ORIGINAL RESEARCH

Correlation of PADI4, GBP1, miR-215, and TMB Expression Levels with Clinical Characteristics and Prognosis of Lung Cancer

Jingyu Chen, MM; Yu Han, MD; Yue Zhao, MD; Weijie Zhu, MD

ABSTRACT

Objective • The study aims to explore the correlation between the expression levels of peptidylarginine deiminase 4 (PADI4), guanylate binding protein 1 (GBP1), miR-215, and tumor mutational burden (TMB) and clinical features and prognosis of lung cancer.

Methods • A total of 156 patients with lung cancer admitted to our hospital from July 2021 to March 2022 were selected. Clinical characteristics of patients were collected and PADI4, GBP1, miR-215, and TMB levels were detected. The correlation between the expression levels of PADI4, GBP1, miR-215, and TMB and the clinical characteristics of lung cancer was analyzed. The predictive value of the expression levels of PADI4, GBP1, miR-215, and TMB for lung cancer prognosis was analyzed by the receiver operator characteristic (ROC) curve.

Results • The expression levels of PADI4 and GBP1 were significantly different with respect to smoking history and histopathological type of lung cancer ($P < .05$). The expression levels of miR-215 and TMB were significantly different in terms of age, smoking history, lymph node metastasis, and tumor node metastasis (TNM) stage of lung cancer ($P < .05$). ROC curve results showed that the area under the curve (AUC) of PADI4, GBP1, miR-215, and TMB combined to predict the prognosis of lung cancer was 0.814 (0.789-0.912), which was higher than the diagnostic efficacy of single biomarker ($P < .05$). Its sensitivity and specificity were 85.75% and 89.34%, respectively.

Conclusions • The expression levels of PADI4, GBP1, miR-215, and TMB are correlated with the clinical characteristics and prognosis of lung cancer, and can be used as prognostic markers. (*Altern Ther Health Med.* 2023;29(5):238-241).

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INTRODUCTION

Lung cancer is the most common malignancy and a major risk factor for disability and premature death.[1] In recent years, the global burden of lung cancer continues to increase. Since the early clinical manifestations of this disease are not specific, most patients progress to the advanced stage when receiving diagnosis and treatment, and the prognosis is poor.[2] Therefore, an early and accurate diagnosis of lung cancer will be of immense clinical significance to improve the prognosis. Biomarkers are important diagnostic tools in clinics, and the diagnostic efficacy of different biomarkers varies greatly. In recent years, peptidylarginine deiminase 4 (PADI4), guanylate binding protein 1 (GBP1), miR-215, tumor mutational burden (TMB), and other novel biomarkers have been proposed. Current studies show that PADI4[3] GBP1,[4] miR-215,[5,6] TMB[7] indexes are closely related to the pathological process of lung disease and the proliferation and growth of tumor cells. There are only a few reports on the efficacy of the combined diagnosis utility of the above indicators which however needs to be further confirmed. This study will attempt to explore the correlation between the expression levels of PADI4, GBP1, miR-215, and TMB and the clinical characteristics and prognosis of lung cancer, aiming to provide patients with more reliable biomarkers for diagnosis and prognosis assessment of lung cancer.

PATIENTS AND METHODS

Patients

A total of 156 lung cancer patients admitted to China-Japan Friendship Hospital from July 2021 to March 2022 were selected. Inclusion criteria were set as follows: (1) Patients meeting the diagnostic criteria for lung cancer[8]: symptoms such as cough, expectoration, and hemoptysis;

(2) Patients with complete clinical data; (3) Patients and their family members aware of the risks and benefits and signed informed consent. Exclusion criteria set as follows: (1) Patients complicated with other malignant tumors, inflammatory liver and kidney diseases, immune diseases, systemic infections, and other serious diseases; (2) Patients who had previously received radiotherapy, chemotherapy, or other adjuvant therapy; (3) Patients with cognitive or mental disorders. The article research protocol was reviewed by the ethics committee of our hospital. This research protocol was in accordance with the Helsinki Declaration.

