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Original Article

High expression level of PGRMC1 correlates to clinicopathological characteristics in patients with lung adenocarcinoma

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Abstract: Previous studies in several cancer types have shown that up-regulation of progesterone receptor membrane component 1 (PGRMC1) was to be involved in cancer tumorigenesis and progression, but the prognostic value of PGRMC1 in lung adenocarcinoma has not been fully elucidated. This study aims to investigate the correlation between PGRMC1 expression level and clinico-pathological characteristics in lung adenocarcinoma. 158 cases of lung adenocarcinoma samples analyzed by Immunohistochemistry (IHC) in tissue microarrays (TMA) to evaluate the expression of PGRMC1 in lung adenocarcinoma. The results suggested that PGRMC1 expression was significantly higher in lung adenocarcinoma tissues than in normal tumor-adjacent lung tissues ($P < 0.001$). High expression level of PGRMC1 was significantly correlated with female ($P = 0.003$), small tumor size ($P = 0.037$), and TNM stage I ($P = 0.022$). Other clinico-pathological characteristics, like age, tumor site, and lymph node metastasis were not associated with PGRMC1 expression level. However, high expression of PGRMC1 had a tendency to associate with tumor grade, but did not reach a statistically significant difference ($P = 0.068$). Kaplan-Meier method as well as multivariate survival analysis found no correlation between PGRMC1 expression level and overall survival of patients with lung adenocarcinoma. In conclusion, high expression level of PGRMC1 was positively correlated with female, smaller tumor volume, and TNM stage I, indicating it was a potential biomarker for female and early TNM stage in patients with lung adenocarcinoma. PGRMC1 expression was not associated with overall survival in this Chinese cohort.

Keywords: Lung adenocarcinoma, PGRMC1, clinico-pathological characteristics, overall survival

Introduction

Lung cancer is the most prevalence malignant tumor and the leading cause of cancer death [1]. In recent decades, the treatment methods of lung cancer have made a lot of development, but the treatment effects are not that satisfactory. In most areas around the world, 5-year survival of lung cancer is less than 20%, whereas in China it is only 18% [2]. According to pathological features, lung cancer is classified into two groups: non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). NSCLC accounts for the major proportion (80%) of lung cancer; meanwhile, lung adenocarcinoma is the important pathologic type of NSCLC. Although in the field of lung adenocarcinoma treatment has developed a large number of genetic diagnosis and targeted therapies but the tumor biomarkers in early diagnosis of lung adenocarcinoma are still rare.

Progesterone receptor membrane component 1 (PGRMC1) is a single transmembrane protein with weight 25-28 kDa which belongs to the membrane-associated progesterone receptor (MAPR) family [3]. Studies show that PGRMC1 is a heme-binding protein which possesses a heme-binding domain, a transmembrane domain, and a N-terminal extracellular domain [4, 5]. PGRMC1 can form complexes with multiple P-450 proteins and epidermal growth factor receptor (EGFR) [6, 7]. PGRMC1 is involved in a wide range of functions such as cholesterol

Table 1. Clinico-pathological characteristics of patients with lung adenocarcinoma

Clinico-pathological characteristics (total)	Patients (*n* %)
Lung adenocarcinoma tissue	158
Normal tumor-adjacent lung tissue	156
Patients with clinical survival data	70
Age (< 60/≥ 60 years old) (*n* = 156)	73 (46.8%)/83 (53.2%)
Gender (male/female) (*n* = 157)	82 (52.2%)/75 (47.8%)
Tumor site (right lung/left lung) (*n* = 156)	86 (55.1%)/70 (44.9%)
Tumor grade (well or moderate/poor) (*n* = 157)	133 (84.7%)/24 (15.3%)
Tumor size (T1/T2/T3/T4) (*n* = 158)	44 (27.8%)/90 (57.0%)/17 (10.8%)/7 (4.4%)
Lymph node metastasis (no/yes) (*n* = 130)	82 (63.1%)/48 (36.9%)
TNM stage (I/II/III/IV) (*n* = 133)	67 (50.4%)/29 (21.8%)/30 (22.5%)/7 (5.3%)

regulation and endocystosis, axonal guidance, and EGFR functions [3, 6]. In addition, PGRMC1 shows moderately high binding affinity with progesterone in porcine liver membranes [8].

