials, greater benefit with anti-PD-1 and anti-PD-L1 treatment was observed with high TMB and PD-L1 expression, suggesting these independent biomarkers can be used together [32–35]. Others, like Wang et al., demonstrated, that PD-L1 had significantly higher expression levels in the higher-TMB subtype than in the lower-TMB subtype of certain cancer types including lung adenocarcinoma [36]. It is also possible that PD-L1 expression assessment will be supplemented in the near future by TMB, but this will require novel techniques to use small biopsy samples and narrow timeframes [37]. TMB was also found to be correlated with Ki-67 proliferation index in breast cancer patients [38], which gives rise to suspicion that higher TMB may be associated with the presence of necrosis, and vice versa.

We found a strong positive correlation between PD-L1 expression of TC and IC, which is in accordance with the study of Jiang et al., where approximately 70% of NSCLC cases had the same PD-L1 staining status on both TC and IC [39]. Jiang et al. also found that PD-L1 expression of TC

positively correlated with tumor grade [39], which was not observed in our cohort. (Initially a very weak positive correlation could be observed ($p < .003$, $R = 0.186$), but it did not remain significant with the use of multiple comparisons correction.) We, however, found a negative correlation between lepidic growth pattern and PD-L1 expression of TC, which may be related to the frequent parallel occurrence of lepidic growth pattern and a favorable tumor grade ($R = 0.339$, $p^* < .001$ in our cohort). This finding is in line with the results of Yeo et al., who also used SP142 clone and found that among 84 LADC cases PD-L1 expression was significantly higher in papillary and solid types than in lepidic and acinar types [7]. Similarly, Toyokawa et al. demonstrated in a recent study that PD-L1 expression correlated with radiologic invasiveness determined by thin-section computed tomography in 292 patients with resected pathologic stage I LADC [8]. Saruwatari et al. also described that lepidic growth pattern correlates with lower PD-L1 expression in LADC, however, they studied highly selected Asian patients as all tumor samples were *EGFR* sensitizing mutation positive [6]. Similarly, in a recent study Kwong et al. demonstrated significant differences in PD-L1 expression of TC in relation to histological patterns, with low/undetectable levels in the lepidic components of non-mucinous adenocarcinomas; however, they did not analyze the PD-L1 and PD-1 expression of ICs [40].

Since previous publications contained data on PD-L1 expression only of TC but not of IC and had no data on PD-1 expression of IC in lepidic growth pattern, our results provide new information on PD-L1 and PD-1 expression of IC in lung adenocarcinomas with lepidic growth pattern, and describe for the first time their negative correlation.

We found a positive correlation between the tumor grade and the PD-1 expression of IC (with an appropriate cutoff level), which is similar to the observation of Yang et al., who found in cervical squamous cell carcinoma (SCC), that increase in PD-1 expression positively correlated with increase in cervical intraepithelial neoplasia grade [41].

In our study, there was no correlation between vascular invasion of the tumor and the PD-L1/PD-1 protein expression of TC and IC. This is in contrast to the result of Yang et al., who found that higher PD-L1 expression of TC was associated with vascular invasion in 163 resected stage I LADC [42].

During recent years, the correlation of PD-L1/PD-1 expression and *EGFR*/*KRAS* mutation status has been extensively investigated. Incecco et al. studied 125 NSCLC cases including 83 LADC, 23 SCC, and 19 other types of NSCLC [43]. They found that PD-1 positivity was significantly associated with current smoking status and with the presence of *KRAS* mutations, whereas PD-L1 positivity of TC was associated with LADC histology and with the presence of *EGFR* mutations. On the contrary, Mu et al. observed that in stage I NSCLC patients the rate of PD-L1 over-expression was 39.9% (65/163) but there was no significant correlation between PD-L1 expression and *EGFR*/*KRAS*/*BRAF*/*ALK* expression [44]. Similarly, Zhang et al. did not find significant relations between PD-L1 expression and *EGFR*/*KRAS* expression in LADC [45]. In a very recent publication, Yoneshima et al.

investigated 80 lung adenocarcinomas including 71 tumors with *EGFR* mutations and nine with *ALK* rearrangements and found no correlation with PD-L1 expression [46]. In a recent publication, Jiang et al. investigated the correlation between PD-L1 expression of TC and IC, PD-1 expression of IC and oncogene drivers including *EGFR*, *KRAS*, *ALK*, *MET*, and *ROS1* in 297 NSCLC including 156 LADC cases. None of the five aforementioned NSCLC driver gene aberrations showed any statistical association with PD-L1 positivity of TC [39]. It is important, however, to emphasize, that within the LADC subgroup the mutation positivity rate was 40.4% for *EGFR*, but only 8.1% for *KRAS*, 5.2% for *MET* amplification, 5.8% for *ALK* rearrangement, and 2.3% for *ROS1* rearrangement [39]. Similarly, in our present study, neither *EGFR* mutation nor *KRAS* mutation showed any correlation with the investigated parameters that remained significant when correcting for multiple comparisons.

In the study of Jiang et al., they also investigated the correlation between PD-L1 expression of TC and clinical parameters of the patients, and found in the LADC subgroup, that PD-L1 expression of TC was significantly higher in males and in smokers [39]. They did not examine, however, the correlation between PD-L1/PD-1 expression and the presence of COPD. In our work, we investigated the correlations between the PD-L1/PD-1 expression and COPD, but found no association. Regarding smoking, we observed that never smokers showed lower and current smokers showed higher PD-1 expression of IC, which is also in line with the study of Incecco et al. [43]. We found, however, no correlations between the PD-L1/PD-1 expression and age or gender.

In summary, this is the first study demonstrating a positive correlation between the presence of tumor necrosis and higher PD-L1 expression of TC and PD-1 expression of IC, as well as a negative correlation between lepidic growth pattern and PD-L1/PD-1 expression of IC in LADC. Further studies are required to determine the clinical relevance of these observations in LADC patients receiving immunotherapy.

Disclosure statement

The authors declare no conflict of interest.

Ethical permissions

Permissions to use the archived tissue have been obtained from the Regional Ethical Committee (ETT-TUKEB No: 510/2013, 86/2015, 77-6/2016), and from the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (SE-TUKEB 241/2016).

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