Methods

Clinical characteristics were collected, including gender, age, smoking history, histopathological types (squamous cell carcinoma, adenocarcinoma), lymph node metastasis, and TNM stage.

All patients had samples tested for levels of PADI4, GBP1, miR-215, and TMB. The levels of PADI4 and GBP1 were detected by western blotting (the primary antibody was Abcam, Cambridge, UK). First, lung cancer and adjacent tissues (normal tissues > 5 cm from the edge of lung cancer tumor) were obtained by fine needle aspiration, fixed in 4% formalin, and routine paraffin-embedded sections were completed. After fixing the tissue samples, xylene I and II were added for dewaxing, anhydrous ethanol hydration lasted for 15 min, and buffer rinse was used (3 times, for 5 min each time). The cells were immersed in 3% hydrogen peroxide at room temperature, incubated, cleaned, dripped with primary antibody (dilution ratio of 1:300) (PADI4 and GBP1 primary antibody were dropped separately), refrigerated overnight (4°C), cleaned, dropped with secondary antibody, cleaned, and re-dyed with horseradish peroxidase, diaminobenzidine (DAB) chromogenic agent, and hematoxylin. The cell content was determined by experienced pathologists from microscopic examination (400 times, 10 visual fields). According to the evaluation of staining intensity (0 to 3 points for none, light yellow, brown yellow, and brown) and the percentage of positive cells (0 to 4 points for ≤5%, 6%-25%, 26%-50%, and ≥51%), the comprehensive score ≤3 was negative, otherwise, it was positive. The real-time quantitative polymerase chain reaction (PCR) method (source: Shanghai Bioengineering Co. Ltd., Shanghai, China) was used to detect miR-215 levels. 4 ml of fasting venous blood of the patient was obtained and placed in an anticoagulant tube (centrifugation operation (10 min, 5000 rpm)) was completed to obtain the upper serum layer. The procedure was followed to add liquid nitrogen to the specimen, grind thoroughly, obtain homogenate, extract total RNA, and PCR reaction (reaction conditions: 95°C for 10 min, 15 s, 60°C for 60 s, 40 cycles). The internal reference gene was U6 and miR-215 expression was calculated. TMB was detected using next-generation sequencing (NGS) (source: Nanjing Shihe Gene Biotechnology Co. Ltd., Nanjing, China). Genomic DNA was extracted from unstained sections using the QIAGEN kit. Hybridization enrichment was completed using xGen locking probe. Amplification enrichment was performed on the Illumina Hiseq4000 NGS platform, and NGS detection was performed to obtain the TMB value.

Observation Indicators

(1) The correlation between the expression levels of PADI4 and GBP1 and the clinical characteristics of lung cancer were analyzed; (2) The correlation between the expression levels of miR-215 and TMB and the clinical characteristics of lung cancer was analyzed: used the optimal cut-off value in the ROC curve as the cut-off value, miR-215 and TMB were divided into low expression group and high expression group. (3) The predictive value of PADI4, GBP1, miR-215, and TMB expression levels on the prognosis of lung cancer was analyzed: The follow-up period was up to September 2022, and the predictive value of single and quadruplex of PADI4, GBP1, miR-215, and TMB biomarkers was analyzed using the receiver operator characteristic (ROC) curve. We used AUC analysis to evaluate the predictive value of PADI4, GBP1, miR-215, and TMB expression levels for lung cancer prognosis, and to stratify patients with early (stage I and II) versus advanced (stage III and IV) disease.

Statistical analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) 23.0 software (IBM, Armonk, NY, USA). The count data were expressed as rate (%), using χ^2 inspection. ROC curve was used to analyze the predictive value of PADI4, GBP1, miR-215, and TMB for lung cancer prognosis (bilateral test, $\alpha = 0.05$), with statistically significant difference ($P<.05$).

RESULTS

Analysis of the correlation between PADI4 and GBP1 expression levels and clinical features of lung cancer

The expression levels of PADI4 and GBP1 were significantly different with respect to smoking history and histopathological type of lung cancer ($P<.05$) (Table 1).

Analysis of the correlation between miR-215 and TMB expression levels and clinical characteristics of lung cancer

The expression levels of miR-215 and TMB were significantly different in terms of age, smoking history, lymph node metastasis, and TNM stage of lung cancer ($P<.05$) (Table 2).

