High Expression of Growth Factor Receptor-bound Protein 14 Predicts Clinical Progression and Poor Prognosis in Patients with Lung Adenocarcinoma

Qixian Chen
Zhuhai Hospital Affiliated with Jinan University
Zhaohui Liu
Guangzhou First People's Hospital
Lintao Zhong
Zhuhai Hospital Affiliated with Jinan University
Zhike Liang
Guangzhou First People's Hospital
Haoyu Song
Zhuhai Hospital Affiliated with Jinan University
Jun Li
Zhuhai Hospital Affiliated with Jinan University
Jian Yang
Zhuhai Hospital Affiliated with Jinan University
Lijun Chen
Zhuhai Hospital Affiliated with Jinan University
Yuqing Weng (1455259814@qq.com)
Zhuhai Hospital Affiliated with Jinan University https://orcid.org/0000-0002-8558-0336

Research

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Abstract

Background: Growth factor receptor-bound protein 14 (Grb14) is an adaptor molecule of the Grb7/10/14 family with a characteristic “between PH and SH2” (BPS) domain that serves to tightly bind tyrosine kinases. Previous studies have demonstrated that Grb14 upregulation may be used as a marker of proliferation, invasion and malignant cell growth in tumors. The overexpression of Grb14 has also been reported to be associated with a poor prognosis in cancer patients. However, the clinical significance of Grb14 in lung adenocarcinoma has not yet been fully elucidated.

Methods: Grb14 protein expression in human lung adenocarcinoma and noncancerous lung tissues was detected by immunohistochemistry analyses. Then, the associations of Grb14 expression with clinicopathological features and clinical outcomes of lung cancer patients were validated by analyzing a microarray-based TCGA dataset at the mRNA level and statistically evaluating the results.

Results: Immunohistochemistry and dataset analyses revealed that Grb14 expression was significantly increased in lung adenocarcinoma tissues compared with noncancerous lung tissues [immunoreactivity score (IRS): lung adenocarcinoma, 6.07±1.01 vs benign, 4.80±1.22, P<0.001]. Additionally, as revealed by analysis of the dataset, the upregulation of Grb14 mRNA expression in the lung adenocarcinoma tissues was significantly correlated with poor overall survival (P<0.001). Furthermore, univariate analysis revealed that higher pathological stage [hazard ratio (HR), 1.925, 95% CI, 0.912-6.301; P<0.05], higher tumor stage (HR, 2.436, 95% CI, 1.659-5.551; P<0.001), higher surgical margin resection status (HR, 3.035, 95% CI, 1.305-37.51; P<0.01) and prior diagnosis status (HR, 0.4893, 95% CI, 0.1818-0.8197; P<0.01) were independent predictors for a shorter survival. Multivariate analysis also revealed that higher pathological stage (HR, 3.730, 95% CI, 1.784-7.796; P<0.01), higher surgical margin resection status (HR, 6.914, 95% CI, 2.063-23.171; P<0.01) and prior diagnosis status were related to a shorter survival.

Conclusion: Our data suggest that elevated Grb14 expression plays an important role in the progression of lung adenocarcinoma. More importantly, the increased expression of Grb14 may efficiently predict poor survival in lung adenocarcinoma patients.

Highlights

According to the TCGA dataset analysis, Grb14 is a potential marker with prognostic implications for lung adenocarcinoma patients.

This study demonstrated that Grb14 expression was significantly increased in lung adenocarcinoma tissues compared with noncancerous lung tissues.

The Kaplan-Meier method demonstrated that lung adenocarcinoma patients with high Grb14 expression had shorter survival times than those with low Grb14 expression.

Univariate analysis and multivariate analysis revealed that high Grb14 expression was associated with poor prognosis in lung adenocarcinoma patients.

