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Association between immunotherapy biomarkers and glucose metabolism from F-18 FDG PET

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Abstract. – OBJECTIVE: To assess associations between parameters derived from F-18 fluorodeoxyglucose (FDG) positron emission tomography (PET) and mRNA expression levels of immune checkpoint biomarkers such as programmed death receptor 1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte antigen 4 (CTLA-4) as well as tumor mutation burden (TMB) in non-small cell lung cancer (NSCLC) patients.

PATIENTS AND METHODS: Integrated data were downloaded from Genomic Data Common Data Portal. Clinical, mRNA-seq, and whole exome-seq data of lung adenocarcinoma and squamous cell carcinoma from The Cancer Genome Atlas (TCGA) database were analyzed. TMB was defined as the total number of somatic missense mutations per megabase of the genome examined. Expression levels of PD-1, PD-L1, CTLA4 mRNA and TMB were collected. Correlations between imaging parameters of glucose metabolism and the expression levels of genomic biomarkers from cancers were evaluated. Bonferroni correction (adjusted $p<0.0027$) was applied to reduce type 1 error.

RESULTS: Of 31 NSCLC cases, 11 cases were adenocarcinoma (LUAD) and 20 were squamous cell carcinoma (LUSC). In linear regression analysis, texture parameters such as low gray-level run emphasis (LGRE, R2=0.48, $p<0.0001$), short run low gray-level emphasis (SRLGE, R2=0.45, $p<0.0001$) and long run low gray-level emphasis (LRLGE, R2=0.41, $p=0.0001$) derived from gray-level run length matrix (GLRLM) showed remarkable correlation with PD-L1 mRNA expression. Expression of PD-1, CTLA-4, and TMB failed to show any significant correlation with parameters of the F-18 FDG PET/CT.

CONCLUSIONS: Texture parameters derived from PET, known to indicate glucose uptake distribution, were correlated with expression of PD-L1 mRNA but not with expression of PD-1, CTLA-4 and TMB. Thus, tumoral heterogeneity could be a surrogate marker for the identification of PD-L1 level in NSCLC.

Key Words:
Lung cancer, PD-L1, Fluorodeoxyglucose, Positron emission tomograph, Texture analysis.

Introduction

Lung cancer is a common cause of cancer-related death across the world. Non-small cell lung cancer (NSCLC) accounts for about 80% of all cases of lung cancer. Surgery combined with chemotherapy or radiotherapy is the main option for treatment so far. However, the clinical outcome of NSCLC with conventional therapy remains poor. Currently, immunotherapy is a promising therapeutic strategy for patients with NSCLC. Novel immunotherapy targeting PD-1, PD-L1, CTLA-4 can activate T cells of hosts and allow the adaptive immune system to cure cancer[1,2].

Therapy with immune checkpoint inhibitor (ICI) therapy such as nivolumab and pembrolizumab has changed the treatment paradigm. In recent years, ICI therapy appears to enhance the overall survival

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compared to conventional therapy in NSCLC patients[3,4]. Food and Drug Administration (FDA) has approved PD-L1 expression by immunohistochemistry (IHC) as a biomarker for ICI therapy since it is deemed useful as a predictive biomarker[5,6]. However, PD-L1 expression by IHC has crucial limitations, such as varying sensitivities with different antibodies and cut-off points of positivity[7,8].

PD-L1 mRNA level is highly correlated with PD-L1 protein expression status[9,10]. Previous reports have suggested that PD-L1 mRNA expression can be a predictive biomarker for disease progression in NSCLC patients. Also, in the field of cancer imaging, there is growing evidence showing that glucose metabolism is positively correlated with PD-L1 expression and that parameters of glucose metabolism, such as maximum standard uptake value (SUVmax), can be predictive biomarker[11-13].

We hypothesized that parameters of glucose metabolism might be correlated with mRNA levels of immune checkpoint biomarkers and TMB as a genomic biomarker for predicting ICI therapy[14]. Hence, our aim was to investigate the possibility that imaging parameters of F-18 FDG PET/CT could be surrogate predictive biomarker for ICI therapy in NSCLC patients.