The predictive value of PADI4, GBP1, miR-215, and TMB expression levels for lung cancer prognosis was analyzed

ROC curve results showed that the AUC of PADI4, GBP1, miR-215, and TMB combined to predict the prognosis of lung cancer was 0.814 (0.789-0.912), which was higher than the diagnostic efficacy of a single biomarker ($P<.05$). Its sensitivity and specificity were 85.75% and 89.34%, respectively (Figure 1 and Table 3).

Table 1. Analysis of the Correlation Between PADI4 and GBP1 Expression Levels and Clinical Features of Lung Cancer

Group	Number of cases	PADI4		χ^2	*P* value	GBP1		χ^2	*P* value
		Positive (n = 105)	Negative (n = 51)			Positive (n = 109)	Negative (n = 47)		
Gender									
Male	93	64	29	0.239	.625	65	28	0.001	.995
Female	63	41	22			44	19		
Age (years)									
≥60	89	58	31	0.431	.512	59	30	1.261	.261
<60	67	47	20			50	17		
Smoking History	76	62	14	13.717	<.001	65	11	17.253	<.001
Histopathological type									
Squamous cell carcinoma	84	67	17	12.829	<.001	69	15	13.019	<.001
Adenocarcinoma	72	38	34			40	32		
Lymph node metastasis	39	24	15	0.789	.375	26	13	0.254	.614
TNM staging									
Phase I, phase II	75	53	22	0.741	.389	55	20	0.822	.365
Stage III, stage IV	81	52	29			54	27		

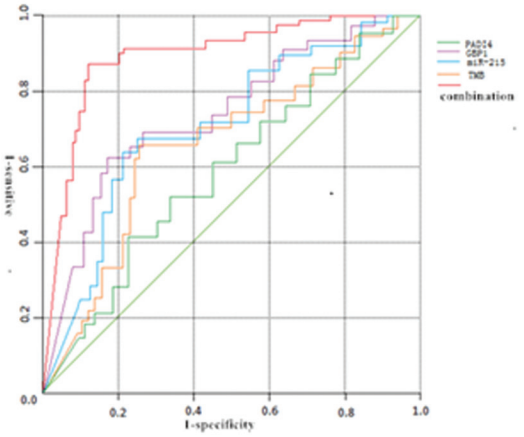
Table 2. Analysis of the Correlation Between miR-215 and TMB Expression Levels and Clinical Characteristics of Lung Cancer (n, %)

Group	Number of cases	miR-215		χ^2	*P* value	TMB		χ^2	*P* value
		Low expression (n = 108)	High expression (n = 48)			Low expression (n = 102)	High expression (n = 54)		
Gender									
Male	93	61	32	1.432	.231	57	36	1.706	.192
Female	63	47	16			45	18		
Age (years)									
≥60	89	53	36	9.116	.003	50	39	7.758	.005
<60	67	55	12			52	15		
Smoking History	78	46	32	7.704	.006	43	33	5.077	.024
Histopathological type									
Squamous cell carcinoma	84	55	29	1.204	.272	52	32	0.974	.324
Adenocarcinoma	72	53	19			50	22		
Lymph node metastasis	39	14	25	27.124	<.001	12	27	27.529	<.001
TNM staging									
Stage I, Stage II	75	44	31	7.568	.006	41	34	7.331	.007
Stage III, Stage IV	81	64	17			61	20		

Table 3. Analyzed the Predictive Value of PADI4, GBP1, miR-215, and TMB Expression Levels for Lung Cancer Prognosis

index	AUC (95%CI)	*P* value	Cut-off value	Sensitivity (%)	Specificity (%)	Jorden index
PADI4	0.529(0.433-0.569)	.025	-	55.23	63.78	0.19
GBP1	0.713(0.695-0.922)	.008	-	77.35	81.63	0.59
miR-215	0.625(0.618-0.726)	.014	1.078	70.15	78.44	0.49
TMB	0.634(0.607-0.712)	.011	6.1 MUTS /Mb	65.29	76.64	0.42
Combined	0.814 (0.789-0.912)	<.001	-	85.75	89.34	0.75