Grb14 could be a potential therapeutic target in lung adenocarcinoma.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide[1] and is mainly divided into small cell and non-small-cell lung cancer[2, 3]. The main histological subtype of non-small-cell lung cancer is adenocarcinoma. Most patients have advanced disease at the time of diagnosis (stage III/IV), and despite significant developments in the oncological management of late-stage lung cancer over recent years, survival remains poor[4]. As a clinically heterogeneous multifocal disease, its natural history is highly variable and difficult to predict[5]. In addition, the mechanisms influencing the progression and prognosis of lung adenocarcinoma are multistep processes[6].

Recent progress in sequencing technologies has enabled us to perform large-scale examinations by whole-genome sequencing, whole-exon sequencing, or RNA sequencing. Several oncogenes, called driver genes, play a significant role in oncogenic activity in lung carcinogenesis[7]. Molecular-targeted therapy targets activated driver oncogene products that promote oncogene addiction in cancer cells[8, 9]. Clinical variables such as CT scan margin status and initial treatment response have been used in various combinations to predict disease outcome[10]. Despite improvements in therapeutic management, the prognosis of lung cancer remains poor, with a 5-year survival rate of less than 15%[11, 12].

There are currently no definitive clinical methods for the diagnosis and determination of the disease outcome. Therefore, it is of great importance to identify novel and effective biomarkers involved in the fundamental aspects of tumor biology to provide valuable information regarding the early diagnosis and tumor progression of lung adenocarcinoma.

In our TCGA dataset analysis, Grb14 was identified as a potential prognostic marker for lung adenocarcinoma patients[13]. Grb14 is an adaptor of the Grb7/10/14 family and has a characteristic “between PH and SH2” (BPS) domain that allows tight binding to tyrosine kinases[14, 15]. It directly binds to the insulin receptor (IR), regulates insulin-induced IR tyrosine phosphorylation and sends signals to IRS-1, Akt and RTK[16, 17]. Accumulating evidence has demonstrated the involvement of Grb14 in various types of human cancer, such as breast cancer, thyroid cancer, and colon cancer[18-21]. The clinical significance of Grb14 in lung adenocarcinoma has not yet been fully elucidated. Thus, the aim of the present study was to investigate the association of Grb14 with tumor progression and prognosis in patients with Grb14.

Materials And Methods

Tissue samples.

For the immunohistochemical analysis, a tissue microarray (TMA, n=150) of 75 primary lung tissues and 75 adjacent noncancerous lung adenocarcinoma tissues was obtained from Shanghai Outdo Biotech Co. Ltd. , (Shanghai, China; CGT NO: HLugA150CS02). Detailed clinical information was also obtained. Patient clinicopathological data, including age, sex, smoking status, TNM stage, histological subtype, and differentiation status, were recorded. For the evaluation of the clinical relevance of Grb14, a publicly available dataset named the TCGA dataset, which includes 230 primary lung adenocarcinoma tissues with messenger RNA (mRNA) microarray expression data, was collected. Patients who received chemotherapy or radiotherapy before surgery were excluded from the study. Detailed information on the clinical characteristics of all the patients in the present study is presented in Table 1.

Immunohistochemistry.

Tissue sections were incubated with an anti-Grb4 antibody (1:100; catalog no. A2742, Abclonal). Background staining was assessed by omitting the primary antibody. The intensity and percentage of positive cells from five fields in each sample were determined independently by two experienced pathologists blinded to the clinical and pathologic data. Staining intensity was assessed using a 4-point scale (0, undetectable; 1, weak; 2, moderate; 3, strong). The percentage of positively stained cells was expressed as one of four categories: 1, 0%–25% stained; 2, 26%–50% stained; 3, 51%–75% stained and 4, 76%–100% stained. Grb14 expression = intensity score + percentage scores.

Statistical analysis.