Patients and Methods

Data Acquisition and Characteristics

Integrated data were downloaded from the Genomic Data Common Data Portal in November 2019. RNA-seq expression [normalized by RNA seq by the Expectation-Maximization (RSEM) method] and clinical information for lung cancer were downloaded from TCGA (https://cancergenome.nih.gov/) database. According to TCGA guidelines, these datasets may be used for publication without restriction or limitation. This process was performed using the 'cgdsr' package in R statistical software. Thirty-one patients in lung cancer cohort (20 LUSC, 11 LUAD) according to TCGA diagnosis criteria were included. Exclusion data were (1) patients with gene expression not available (NA) or – infinite (-Inf) (2) patients with insufficient clinical information, (3) patients who did not have pre-treatment F-18 FDG PET/CT image. Expression data of PD-1, PD-L1 and CTLA-4 mRNA were collected As TCGA has already received Ethics Committee Approval, this study did not require additional approval.

Tumor mutational burden was defined as the total number of somatic missense mutation per megabase of genome examined. Whole exome sequencing data of a total of 31 lung cancer patients were downloaded from The Cancer Genome Atlas database using the TCGAbiolinks Bioconductor package in R. All analyses were performed using R software version 3.5.2 (RStudio Team 2016, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, URL http://www.rstudio.com/).

Imaging Analysis

TCGA-TCIA patient database was used to explore correlations between tissue genotype and imaging phenotype. Genomic information and PET data of 31 lung cancers were available. They were used to perform correlation analysis. Of PET imaging datasets, the volume of interest was drawn over primary tumor uptake in the lung using a threshold of standard uptake value (SUV) with a cut-off value of 2.5. All parameters were extracted using LIFEx software (http://www.lifexosft.org). In each VOI, two conventional indices (SUVmax and MTV) and 20 texture parameters were calculated. Voxel intensity was resampled with 64 grey levels and normalized by absolute resampling bounds between 0 and 20 SUV units. Tumoral heterogeneity was presented by texture parameters from first-order and higher-order matrices. First-order texture parameters included skewness, kurtosis, entropy and energy based on intensity histogram. Sixteen texture parameters were extracted from higher-order matrices, including five parameters (homogeneity, energy, contrast, entropy and dissimilarity) for grey level co-occurrence matrix (GLCM), and 11 parameters (SRE, LRE, LGRE, HGRE, SRLGE, SRHGE, LRLGE, LRHGE, GLNU, RLNU and RP) for grey-level run length matrix (GLRLM). These parameters of GLRLM and their abbreviations are summarized in Table I. Formula from texture parameters can be found in a published study[15].

Table I. Treatment characteristics.

SRE	Short-run emphasis
LRE	Long-run emphasis
LGRE	Low grey-level run emphasis
HGRE	High grey-level run emphasis
SRLGE	Short-run low grey-level emphasis
SRHGE	Short-run high grey-level emphasis
LRLGE	Long-run low grey-level emphasis
LRHGE	Long-run high grey-level emphasis
GLNU	Grey-level non-uniformity for run
RLNU	Run-length non-uniformity
RP	Run percentage

Statistical Analysis

To investigate relationships between parameters of F-18 FDG PET/CT and mRNA $\log_2$ transformation values, Mann Whitney U test and linear regression were used. The median PD-L1 mRNA level was used as the cut-off value to define low and high groups of PD-L1 gene expression. Bonferroni correction was used to adjust *p*-value of multiple comparisons. An adjusted *p* value of 0.0027 (=0.05/22) was applied. Regression line with mRNA expression as dependent variables and parameters of F-18 FDG PET/CT as independent variables was described. The Spearman's rank correlation was used to analyze the possibility of inter-correlation among texture parameters. All statistical analyses were performed with MedCalc software (MedCalc, Mariakerke, Belgium).

Results

Patient Characteristics

From TCGA lung cancer data, clinical and gene expression data were analyzed. Table II shows clinico-pathologic characteristics of patients. A total of 31 cases were included in this study. Thirteen (41.9%) patients were males. Median age of all patients was 72 years (range 45-83 years). All patients had pathologic data. According to the pathology, 11 cases were adenocarcinoma and 20 cases were squamous cell carcinoma. Numbers of cases for stages I, II, III and IV accounted for 14 (45.2%), 9 (29.0%), 7 (22.6%), and 1 (3.2%), respectively. Nineteen (61.3%) patients. Twelve (38.7%) patients were non-smokers.

Table II. Patient characteristics.

