Correlation analysis of RBM3 expression in tumor stroma and prognosis of patients with non-small cell lung cancer

Ling Chen

Qingdao Municipal Hospital

Dongshuai Ling

Qingdao Municipal Hospital

Yi Chen

Qingdao Municipal Hospital

Kai Gai

Qingdao Municipal Hospital

Yang Liu

Qingdao Municipal Hospital

Chengye Guo

Qingdao Municipal Hospital

Tenglong Zhang (✉ Zhangtenglong123@163.com)

Qingdao Municipal Hospital

Research article

Keywords: non-small cell lung cancer, RBM3 protein, AKT, OS, PFS

Posted Date: September 18th, 2019

DOI: <https://doi.org/10.21203/rs.2.14630/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective: To investigate the correlation between the expression of RNA binding motif protein 3 (RBM3) in non-small cell lung cancer (NSCLC) pathological tissue and known risk factors.

Methods: A retrospective analysis of the clinical and pathological features of 128 patients with NSCLC from August 1, 2014 to August 1, 2015 was performed. The expression of RBM3 and protein kinase B (AKT) in the tumor stroma was examined by immunohistochemistry and pathological enumeration, followed by analysis of its correlation with prognosis. The survival status of patients was followed up. The relationships between RBM3 and AKT protein expression in tumor stroma with overall survival (OS) and progression-free survival (PFS) were evaluated.

Results: The expression of RBM3 was significantly correlated with tumor differentiation ($P = 0.012$), lymph node metastasis ($P = 0.02$), T staging ($P = 0.041$), and AKT expression ($P = 0.021$). Univariate Kaplan-Meier analysis showed that high expression of RBM3, lymph node metastasis, T staging, and high expression of AKT were prognostic factors in NSCLC ($P < 0.05$). Multivariate analysis of Cox proportional hazard model showed that high RBM3 expression, lymph node metastasis, and high AKT expression were independent risk factors for prognosis ($P < 0.05$).

Conclusion: RBM3, AKT, and lymph node metastasis are independent prognostic factors for NSCLC, significantly affecting the prognosis of patients possibly through the classical signaling pathway AKT. RBM3 may be a prognostic marker for the overall survival rate of NSCLC and a candidate for the treatment of NSCLC, with potential therapeutic prospects.

Background

Lung cancer is one of the most malignant tumors with the highest morbidity and mortality[1], which seriously endangers human health, of which non-small cell lung cancer (NSCLC) accounts for about 85% [2]. The main treatment methods include surgery, radiotherapy, chemotherapy, and combined chemotherapy with radiotherapy and chemotherapy. Molecularly targeted therapies are now being accepted. However, the 5-year survival rate of NSCLC is only 15%. Therefore, further study on the occurrence, development, and mechanism of lung cancer is the focus of current research.

In recent years, studies have demonstrated that the cold shock protein family plays an important role in the occurrence and development of tumors, including RNA binding motif protein 3 (RBM3). RBM3 is highly expressed in most human tumors[3, 4], and the earliest gene-based approach clarified the role of RBM3 as a tumor marker. The involvement of RBM3 in tumorigenesis and clinical outcome prediction has been extensively studied and has reached different conclusions in many cancers, including breast cancer, urothelial cancer, prostate cancer, colorectal cancer, and gastric cancer[5–9]. However, the role of RBM3 in NSCLC has not been reported. Besides, over-activation of protein kinase B (AKT) pathway was found to promote proliferation of lung cancer cells. This study focused on the analysis of the relationship between

the expression of RBM3 in NSCLC pathological tissues and known high-risk factors (including AKT), providing a theoretical basis for the clinical treatment of NSCLC patients.

Methods

Clinical data

In this study, 128 non-small cell lung cancer (NSCLC) patients treated in the Department of Thoracic Surgery, Qingdao City Hospital from August 1, 2014 to August 1, 2015 were enrolled. All patients were followed up. None of the selected patients had any anti-tumor treatment before surgery. There were 34 males and 94 females, aged 42 to 75 years old. RNA binding motif protein 3 (RBM3) was highly expressed in 55 cases and lowly expressed in 73 cases. Staging was performed according to the 7th Edition tumor node metastasis (TNM) staging criteria from the American Joint Committee on Cancer (AJCC).

Specimen examination

Immunohistochemistry two-step method for detecting gene expression in lung cancer tissues

All paraffin sections were made into 4-um pieces and then stained in strict accordance with the reagent instructions. The EDTA repair solution was used for antigen retrieval. Main reagents: rabbit anti-human RBM3 monoclonal antibody (working concentration: 1:100, purchased from Beijing Boaosan Biotechnology Co., Ltd.); mouse anti-human AKT monoclonal antibody (working concentration: 1:400, purchased from Abcam).