The Kolmogorov-Smirnov test was used to test for normality of the distribution of Grb14 expression levels. The Mann-Whitney U and Kruskal-Wallis H tests were performed to examine the association between Grb14 and the clinicopathologic characteristics of lung adenocarcinoma patients in the TCGA dataset. The Kaplan-Meier method was used for survival analysis, and Cox regression analysis was used for univariate and multivariate analyses. Independent Student's *t*-test was used to analyze the results, and data are expressed as the mean ± SD. Statistical analysis was performed using Fisher's exact test for any 2x2 tables and the Pearson $\chi 2$ test for non-2x2 tables. Differences were considered statistically significant if the P-value was less than 0.05.

Results

Grb14 protein expression is upregulated in lung adenocarcinoma clinical specimens.

The clinical characteristics of all patients are shown in Table 1. Grb14 protein expression in the TMA was detected by IHC. In this TMA, the expression profile and localization of Grb14 in 75 lung adenocarcinoma and 75 adjacent lung adenocarcinoma tissues were examined by immunohistochemical analysis (Figure 1A). The expression level of Grb14 in the lung adenocarcinoma tissues was significantly higher than that in the noncancerous lung adenocarcinoma tissues (lung adenocarcinoma 6.07±1.01 vs. benign, 4.80±1.22; P<0.01) (Figure 1B). Immunohistochemical staining revealed that Grb14 immunostaining occurred mainly in the cytoplasm of the cells from the lung adenocarcinoma tissue (Figure 1C and D).

The intensity of Grb14 staining was high in the cytoplasm of cancer cells (Figure 1E), while it was low in the cytoplasm of normal lung tissue samples Figure 1F).

Table 1. Clinical characteristics of all patients

Clinical characteristics	TMA	TCGA
Lung adenocarcinoma (cases)	75	230
Age (mean)		
< 65	48	84
≥ 65	27	122
Pathological stage		
T1	20	60
T2	41	130
T3	11	17
T4	3	14
Metastasis		
NO	71	158
YES	4	8
Tumor stage		
Stage I	37	115
Stage II	18	47
Stage III	16	46
Stage IV	4	9

TMA, tissue microarray (tissues analyzed by immunohistochemistry). The TCGA dataset was contributed by EA et al[13]. All 230 patients in the TCGA dataset were given a follow-up examination ranging from 0 to 224 months (median, 13.35 months). The primary analysis endpoint for the cohort of patients was overall survival. All patients who succumbed to diseases other than lung adenocarcinoma or unexpected events were excluded from the cohort.

High Grb14 mRNA levels in the TCGA dataset are associated with disease progression and poor prognosis in lung adenocarcinoma. To further assess the prognostic value of high Grb14 expression in lung adenocarcinoma patients, Kaplan-Meier analysis was performed to compare prognosis between patients with high and low Grb14 expression levels (Figure 2). The mean Grb14 expression level in the TCGA dataset was used as a cut-off to divide cases into high and low Grb14 expression groups. Interestingly, overall survival in the high and low Grb14 expression groups was 41.59 ± 5.2 and 88.67 ± 16.69 months, respectively, indicating that lung adenocarcinoma patients with high Grb14 expression experienced shorter survival times than the low expression group (log rank = 7.714, P = 0.005). Association of Grb14 protein and mRNA expression with the clinicopathological characteristics of patients with lung adenocarcinoma. As shown in Table 2, the results from immunostaining with the limited clinical information from the TMA failed to reveal any significant association between Grb14 expression and the clinicopathological characteristics of the patients with lung adenocarcinoma tissues. Since the increased expression of Grb14 protein in lung adenocarcinoma tissues did not correlate with

the clinicopathological characteristics of our TMA cohort, we proceeded to analyze Grb14 expression at the mRNA level. However, we failed to find any significant association between Grb14 expression and the clinicopathological characteristics of the patients with lung adenocarcinoma in the TCGA dataset. The reason may be related to the limited amount of clinical information.