Characteristic	n (%)
Gender	
Male	13 (41.9)
Female	18 (58.1)
Age	
> 65	21 (67.8)
≤ 65	10 (32.2)
Histology	
Adenocarcinoma	11 (35.4)
Squamous cell carcinoma	20 (64.5)
T	
T1	6 (19.4)
T2	18 (58.1)
T3	5 (16.1)
T4	2 (6.5)
N	
N0	19 (61.3)
N1	8 (25.8)
N2	4 (12.9)
N3	0
Smoking status	
Smoker	19 (61.3)
Never-smoker	12 (38.7)
Stages	
IA	6 (19.4)
IB	8 (25.8)
IIA	3 (9.7)
IIB	6 (19.4)
IIIA	6 (19.4)
IIIB	1 (3.2)
IV	1 (3.2

Correlations of Metabolic Parameters With PD-1, PD-L1 and CTLA-4 mRNA Expression As Well As TMB

In linear regression analysis, mRNA expression levels of PD-1 and CTLA-4 in tumor tissues failed to show significant correlation with parameters of F-18 FDG PET/CT (Table III). Genomic biomarker TMB did not show any significant correlation with parameters. Only PD-L1 mRNA expression was correlated with texture parameters (LGRE, $R^2 = 0.489$, $p < 0.0001$; SRLGE, $R^2 = 0.453$, $p < 0.0001$; LRLGE, $R^2 = 0.417$, $p = 0.0001$) (Figure 1). The same trend was observed between mRNA level of PD-L1 and some texture parameters (skewness-histogram, $R^2 = 0.143$, $p = 0.035$; kurtosis-histogram, $R^2 = 0.187$, $p = 0.015$; entropy-histogram, $R^2 = 0.133$, $p = 0.043$; entropy-GLCM, $R^2 = 0.129$, $p = 0.046$; SRE, $R^2 = 0.136$, $p = 0.040$; RP, $R^2 = 0.130$, $p = 0.046$). In Mann Whitney U test, 20 of 22 texture parameters (skewness-histogram, $p = 0.033$; kurtosis-histogram, $p = 0.017$; entropy- histogram, $p = 0.007$; energy- histogram, $p = 0.026$; contrast-GLCM, $p = 0.040$; entropy-GLCM, $p = 0.005$; dissimilarity-GLCM, $p = 0.021$; SRE, $p = 0.024$; LGRE, $p = 0.005$; HGRE, $p = 0.006$; SRLGE, $p = 0.012$; SRHGE, $p = 0.007$; LRLGE, $p = 0.019$; LRHGE, $p = 0.004$; RLNU, $p = 0.019$; RP, $p = 0.026$) exhibited low correlation values with PD-L1 mRNA expression. This correlation statistically insignificant after Bonferroni correction (Table IV) (Figure 2).

Inter-Correlation Among Texture Parameters

After applying Bonferroni-adjusted p value, three texture parameters (LGRE, SRLGE, and LRLGE) had significant correlations with genomic PD-L1 mRNA level. However, significant inter-correlations among identified parameters were noted. LGRE correlated very strongly with SRLGE (rho = 0.984, $p < 0.0001$) and LRLGE (rho = 0.913, $p < 0.0001$). SRLGE showed strong correlation with LRLGE (rho = 0.844, $p < 0.0001$) (Figure 3).

Table III. Linear regression of immunotherapy biomarkers and imaging parameters.

		PD-1	PD-L1	CTLA-4	TMB
SUV_{max}	R^2	0.081	0.036	0.095	0.055
	p	0.120	0.305	0.091	0.202
MTV	R^2	0.000	0.064	0.068	***0.188***
	p	0.949	0.166	0.154	***0.014****
Skewness-histogram	R^2	0.083	***0.143***	0.016	0.584
	p	0.115	***0.035****	0.494	0.190
Kurtosis-histogram	R^2	0.106	***0.187***	0.007	0.076
	p	0.072	***0.015****	0.636	0.131
Entropy-histogram	R^2	0.114	***0.133***	0.109	0.063
	p	0.063	***0.043****	0.069	0.171
Energy-histogram	R^2	0.097	0.118	0.090	0.017
	p	0.086	0.058	0.099	0.480
Homogeneity-GLCM	R^2	0.095	0.115	0.045	0.012
	p	0.090	0.061	0.249	0.556
Energy-GLCM	R^2	0.067	0.098	0.052	0.000
	p	0.158	0.085	0.215	0.868
Contrast-GLCM	R^2	0.062	0.073	0.012	0.023
	p	0.175	0.139	0.553	0.410
Entropy-GLCM	R^2	0.117	***0.129***	0.115	0.052
	p	0.059	***0.046****	0.061	0.213
Dissimilarity-GLCM	R^2	0.074	0.083	0.030	0.025
	p	0.138	0.115	0.348	0.390
SRE	R^2	0.099	***0.136***	0.022	0.003
	p	0.083	***0.040****	0.425	0.753
LRE	R^2	0.078	0.118	0.013	0.000
	p	0.126	0.058	0.538	0.960
LGRE	R^2	0.073	***0.489***	0.015	0.050
	p	0.139	***<0.0001****	0.503	0.223
HGRE	R^2	0.046	0.116	0.082	0.082
	p	0.245	0.060	0.117	0.118
SRLGE	R^2	0.068	***0.453***	0.038	0.086
	p	0.154	***<0.0001****	0.288	0.180
SRHGE	R^2	0.045	0.115	0.080	0.080
	p	0.249	0.060	0.122	0.123
LRLGE	R^2	0.068	***0.417***	0.038	0.086
	p	0.154	***0.0001****	0.288	0.108
LRHGE	R^2	0.050	0.123	0.092	0.093
	p	0.224	0.053	0.096	0.094
GLNU	R^2	0.017	0.000	0.016	0.078
	p	0.475	0.884	0.490	0.127
RLNU	R^2	0.008	0.080	0.083	***0.148***
	p	0.624	0.123	0.115	***0.032****
RP	R^2	0.091	***0.130***	0.021	0.000
	p	0.098	***0.046****	0.429	0.896