Immunohistochemical staining procedures

The negative control group was also immunohistochemically stained. The procedure was the same as the experimental group, and only the primary antibody was replaced with PBS buffer. The counting process was performed by two pathologists, and the tissue sections were counted separately by double-blind method. When the results of the grouping were inconsistent, the third pathologist recounted and grouped. According to the result determination method, all the slices were grouped, and the obtained data was used for statistical analysis.

Result judgment

For RBM3 staining, 5 high-magnification fields were randomly selected, and 100 cells were counted. The depth of coloration and the number of positive cells were determined. Staining intensity scores: 3 for

sepia, 2 for brown, 1 for pale yellow, and 0 for colorless. Positive cell percentage scoring criteria: 25% of positive staining cells were the low expression, and >25% were the high expression. RBM3 immunostaining was independently evaluated by two pathologists in a blinded way.

For AKT staining, 5 high-magnification fields were randomly selected, and 200 cells were counted. The staining intensity of cancer cells and the proportion of stained cells were comprehensively scored. The film was read by a pathologist in a blinded way. Once the cytoplasm and/or nucleus were stained, it was judged to be a positive cell. Staining intensity: 3 points for sepia, 2 points for brown yellow, 1 point for pale yellow, 0 for unresponsive cells. The percentage of stained cells indicated: $\geq 50\%$ was 3 points, $<10\%$ was 0 points, $<50\%$ was 2 points, $<20\%$ was 1 point. The staining intensity multiplied by the percentage of the stained cells was used to evaluate the AKT expression. The integral 0 to 2 and 3 to 9 were classified into negative and positive expression, respectively.

Statistical methods

Statistical analysis was performed using SPSS 21.0 software. Correlation comparisons were performed using a chi-square test. Survival analysis was performed using univariate Kaplan-Meier analysis and multivariate Cox regression analysis. The difference was statistically significant at $P < 0.05$.

Results

Relationship between RBM3 expression and clinicopathological parameters

The results of immunohistochemical staining are shown in Figure 1. There were 31 cases of high expression of AKT and 24 cases of low expression of AKT in 55 patients with high expression of RBM3, and 47 cases of low expression of AKT and 26 cases of high expression of AKT in 73 cases of low RBM3 expression. The difference was statistically significant ($P = 0.021$), indicating that the expression of AKT in most patients with high expression of RBM3 was also highly expressed, and the expression of RBM3 was significantly correlated with the expression of AKT (Table 1). Therefore, RBM3 may promote the proliferation of lung cancer cells through the PI3K/AKT signaling pathway.

Univariate analysis of RBM3 and OS, PFS

RBM3 expression was significantly associated with tumor differentiation ($P = 0.012$), lymph node metastasis ($P = 0.02$), T staging ($P = 0.041$), and AKT expression ($P = 0.021$) (Table 1). Moreover, univariate analysis of Kaplan-Meier analysis risk model showed that RBM3 expression, lymph node metastasis, T staging, and high AKT expression were the prognostic factors affecting NSCLC ($P < 0.05$) (Table 2).

Multivariate analysis of RBM3 and OS, PFS

We then performed the multivariate analysis of Cox proportional hazard model and found significant differences in overall survival (OS) and progression-free survival (PFS) between patients with high expression of RBM3 and those with low expression of RBM3 ($P = 0.000$). In other words, patients with high RBM3 expression had a worse prognosis than those with low RBM3 expression, indicating that high expression of RBM3 is an independent risk factor for the prognosis of NSCLC, which was not affected by age, gender, lymph node metastasis, smoking or not, and the degree of differentiation.

There were significant differences in OS and PFS between patients with lymph node metastasis and those without lymph node metastasis ($P = 0.000$). Therefore, patients with lymph node metastasis had a worse prognosis than those with non-lymph node metastasis, suggesting that lymph node metastasis is an independent risk factor for NSCLC prognosis, which was not affected by age, gender, smoking or differentiation.

There were significant differences in OS ($P = 0.003$) and PFS ($P = 0.005$) between the patients with low AKT expression and those with high AKT expression. Patients with low AKT expression had a better prognosis when comparing to those with high AKT expression, indicating that high expression of AKT is also an independent risk factor for NSCLC prognosis.

Taken together, we found that high RBM3 expression, lymph node metastasis, and high AKT expression were independent risk factors for prognosis (Table 3).