Table 2. Correlation of GRB14 expression with the clinicopathological characteristics of lung adenocarcinoma

linical characteristics	TMA			Grb14 expression in TCGA dataset		
	Cases	Mean ± SD	P-value	Cases	Mean ± SD	P-value
rb14 expression						
enign	75	4.80±1.22	<0.01		-	
ancer	75	6.07±1.01			-	
ge (years)						
65	48	6.13±1.02	0.533	84	137.71±205.06	0.112
65	27	5.96±1.16		122	185.88±218.41	
[etastasis						
O	71	6.06±1.08	0.727	158	177.49±317.71	0.672
ES	4	6.25±0.96		8	129.55±88.93	
athological stage						
T3A	61	6.00±1.11	0.263	207	175.21±304.08	0.633
T3A	14	6.36±0.84		14	215.18±275.39	
verall survival						
live	-			155	175.98±335.78	0.941
eceased	-			67	179.27±202.98	

-, indicates lack of relative information of patients. TCGA dataset contributed by Collisson EA et al[13]*.*

Prognostic implications of Grb14 expression in lung adenocarcinoma.

As shown in Table 3, univariate analysis and multivariate analysis revealed that there was a significant difference in the survival rates between the patients with high Grb14 expression and those with low Grb14 expression [hazard ratio (HR), 1.960; 95% confidence interval (CI), 1.212-3.299; P<0.001] and (HR 3.418; 95% CI 1.610-7.528). In addition, univariate analysis revealed that higher pathological stage [hazard ratio (HR), 1.925, 95% CI, 0.912-6.301; P<0.05], higher tumor stage (HR, 2.436, 95% CI, 1.659-5.551; P<0.001), higher surgical margin resection status (HR, 3.035, 95% CI, 1.305-37.51; P<0.01) and prior diagnosis status (HR, 0.4893, 95% CI, 0.1818-0.8197; P<0.01) were independent predictors for poor survival. Multivariate analysis also revealed that higher pathological stage (HR, 3.730, 95% CI, 1.784-7.796; P<0.01), higher surgical margin resection status (HR, 6.914, 95% CI, 2.063-23.171; P<0.01) and prior diagnosis status were related to poor survival.

Table 3. Prognostic value of Grb14 expression for overall survival by Cox proportional hazards model.

	Overall survival			Overall survival	
	Hazard ratio (95% CI)	P-value		Hazard ratio (95% CI)	P-value
Univariate analysis			Multivariate analysis		
Grb14	1.960 (1.212-3.299)	<0.001	Grb14	3.418 (1.610-7.258)	<0.001
Age		0.079	Age		0.088
Metastasis	1.271 (0.474-3.626)	0.625	Metastasis	0.292 (0.093-0.913)	0.532
Pathological stage	1.925 (0.912-6.301)	<0.05	Pathological stage	3.730 (1.784-7.796)	<0.01
Tumor stage	2.436 (1.659-5.551)	<0.001	Tumor stage	-	<0.01
Surgical margin resection status	3.035 (1.305-37.51)	<0.05	Surgical margin resection status	6.914 (2.063-23.171)	<0.01
Prior diagnosis	0.4893 (0.1818-0.8197)	<0.01	Prior diagnosis	0.449 (0.200-1.008)	<0.05

Discussion

Patients with similar clinicopathological characteristics may also have different outcomes, such as cancer recurrence, metastasis or death after surgical resection[22]. In other words, the tumor behavior of lung adenocarcinoma cannot be reliably predicted by current diagnostic markers. In recent years, an increasing number of scholars have emphasized new biomarkers, targeting sites and pathways in lung adenocarcinoma via transcriptomics, which may help establish personalized treatments and lessen the morbidity of patients with lung adenocarcinoma[23]. Thus, the identification of predictive and prognostic biomarkers and the potential application of personalized drugs are important for the rehabilitation of patients with lung adenocarcinoma.

The present study suggests that Grb14 is an oncogene that plays an important role in in lung adenocarcinoma progression. Moreover, the increased expression of Grb14 may efficiently predict survival in patients with lung adenocarcinoma.