PGRMC1 contributes to mitotic progression, tumor growth, and suppresses apoptosis in ovarian cancer [9-11]. PGRMC1 also promotes lung cancer cells on proliferation, migration, and metastasis [12]. PGRMC1 is elevated in many tumor types such as ovarian cancer, breast cancer, and lung cancer [10, 13, 14]. Although highly expressed in lung cancer, the prognostic value of PGRMC1 in lung adenocarcinoma has not been fully elucidated. Thus, in the present study, PGRMC1 expression was evaluated by immunohistochemistry (IHC) in a retrospective cohort of lung adenocarcinoma patients who had done curative surgical resection. Besides, we evaluate whether it is correlated to the clinico-pathological characteristics of patients and whether it is useful to predict postoperative survival.

Materials and methods

Specimens and clinico-pathological characteristics

Lung adenocarcinoma and the relevant normal tumor-adjacent lung tissue microarrays (TMAs) were purchased from the National Engineering Center for BioChips (NECB) (shanghai, China), and clinico-pathological characteristics information also provided by the NECB. This study has a total of 158 lung adenocarcinoma specimens. All of these specimens had tumor size information, 157 cases had gender or tumor grade information, 156 specimens have paired normal tumor-adjacent lung tissue or registered age, and tumor site information, 133 patients have TNM stage information, 130 cases have lymphatic metastasis data, and 70 cases have clinical survival data. The clinico-pathological characteristics of the lung adenocarcinoma TMAs are shown in **Table 1**.

Immunohistochemical staining

Lung adenocarcinoma TMA slides were baked at 65°C for 30 minutes and then deparaffinised with xylene, used graded ethanol to rehydrating. For antigen retrieval, TMA slides were heated by microwave for 20 minutes in citrate buffer. To quench the endogenous peroxidase, specimens were treated with aqueous 3% H_2O_2 for 20 minutes in room temperature. We used 1% bovine serum albumin blocked the non-specific antigen binding. PGRMC1 primary antibody (ab80941, Abcam Co., Ltd.; dilution: 1:450) was incubated with the slides at 4°C for 12 h, and then incubated with biotinylated anti-rabbit IgG for 30 minutes. In the end, slides were stained by DAB chromogenic reagent and hematoxylin.

Scoring of PGRMC1 immunohistochemical staining

PGRMC1 expression was evaluated independently by 2 pathologists blinded to the outcome and clinicopathologic characteristics of the specimens. The staining was evaluated in high magnification (200 ×). Specimens will be re-examined if a disagreement occurred. The staining intensity was visualized as follows (I): "0" meant negative, "1" meant weak, "2" meant moderate, and "3" meant strong. Staining was also scored into five grades depended on the percentage of positive cells (P): < 1% = 0, 1-25% = 1, 26-50% = 2, 51-75% = 3, and > 75% = 4. The overall histological score was calculated as