Discussion

Lung cancer is one of the most common malignant tumors in China, ranking first in cancer death worldwide[1]. Among them, NSCLC accounts for about 85%[2]. In the treatment of NSCLC, it can be surgically removed at an early stage, but approximately 70% of the patients are diagnosed as locally advanced or metastatic when initially diagnosed[10]. The prognosis of advanced NSCLC is extremely poor, with a median survival time of about 1 year and a 5-year survival rate of less than 20%[11–14]. In this study, we investigated the relationship between RBM3 expression in the pathological tissues of NSCLC patients and OS, PFS, and indicated that RBM3 may promote the proliferation of lung cancer cells via the PI3K/AKT signaling pathway. Therefore, it is possible to further understand or clarify the possible mechanisms of tumor development to find more effective methods of diagnosis and treatment to improve the therapeutic effect of lung cancer.

RBM3 is a highly conserved cold-inducing RNA-binding protein that is one of three X-chromosome-associated RBM genes (RBMX, RBM3, RBM10), localized to Xp11.23, and transcriptionally upregulated in stress reaction[15]. RBM3 is involved in the biological action of mRNA, stimulates protein synthesis, promotes cell proliferation, and exerts anti-apoptotic effects. RBM3 is expressed in various tissues such as the cerebellum, lung, spleen, small intestine, and uterus, and its expression is increased in response to cold shock. With the deepening of RBM3 research, the role of RBM3 protein in the occurrence and

development of tumors has been paid more and more attention. More and more immunohistochemical studies have shown that RBM3 acts as a proto-oncogene and is associated with the progression and metastasis of various cancers[16]. The main reasons for RBM3 acting as a proto-oncogene were listed as follows. First, the expression level of RBM3 was related to the stage of the tumor, suggesting that it may be related to tumorigenesis. Second, RBM3 silencing increased the number of G2/M-phase cells, and ultimately led to apoptosis; G2 phase control by RBM3 was found in RBM3-silencing A2780 cells[17] and RBM3 knockout mice [18]. Finally, RBM3 can improve the stabilization and translation of mRNAs of cyclooxygenase-2, IL-8, and vascular endothelial growth factor [3, 19, 20]. Recently, some studies suggest that RBM3 may influence tumor progression by altering the level of cellular microRNA control of globulin expression [21]. Although it has been confirmed that the carcinogenic factors of lung cancer include smoking, air pollution, environmental factors, ionizing radiation, ras, myc gene family, etc., there are few studies on the role of RBM3 in the occurrence, development, and mechanism of lung cancer. In this study, we found that the high expression of RBM3 was associated with a poor prognosis of NSCLC patients, making itself an independent prognostic marker for NSCLC.

AKT is a central mediator that inhibits the sensitivity of chemotherapeutic drugs that induce apoptosis. A growing number of experiments have shown that inhibition of Akt expression can block the PI3K/Akt classical signaling pathway to improve the treatment effect. In a recent study of nasopharyngeal carcinoma (NPC) [22], shRNA-mediated knockdown of RBM3 was found to reduce AKT phosphorylation, and direct interaction between RBM3 and PI3K was also detected.

Furthermore, they demonstrated that RBM3 was involved in NPC radioresistance via the PI3K/AKT/Bcl-2 signaling pathway. Therefore, RBM3 may be a new candidate for predicting the development of radioresistance and a potential therapeutic target for NPC. In this study, we also found that RBM3 might affect the prognosis of patients through the AKT signaling pathway.

The invasion and metastasis of malignant tumors is an extremely complex multi-step, multi-stage development process, including degradation of extracellular matrix, invasion, circulating diffusion, distant cloning, and angiogenesis. The expression of RBM3 was detected in 88 cases of benign and malignant prostate tumors by immunohistochemistry and tissue microarray and it was found that RBM3 was rarely expressed in the tissues of surgically resected benign bladder tumors but significantly increased in bladder malignant tumors, with higher expression in distant invasive bladder cancer [23]. These findings indicate that RBM3 is highly expressed in bladder transitional cell carcinoma and is more closely related with distant metastasis than *in situ* invasiveness. In this study, RBM3 was highly expressed in NSCLC with lymph node metastasis, while in NSCLC without lymph node metastasis, RBM3 expression was low, also suggesting that RBM3 plays a role in distant metastasis of the malignant tumor.

In summary, the expression of RBM3 was significantly correlated with the degree of tumor differentiation, lymph node metastasis, T staging, and AKT expression. High RBM3 expression, high AKT expression, and lymph node metastasis were independent risk factors for the prognosis of NSCLC. RBM3 indirectly affects the survival prognosis of NSCLC by affecting the classical AKT signal transduction pathway and

RBM3 may become an important clinical prognostic marker for the treatment of NSCLC (immunohistochemical staining).

Conclusion

RBM3, AKT, and lymph node metastasis are independent prognostic factors for NSCLC, significantly affecting the prognosis of patients possibly through the classical signaling pathway AKT.