It has been demonstrated that Grb14 is upregulated in primary cancers[24] and that Grb14 is overexpressed in tumor tissue compared to nontumor tissue[19]. Silencing Grb14 can decrease the ability of tumor cells to proliferate, form colonies and induce tumor formation in nude mice[19.] In the present study, we found via immunohistochemistry that the expression of Grb14 protein in lung cancer tissues was significantly higher than that in paired adjacent tissues. The survival of patients with lung adenocarcinoma with high Grb14 expression was significantly shorter than that of patients with low Grb14 expression. These findings were consistent with the results from previous studies on other tumor types. Thus, we hypothesized that high Grb14 expression is associated with poor prognosis in lung cancer. Grb14 acts by increasing the ability of lung adenocarcinoma cells to proliferate, invade, migrate and form colonies. However, further studies are needed to validate this hypothesis.

Some studies have revealed that the upregulation of Grb14 is also significantly associated with high tumor grade and poor overall survival in patients with primary breast cancer, colon cancer and thyroid cancer. Our results indicate that high Grb14 expression is an independent predictor of shorter survival. Kaplan-Meier plots and the Cox proportional hazards model were used to evaluate the association of Grb14 expression with the outcome of surgical treatment in the TCGA dataset. Statistical analysis revealed that the increased expression of Grb14 was correlated with shorter survival. However, we failed to find any significant association of Grb14 expression with the clinicopathological characteristics of patients with lung adenocarcinoma in the TMAs and the TCGA dataset, which could be attributed to the limited amount of clinical information.

Further analyses with more clinical information are required to better understand the different correlations of Grb14 expression with the clinicopathological characteristics of patients with lung adenocarcinoma, which is a heterogeneous multifocal disease.

In conclusion, the findings from the present study emphasize the prognostic significance of Grb14 in lung adenocarcinoma. The results not only provide new insights into the progression of lung adenocarcinoma but also aid clinicians in designing more personalized treatments for patients with lung adenocarcinoma.

Abbreviations

Grb14: growth factor receptor-bound protein 14; BPS: between PH and SH2; TCGA: The Cancer Genome Atlas; IRS: immunoreactivity score; NSCLC: non-small-cell lung cancer; TMA: tissue microarray; OS: overall survival; mRNA: messenger RNA; IHC: immunohistochemistry; HR: hazard ratio; IR: insulin receptor

Declarations

Ethics approval and consent to participate

The research was approved by the Ethics Committee of Zhuhai People's Hospital.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

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Competing interests

The authors declare that they had no competing interests.

Authors' contributions

Qixian Chen wrote the paper, performed the experiments and analyzed the data, Zhaohui Liu , Lintao Zhong, Zhike Liang performed the experiments and analyzed the data, Haoyu Song and Lijun Chen analyzed the data and wrote the paper, Jun Li and Jian Yang designed the research, Yuqing Weng designed the research and wrote the paper. All authors read and approved the final manuscript.

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Author details

[1] Zhuhai People's Hospital, Zhuhai Hospital Affiliated with Jinan University, Zhuhai 519000, Guangdong, China. [2] Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou 510180, Guangdong, China