*$p<0.05$ (unadjusted); **$p<0.0027$ (Bonferroni-adjusted). Italicized entries indicate $p<0.05$ (unadjusted).

Discussion

From TCGA-TCIA datasets, we could examine the association between tumor genotype and imaging phenotype on F-18 FDG PET/CT in NSCLC patients. This preliminary study presented a link between tumor heterogeneity, analyzed by texture analysis, and PD-L1 gene expression at mRNA level. This association might justify effort to explore usefulness of the texture parameters as biomarkers for ICI therapy. To the best of our knowledge, this study is the first one to address

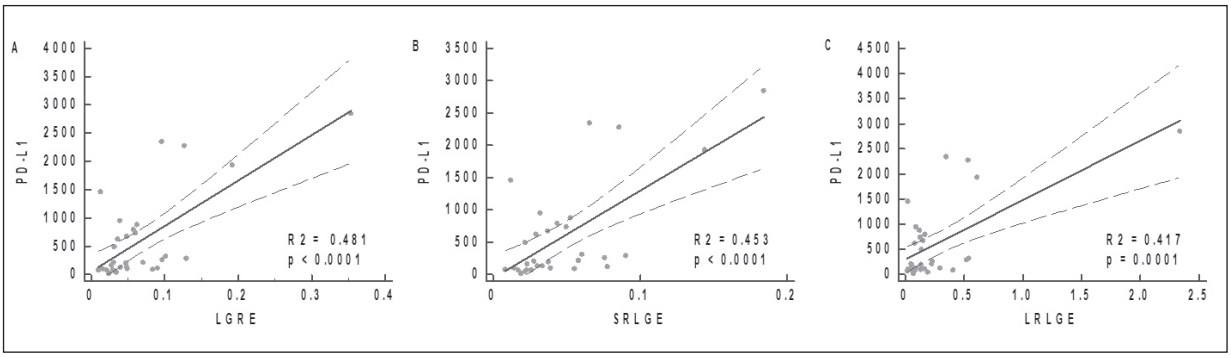

Figure 1. Linear regression analysis of texture parameters with mRNA expression of PD-L1. (**A**) LGRE, (**B**) SRLGE, and (**C**) LRLGE.

a correlation between PD-L1 mRNA expression and tumor heterogeneity on F-18 FDG PET/CT. In addition, this study showed that imaging parameters of glucose metabolism were not related to PD-1 or CTLA-4 mRNA level or TMB.

Although PD-L1 expression in the tumor is routinely conducted by IHC before initiating an ICI therapy, it has a few drawbacks[7,8]. First, PD-L1 does not fully predict patient response to ICI therapy according to cancer type. Second, there was no validated method for determining PD-L1 level. Few studies have noted a correlation between PD-L1 expression by IHC and PD-L1 mRNA level[8-10]. Two previous studies have reported that higher PD-L1 mRNA expression in NSCLC is associated with better survival[10-16]. Therefore, PD-L1 mRNA expression can be used not only as a patient selection biomarker, but also as a prognostic predictive biomarker. Assuming PD-L1 mRNA is a possible alternative biomarker to IHC, other

Table IV. Mann Whitney U test of immunotherapy biomarkers and imaging parameters.