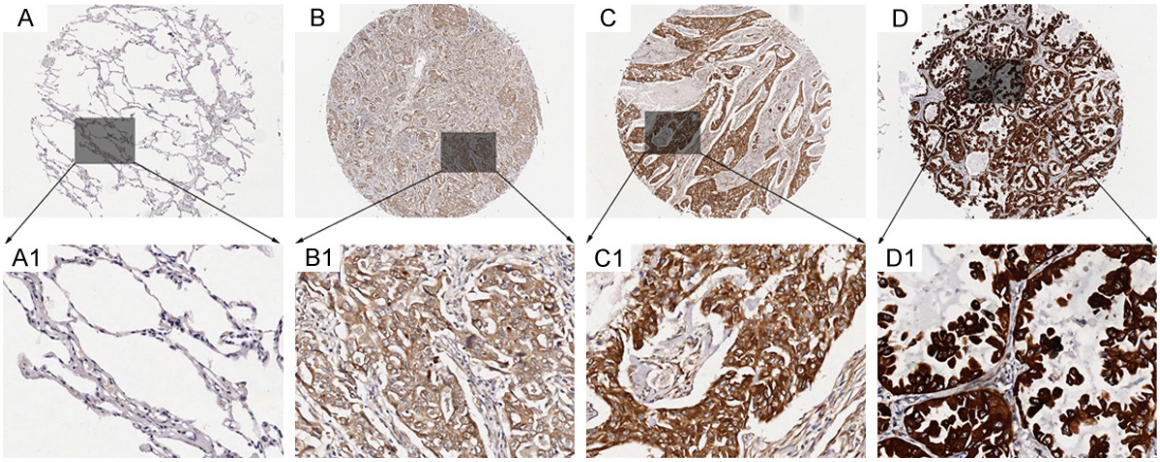

Figure 1. Representative TMA images of PGRMC1 protein expression in lung adenocarcinoma specimens (B-D) and normal tumor-adjacent lung specimens (A) by IHC staining. (A, A1) Negative expression of PGRMC1. (B, B1) Weak expression of PGRMC1. (C, C1) Moderate expression of PGRMC1. (D, D1) Strong expression of PGRMC1. Original magnification × 40 in A-D); × 200 in (A1, B1, C1, D1).

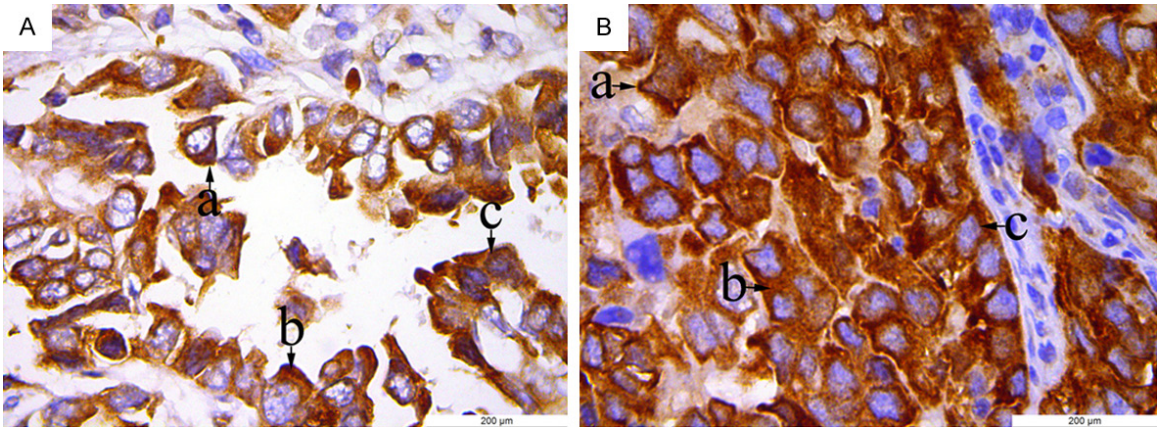

Figure 2. Immunohistochemical localization of PGRMC1 expression in lung adenocarcinoma cells. The *arrows* mark the plasma membrane location; the *b arrows* mark the cytoplasm location; the *c arrows* mark the location closed to cell nucleus. Images (A and B) are taken from different specimens. Original magnification × 1000.

the following formula: overall scores = I × P [15]. According to the overall score, the specimens were graded as three groups: negative "0-1", low expression "2-8", and high expression "9-12". To the same specimen, the highest score tissue spot was selected for statistical analysis [16].

Statistical analysis

The SPSS (17.0 version) was adopted for statistical analysis. The difference of PGRMC1 expression score between tumor and normal tumor-adjacent lung tissues was evaluated by Kruskal-Wallis test and Wilcoxon Signed Ranks Test. Chi-square test used to analyze the association of PGRMC1 score and clinico-pathological characteristics. The impact of PGRMC1 score on survival was determined by log-rank test and assessed by Kaplan-Meier method. Cox regression analyzed various clinico-pathological variables on the influence of survival. *P* value less than 0.05 was considered to be statistically significant.

Results

Immunohistochemical localization of PGRMC1 in lung adenocarcinoma

The expression intensity of PGRMC1 from different specimens was various (**Figure 1**). At high magnification microscopy (1000 ×), PGRMC1 was detected in virtually all the lung

Table 2. Expression levels of PGRMC1 in lung adenocarcinoma specimens and normal tumor-adjacent lung specimens

Histologic types	Case number	PGRMC1 expression			Chi-square	*P* value
		Negative	Low	High		
Tumor	158	0	61 (38.6%)	97 (61.4%)	139.5	< 0.001
Normal tumor-adjacent lung tissue	156	3 (1.9%)	153 (98.1%)	0		

Table 3. Correlation of PGRMC1 expression with clinico-pathological characteristics of lung adenocarcinoma