References

1. Freddie, Bray, Jacques, Ferlay, Isabelle, Soerjomataram, Rebecca, Siegel, Lindsey. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 2018.
2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008; 359(13): 1367-1380.
3. Nakamura H, Saji H. A worldwide trend of increasing primary adenocarcinoma of the lung. Surgery Today 2014, 44(6): 1004-1012.
4. Walters S, Maringe C, Coleman MP, Peake MD, Butler J, Young N, et al. Lung cancer survival and stage at diagnosis in Australia, Canada, Denmark, Norway, Sweden and the UK: a population-based study, 2004–2007. Thorax 2013, 68(6): 551-564.
5. Välk K, Vooder T, Kolde R, Reintam MA, Petzold C, Vilo J, Metspalu A. Gene expression profiles of non-small cell lung cancer: survival prediction and new biomarkers. Oncology 2010, 79(3-4): 283-292.
6. Denisenko TV, Budkevich IN, Zhivotovsky B. Cell death-based treatment of lung adenocarcinoma. Cell Death & Disease 2018,9(2): 117.
7. Saito M, Suzuki H, Kono K, Takenoshita S, Kohno T. Treatment of lung adenocarcinoma by molecular-targeted therapy and immunotherapy. Surg Today 2018,48(1): 1-8.
8. Gately K, O'Flaherty J, Cappuzzo F, Pirker R, Kerr K, O'Byrne K. The role of the molecular footprint of EGFR in tailoring treatment decisions in NSCLC. Journal of Clinical Pathology 2012, 65(1): 1-7.
9. Meng F, Zhang L, Ren Y, Ma Q. The genomic alterations of lung adenocarcinoma and lung squamous cell carcinoma can explain the differences of their overall survival rates. J Cell Physiol 2019,234(7): 10918-10925.
10. Blandin Knight S, Crosbie PA, Balata H, Chudziak J, Hussell T, Dive C. Progress and prospects of early detection in lung cancer. Open Biol 2017, 7(9):170070
11. Behera M, Steuer C, Gal A, Owonikoko T, Khuri F, Ramalingam S, et al. Lung Adenocarcinoma Staging Based on 2011 IASLC/ATS/ERS Classification: A Pooled Analysis of Adenocarcinoma In Situ (AIS) and Minimally Invasive Adenocarcinoma (MIA). Clinical Lung Cancer 2016, 17(5): e57-e64.
12. Hong SR, Hur J, Moon YW, Han K, Chang S, Kim JY, et al. Predictive factors for treatment response using dual-energy computed tomography in patients with advanced lung adenocarcinoma. European Journal of Radiology 2018, 101: 118-123.
13. Collisson EA, Campbell JD, Brooks AN, Berger AH, Lee W, Chmielecki J, et al. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014, 511(7511): 543-550.
14. Han DC, Shen TL, Guan JL. The Grb7 family proteins: structure, interactions with other signaling molecules and potential cellular functions. Oncogene 2001, 20(44): 6315-6321.
15. Cariou B, Bereziat V, Moncoq K, Kasus-Jacobi A, Burnol AF. Regulation and functional roles of Grb14. Frontiers in Bioscience A Journal & Virtual Library 2004,9(5): 1626-1636.

16. Holt LJ, Lyons RJ, Ryan AS, Beale SM, Andrew W, Cooney GJ, et al. Dual Ablation of Grb10 and Grb14 in Mice Reveals Their Combined Role in Regulation of Insulin Signaling and Glucose Homeostasis. Molecular Endocrinology 2009,23(9): 1406–1414.
17. Kairouz R, Parmar J, Lyons RJ, Swarbrick A, Musgrove EA, Daly RJ. Hormonal regulation of the Grb14 signal modulator and its role in cell cycle progression of MCF-7 human breast cancer cells. Journal of Cellular Physiology 2005,203(1): 85-93.
18. Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nature Medicine 2008,14(5): 518-527.
19. Balogh K, Asa SL, Zheng L, Cassol C, Cheng S, Ezzat S. The insulin resistance Grb14 adaptor protein promotes thyroid cancer ret signaling and progression. Oncogene 2012,31(36): 4012-4021.
20. Reis-Filho JS, Simpson PT, Turner NC, Lambros MB, Ashworth A. FGFR1 Emerges as a Potential Therapeutic Target for Lobular Breast Carcinomas. Clinical Cancer Research An Official Journal of the American Association for Cancer Research 2006,12(22): 6652-6662.
21. Yang P, Wei J, Li W, He F, Zeng S, Zhang T, et al. High expression of growth factor receptor-bound protein 14 predicts poor prognosis for colorectal cancer patients. Biotechnol Lett 2016, 38(6): 1043-1047.
22. Shao W, Wang D, He J. The role of gene expression profiling in early-stage non-small cell lung cancer. Journal of Thoracic Disease 2010,2(2): 89-99.
23. Beer DG, Kardia SLR, Huang C-C, Giordano TJ, Levin AM, Misek DE, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nature Medicine 2002,8(8): 816-824.
24. Ou H, Min J, Xi Z, Xie Z, Chen X, Wu J, et al. Grb14 as an Independent Good Prognosis Factor for Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. Japanese Journal of Clinical Oncology 2013,43(11):1064-72