		PD-1	PD-L1	CTLA-4	TMB
SUV_{max}	*p*	0.101	***0.029****	0.119	0.231
MTV	*p*	0.736	0.089	***0.037****	0.220
Skewness-histogram	*p*	0.519	***0.033****	0.093	0.318
Kurtosis-histogram	*p*	0.598	***0.017****	0.085	0.175
Entropy-histogram	*p*	0.119	***0.007****	***0.049****	0.175
Energy-histogram	*p*	0.162	***0.010****	***0.029****	0.299
Homogeneity-GLCM	*p*	0.085	***0.019****	0.139	0.299
Energy-GLCM	*p*	0.110	***0.026****	***0.015****	0.516
Contrast-GLCM	*p*	0.400	***0.040****	0.216	0.188
Entropy-GLCM	*p*	0.119	***0.005****	***0.029****	0.264
Dissimilarity-GLCM	*p*	0.400	***0.021****	0.216	0.231
SRE	*p*	0.093	***0.024****	0.162	0.446
LRE	*p*	0.216	***0.054***	0.202	0.545
LGRE	*p*	0.358	***0.005****	0.085	0.264
HGRE	*p*	0.175	***0.006****	0.071	0.129
SRLGE	*p*	0.379	***0.012****	0.093	0.264
SRHGE	*p*	0.151	***0.007****	0.071	0.139
LRLGE	*p*	0.400	***0.019****	0.101	0.571
LRHGE	*p*	0.188	***0.004****	0.101	0.129
GLNU	*p*	0.446	***0.984***	0.162	0.984
RLNU	*p*	0.545	***0.019****	***0.017****	0.281
RP	*p*	0.119	***0.026****	0.151	0.545

Italicized entries are *$p<0.05$ values

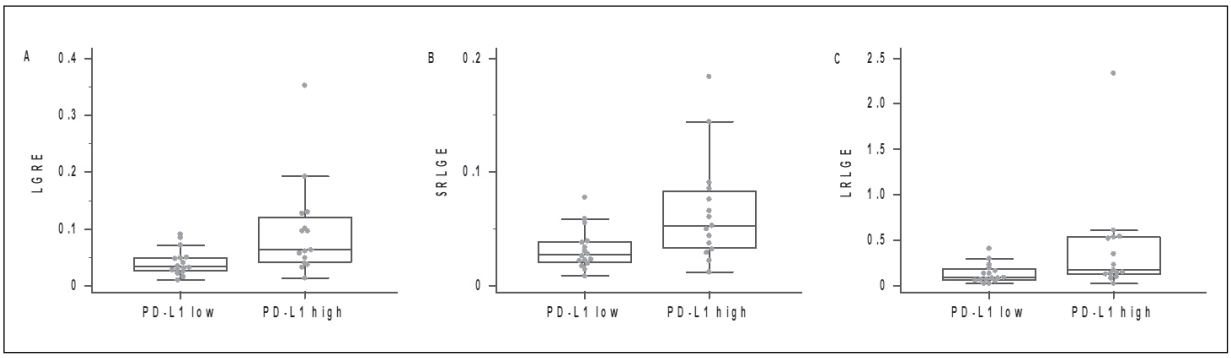

Figure 2. Comparison of texture parameters between patients with high and low PD-L1 mRNA expression levels. (**A**) LGRE, (**B**) SRLGE, and (**C**) LRLGE.

non-invasive imaging biomarkers related to PD-L1 mRNA need to be taken into consideration. In this study, we analyzed possible correlations between immunotherapy biomarkers (PD-1, PD-L1, and CTLA-4 mRNA expression and TMB) and parameters of glucose metabolism.

There have been correlation studies focusing on PD-L1 protein and imaging parameters. Takada et al[18] have studied the correlation of PD-L1 protein level with SUVmax, the most commonly used and validated conventional parameter on F-18 FDG PET/CT [17]. They found that SUVmax was significantly higher in patients with high PD-L1 protein expression. Jreige et al[19] have recently reported that PD-L1 expression has a strong correlation with novel parameter called metabolic to morphological volume ratio (MMVR). It has been reported that necrotic tumor is associated with higher PD-1/PD-L1 expression and that MMVR is a parameter reflecting tumor necrosis or apoptosis.