Clinico-pathological characteristics (total)	Case number	PGRMC1 expression		*P* value
		Low	High	
Age (years) (*n* = 156)				
< 60	73	29 (39.7%)	44 (60.3%)	0.870
≥ 60	83	31 (37.3%)	52 (62.7%)	
Gender (*n* = 157)				
Male	82	41 (50.0%)	41 (50.0%)	0.003
Female	75	20 (26.7%)	55 (73.3%)	
Tumor site (*n* = 156)				
Right	86	31 (36.0%)	55 (64.0%)	0.623
Left	70	28 (40.0%)	42 (60.0%)	
Tumor grade (*n* = 157)				
Well/moderate	133	56 (42.1%)	77 (57.9%)	0.068
Poor	24	5 (20.8%)	19 (79.2%)	
Tumor size (*n* = 158)				
T1	44	9 (20.5%)	35 (79.5%)	0.037
T2	90	41 (45.6%)	49 (54.4%)	
T3	17	8 (47.1%)	9 (52.9%)	
T4	7	3 (42.9%)	4 (57.1%)	
Lymph node metastasis (*n* = 130)				
No	82	29 (35.4%)	53 (64.6%)	0.139
Yes	48	24 (50.0%)	24 (50.0%)	
TNM stage (*n* = 133)				
I	67	20 (30.0%)	47 (70.0%)	0.084
II	29	16 (55.2%)	13 (44.8%)	
III	30	13 (43.3%)	17 (56.7%)	
IV	7	4 (57.1%)	3 (42.9%)	
TNM stage: divided into two groups (*n* = 133)				
I	67	20 (30.0%)	47 (70.0%)	0.022
II/III/IV	66	33 (50.0%)	33 (50.0%)	

adenocarcinoma cells, and PGRMC1 protein expressed in plasma membrane, cytoplasm, and around the nucleus (**Figure 2**).

PGRMC1 is highly expressed in lung adenocarcinoma

In the lung adenocarcinoma tissues, we found that 97/158 (61.4%) showed PGRMC1 high expression, 61/158 (38.6%) showed PGRMC1 low expression, and PGRMC1 negative expression was none. In the normal tumor-adjacent lung tissues, 153/156 (98.1%) showed PGRMC1 low expression, 3/156 (1.9%) showed PGRMC1 negative expression, and PGRMC1 high expression was none. For PGRMC1 expression levels between lung adenocarcinoma and normal tumor-adjacent lung tissues, both Kruskal-Wallis test and Wilcoxon Signed Ranks Test demonstrated statistically significant differences ($P < 0.001$) (**Table 2**).

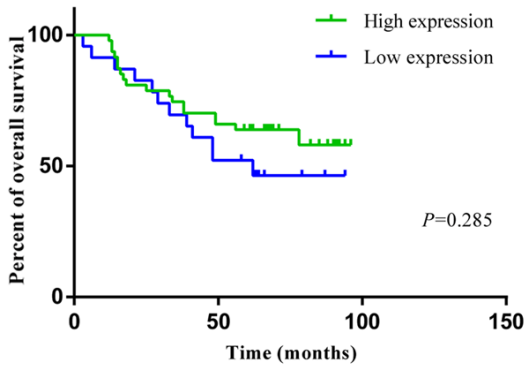

Figure 3. Kaplan-Meier curves of overall survival according to the expression levels of PGRMC1.

Correlation between PGRMC1 expression and clinicopathologic factors in patients with lung adenocarcinoma

High PGRMC1 expression was significantly correlated with gender ($P = 0.003$), tumor size ($P = 0.037$). Other clinico-pathological characteristics, like age ($P = 0.870$), tumor site ($P = 0.623$), lymph node metastasis ($P = 0.139$) were not associated with PGRMC1 expression level. However, high expression of PGRMC1 has a tendency to associate with tumor grade and TNM stage, but did not reach a statistically significant difference ($P = 0.068$ and $P = 0.084$, respectively). When changed the way of TNM stage grouping, we found that high PGRMC1 expression was significantly associated with TNM stage I ($P = 0.022$) (**Table 3**).

Survival analysis of PGRMC1 expression

Seventy patients registered follow-up data (**Table 1**), the period was 3-96 months (median, 61 months), thirty patients had termination event (death), and forty patients reached the end time. The Kaplan-Meier method showed that patients with high PGRMC1 expression and low PGRMC1 expression had no different overall survival ($P = 0.285$) (**Figure 3**). Multivariate analysis found that other clinicopathologic variables such as tumor size ($P = 0.043$), lymph node metastasis ($P = 0.025$) and TNM stage ($P = 0.036$) were significantly associated with survival.