Figures

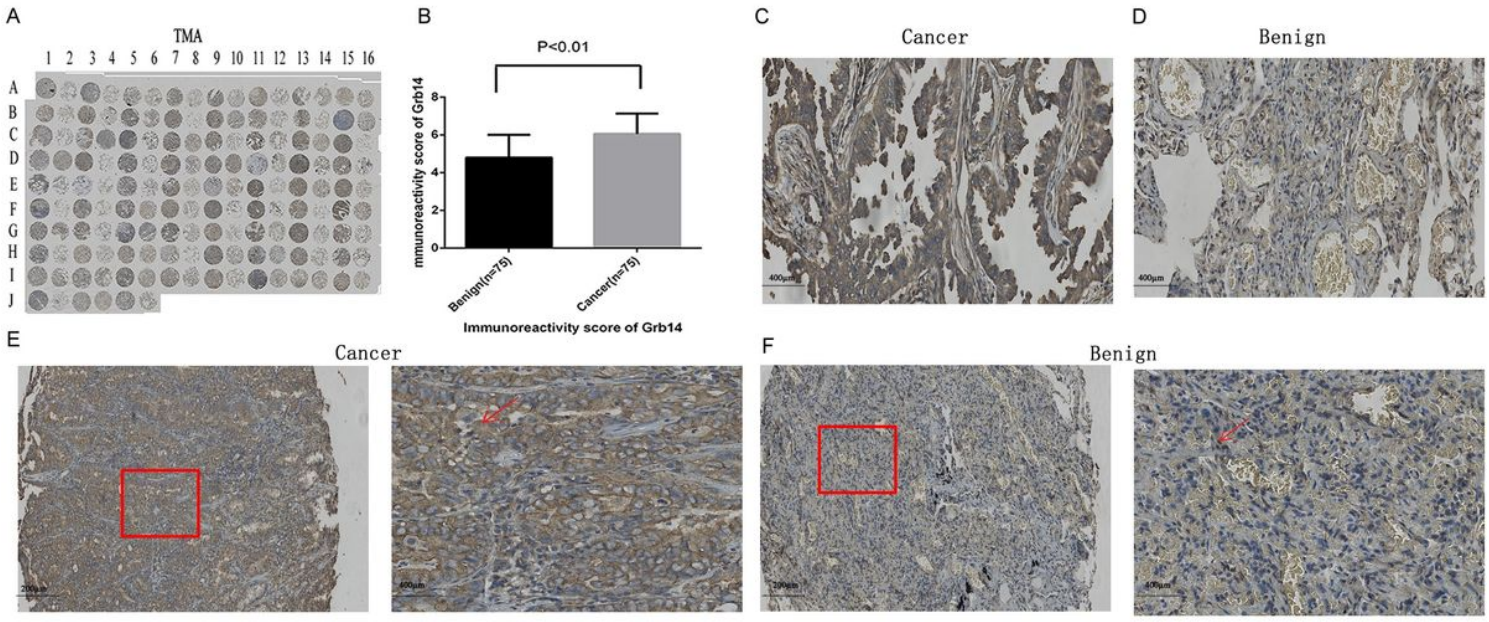

Figure 1

Immunohistochemical staining for Grb14 in lung adenocarcinoma tissues and adjacent noncancerous lung tissues in our TMA. (A) Immunohistochemical staining for Grb14 in the TMA. (B) The expression level of Grb14 in the lung adenocarcinoma tissues was significantly higher than that in the noncancerous lung adenocarcinoma tissues (lung adenocarcinoma 6.07±1.01 vs. benign 4.80±1.22; P<0.01). (C and D) Immunohistochemical staining of Grb14 occurred in the stroma between cancer cells, but moderate staining was observed in the benign