Figure 1. ROC Curve Analysis of the Prognostic Value of PADI4, GBP1, miR-215, and TMB in Lung Cancer



DISCUSSION

PADI4 can be involved in the pathogenesis of pneumonia by catalyzing the citrullination of histones and formation of neutrophil external traps.[9] Other studies have shown that PADI4 can transform histone arginine residues into citrulline, and then participate in the regulation of chromatin organization and gene expression, resulting in increased oncogene mRNA.[10] GBP1 can promote the malignant progression of lung cancer by promoting the exocrine secretion of indoleamine 2, 3-dioxygenase, and participates in the invasion and migration of lung cancer cells at a high expression level. It has been reported that GBP1 is involved in the proliferation, growth, and invasive regulation of tumor cells, and its expression level is closely correlated with the prognosis of patients with glioma cells.[11] In recent years, studies have confirmed that miRNA can be used as a potential therapeutic target in molecular diagnosis and prognosis prediction of lung cancer, and play a key role in the proliferation, migration, invasion, and metastasis of lung cancer cells. miR-215 is a common subtype, which can mediate the inhibition of lung cancer proliferation, migration, and invasion by matrix metalloproteinase-16. Husing et al[12] reported that miR-215 can regulate acidosis and influence the migration and adhesion function of tumor cells. TMB is a recently proposed predictive biomarker for immunotherapy of solid tumors, indicating the number of somatic mutations within the coding region of the tumor genome, with higher levels of expression indicating greater tumor immunogenicity and a better response to immune response. Boumber[13] reported that TMB could be used as a biomarker for immunotherapeutic response in small-cell lung cancer.

The results of this study showed that there were statistical differences between the expression levels of PADI4 and GBP1 with respect to smoking history and histopathological type of lung cancer (P<0.05). The expression levels of miR-215 and TMB were also significantly different with respect to age, smoking history, lymph node metastasis, and TNM stage of lung cancer (P<0.05). The reason may be that the tumor growth process stimulates the massive expression of PADI4 and GBP1 to relieve the physiological damage caused by the tumor. Tumor patients with high mutation load can generate more tumor-specific mutation antigens, which can help cytotoxic T cells recognize tumor cells and kill them effectively. Therefore, TMB can be used as a potential marker to predict lung cancer and evaluate efficacy and prognosis. miR-215 also plays an important role in lung cancer cell proliferation. ROC curve results showed that the AUC of PADI4, GBP1, miR-215, and TMB combined to predict the prognosis of lung cancer was 0.814 (0.789-0.912), which was higher than the diagnostic efficacy of single biomarker (P<0.05). Its sensitivity and specificity were 85.75% and 89.34%, respectively. The reason is that all types of biomarkers are correlated with the diagnosis and prognosis assessment of lung cancer, but there are still some risks of misdiagnosis and missed diagnosis. For example, patients with low TMB may also respond to immunotherapy, thereby affecting the accuracy of prognosis results. Reasonable use of combined technology can further improve the diagnosis and prognosis assessment ability of lung cancer.

The study has a few limitations. The study only includes patients from a single hospital over a relatively short period of time (July 2021 to March 2022). This may limit the generalizability of the findings to other populations or settings. The study design is cross-sectional, implying that the data was collected at a single time point. This limits the ability to establish causality or to track changes in the biomarker expression levels over time. Furthermore, the potential confounding effects of other clinical or environmental factors, such as exposure to air pollution or occupational hazards, that may affect the biomarker expression levels have not been examined, thus warranting the need for such studies in the future.

CONCLUSION

In conclusion, the expression levels of PADI4, GBP1, miR-215, and TMB are correlated with the clinical characteristics and prognosis of lung cancer, and can be used as prognostic markers.

AUTHOR DISCLOSURE STATEMENT

The authors have no potential conflicts of interest to report relevant to this article.

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JC and YH designed the study and performed the experiments, YZ collected the data, WZ analyzed the data, and JC prepared the manuscript. All authors read and approved the final manuscript.

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