Discussion

Lung adenocarcinoma is a common pathological type of lung cancer. In recent years, with the development of molecular pathology, some lung adenocarcinoma molecular markers have already been found [17, 18]. The discovery of these molecular markers provides an important reference value for the treatment and prognosis of lung adenocarcinoma [19]. Because lung adenocarcinoma is more common in female patients than male patients [20, 21], finding the molecular markers of lung adenocarcinoma on female is of great significance.

Studies have shown that PGRMC1 can play a role in tumor promotion through mediating progesterone or medroxyprogesterone acetate [22, 23], inhibiting or down-regulation PGRMC1 display a anti-tumor effect [10, 12, 22], indicating PGRMC1 could be a potential target for cancer therapy. PGRMC1 expression in cancer tissues was significantly higher than the adjacent tissues or normal tissues, and PGRMC1 expression levels can be used as a predictor of overall survival in cancer patients after surgery [14, 24], suggesting PGRMC1 is also a potential tumor biomarker and prognostic indicator.

In this study, we found that PGRMC1 protein expressed in plasma membrane, cytoplasm, and around the nucleus in lung adenocarcinoma cells. The expression of PGRMC1 in lung adenocarcinoma tissues was significantly higher than in normal tumor-adjacent lung tissues. Results analysis found that PGRMC1 expression in lung adenocarcinoma has obvious gender difference, high expression rate in female patients was significantly higher than male patients. PGRMC1 expression was significantly correlated with tumor size, high expression of PGRMC1 may indicate smaller tumor volume. And the expression of PGRMC1 has a tendency to associate with clinical TNM staging, seems that early stage lung adenocarcinoma has the higher PGRMC1 expression level. Further analysis found that there has no correlation between the PGRMC1 expression level and overall survival of lung adenocarcinoma patients after curative surgical resection.

A study had found that PGRMC1 expressed in the perinuclear location of breast cancer cells [13], and another study found PGRMC1 expressed in the cytoplasm and plasma membrane of ovarian cancer cells [10]. By immunohistochemistry staining, we revealed PGRMC1 protein located not only in close to cell nucleus

but also in plasma membrane and the whole cytoplasm of lung adenocarcinoma cells, which suggest the localization of PGRMC1 in cancer cells differ with different cancer types. Some studies showed that the expression of PGRMC1 in cancer tissues was significantly higher than in normal tumor-adjacent tissue or normal tissues [14, 24]. In this study, we also found the expression level of PGRMC1 was significantly higher than in normal tumor-adjacent lung tissue, indicating PGRMC1 is also a potential biomarker of lung adenocarcinoma. A small sample group study (15 patient specimens) found that all of the lung adenocarcinoma specimens expressed PGRMC1, and survival analysis showed the expression level of PGRMC1 was correlated with the overall survival of patients with lung adenocarcinoma, which high expression level of PGRMC1 indicated poor overall survival [14]. Our study also found PGRMC1 expressed in all the lung adenocarcinoma specimens, but survival analysis showed the expression level of PGRMC1 was not associated with the overall survival of patients with lung adenocarcinoma, we consider the imparity is related with the difference between cohorts.

However, our study has several limitations and need further discussion. This study did not collected postoperative disease-free survival data, if we can analyze the relationship between expression level of PGRMC1 and disease-free survival of lung adenocarcinoma, we will understand the role of PGRMC1 in the recurrence process better. We have obtained the correlation with clinical pathological features of patients with lung adenocarcinoma and expression levels of PGRMC1, it needs to be validated in other cohorts in the future. The molecular mechanisms of PGRMC1 in lung adenocarcinoma remains to be further elucidated in order to make us a better understanding of the role of PGRMC1 in the development of lung adenocarcinoma.

In conclusion, the current study on specimens of lung adenocarcinoma demonstrated that high expression level of PGRMC1 was positively correlated with female, smaller tumor volume, and TNM stage I, indicating it was a potential biomarker for female and early TNM stage in patients with lung adenocarcinoma. PGRMC1 expression was not associated with overall survival in this Chinese cohort.

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Disclosure of conflict of interest

None.

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