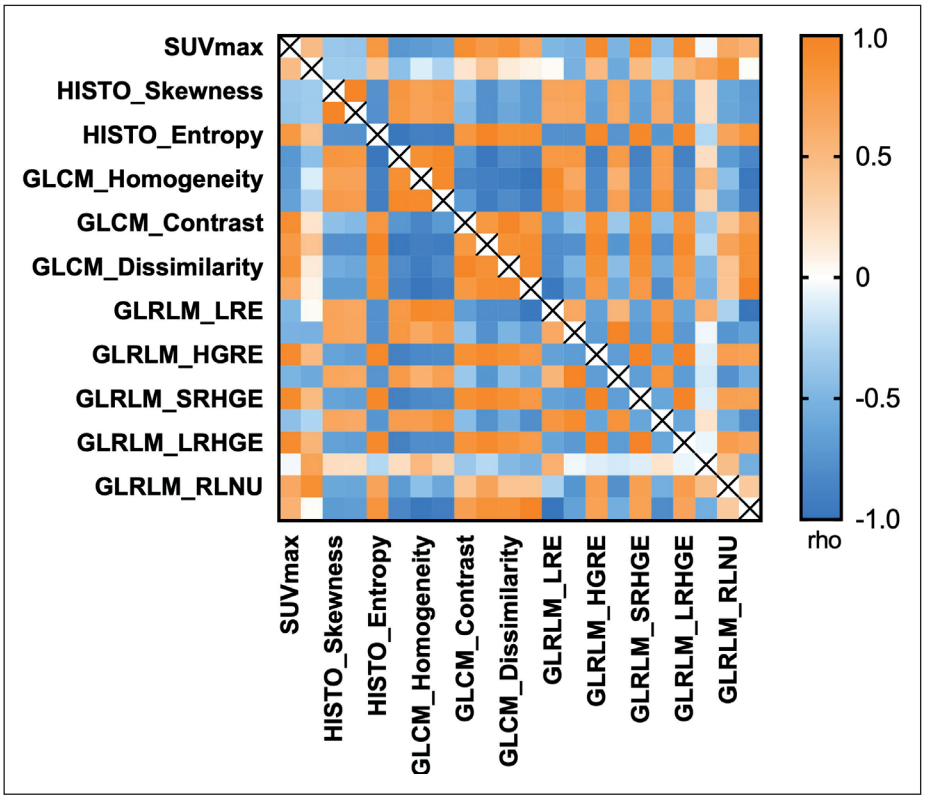

Figure 3. Heat map illustrating correlation coefficients between pairs of parameters of glucose metabolism.

The focus of the present study was to determine associations of imaging parameters with biomarkers at mRNA level. We included not only conventional parameters (SUVmax and MTV), but also texture parameters as potential parameters because texture analysis of PET images could be used to characterize tumoral biologic processes such as metabolism, hypoxia, and necrosis[20-22]. Three texture parameters (LGRE, SRLGE, and LRLGE) showed a significant correlation with PD-L1 mRNA level in the current study, suggesting that tumor heterogeneity measured with F-18 FDG PET/CT could reveal underlying tumor necrosis biology. A recent study has shown that TNFα can directly increase mRNA level of PD-L1[23]. This mechanism might provide an explanation to intercorrelations among tumor necrosis, mRNA level, and tumoral heterogeneity on F-18 FDG PET/CT. Additional prospective studies are needed to confirm this supposition.

This study has some limitations. First, the number of subjects was small. In addition, subjects were heterogeneous from the TCGA-TCIA database, thus providing insufficient power of statistical analysis. Only 31 NSCLC cases were sampled from the TCGA-TCIA database. They had varying treatment protocols[24]. Second, textural parameters depend on various factors such as image acquisition, reconstruction, processing and segmentation[21]. TCGA-TCIA database technically provides heterogeneous and non-standardized image data. Thus, texture analysis based on PET image following a standardized protocol is necessary in the future.

Conclusions

This preliminary study suggests that texture parameters of tumors known to represent glucose uptake distribution may predict PD-L1 expression in patients with NSCLC. Texture parameters (LGRE, SRLGE, and LRLGE) on F-18 FDG PET/CT could be potential biomarkers to select patients for ICI therapy. Further investigation into the role of such predictive biomarkers is needed.

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Author Contributions

Conception and design: HJH, YHK. Acquisition of data: JK, SJ, HK. Analysis of data and writing: BSK, JK. Interpretation of data, review of the manuscript: KP, GHK. Study supervision: HJH, YHK. All authors have read and approved the final version of this manuscript.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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