lung tissues. (E) High Grb14 staining was observed in the cytoplasm of cancer cells. (F) Low Grb14 staining was observed in the cytoplasm of normal lung tissue samples. (E) and (F) are enlarged images of the D3 and C4 fields. The red arrows in (E) and (F) indicate positively stained cells.

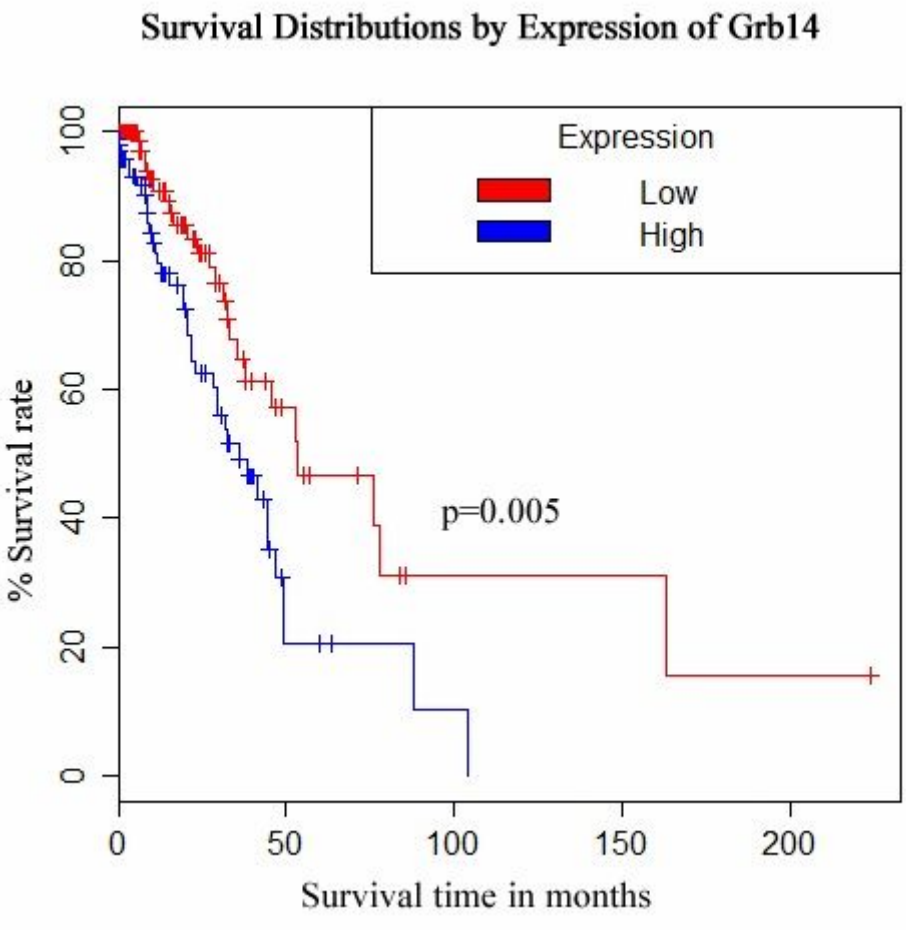

Figure 2

Kaplan-Meier survival curves indicating the overall survival of lung adenocarcinoma patients with high and low Grb14 expression. Overall survival in the high and low Grb14 expression groups was 41.59 ± 5.2 and 88.67 ± 16.69 months, respectively.