Abbreviations

RNA binding motif protein 3 (RBM3)

non-small cell lung cancer (NSCLC)

tumor node metastasis (TNM)

American Joint Committee on Cancer (AJCC)

overall survival (OS)

progression-free survival (PFS)

nasopharyngeal carcinoma (NPC)

Declarations

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Department of Oncology, Qingdao Municipal Hospital.

Consent for publication

Written informed consent was obtained from all participants.

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Availability of data and material

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Competing interests

None.

Funding

None.

Authors' contributions

LC and DSL collected the data and drafted the manuscript. YC participated in collecting the data and analyzed the data. KG and YL participated in collecting the data. CYG and TLZ conceived of the study, designed the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. De Angelis, R., et al., *Cancer survival in Europe 1999–2007 by country and age: results of EURO CARE–5 –a population-based study*. The lancet oncology, 2014. 15(1): p. 23–34.
2. Shi, Y. and Y. Sun, *Manual Of Medical Oncology*, ed. P.s.M. P. House. 2015: Ren min wei sheng chu ban she.
3. Sureban, S., et al., *Translation regulatory factor RBM3 is a proto-oncogene that prevents mitotic catastrophe*. Oncogene, 2008. 27(33): p. 4544.
4. O'connell, R. M., et al., *Physiological and pathological roles for microRNAs in the immune system*. Nature Reviews Immunology, 2010. 10(2): p. 111.
5. Jonsson, L., et al., *High RBM3 expression in prostate cancer independently predicts a reduced risk of biochemical recurrence and disease progression*. Diagnostic pathology, 2011. 6(1): p. 91.
6. Yang, G., et al., *RNA-binding protein quaking, a critical regulator of colon epithelial differentiation and a suppressor of colon cancer*. Gastroenterology, 2010. 138(1): p. 231–240. e5.
7. Schneider, J., et al., *mRNA expression of the putative antimetastatic gene BRMS1 and of apoptosis-related genes in breast cancer*. Cancer Genomics-Proteomics, 2011. 8(4): p. 195–197.

- 8.Boman, K., et al., *Decreased expression of RNA-binding motif protein 3 correlates with tumour progression and poor prognosis in urothelial bladder cancer*. BMC urology, 2013. 13(1): p. 17.
- 9.Ye, F., et al., *High RNA-binding motif protein 3 (RBM3) expression is independently associated with prolonged overall survival in intestinal-type gastric cancer*. Medical science monitor: international medical journal of experimental and clinical research, 2017. 23: p. 6033.
- 10.Molina, J. R., et al. *Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship*. in *Mayo Clinic Proceedings*. 2008. Elsevier.
- 11.Tas, F., et al., *Age is a prognostic factor affecting survival in lung cancer patients*. Oncology letters, 2013. 6(5): p. 1507–1513.
- 12.Segal, R., K. Miller, and A. Jemal, *Cancer statistics, 2018*. CA Cancer J Clin, 2018. 68: p. 7–30.
- 13.Chen, K., et al., *Comparison of the efficacy of concurrent chemoradiotherapy plus consolidation chemotherapy and sequential chemoradiotherapy in the treatment of elderly patients with stage III non-small cell lung cancer*. Chinese Journal of Gerontology, 2014. 34(9): p. 2420–2421.
- 14.HU, H.-t., et al., *Clinical observation on concurrent chemotherapy plus radiotherapy in treatment of stage II non-small cell lung cancer [J]*. Journal of Regional Anatomy and Operative Surgery, 2011. 1.
- 15.Derry, J. M., J. A. Kerns, and U. Francke, *RBM3, a novel human gene in Xp11. 23 with a putative RNA-binding domain*. Human molecular genetics, 1995. 4(12): p. 2307–2311.
- 16.Zhou, R.-B., et al., *RNA binding motif protein 3: a potential biomarker in cancer and therapeutic target in neuroprotection*. Oncotarget, 2017. 8(13): p. 22235.
- 17.Ehlén, Å., et al., *Expression of the RNA-binding protein RBM3 is associated with a favourable prognosis and cisplatin sensitivity in epithelial ovarian cancer*. Journal of translational medicine, 2010. 8(1): p. 78.
- 18.Matsuda, A., et al., *Generation of mice deficient in RNA-binding motif protein 3 (RBM3) and characterization of its role in innate immune responses and cell growth*. Biochemical and biophysical research communications, 2011. 411(1): p. 7–13.
- 19.Zeng, Y., et al., *Down-regulating cold shock protein genes impairs cancer cell survival and enhances chemosensitivity*. Journal of cellular biochemistry, 2009. 107(1): p. 179–188.
- 20.Cok, S. J., et al., *Identification of RNA-binding proteins in RAW 264.7 cells that recognize a lipopolysaccharide-responsive element in the 3-untranslated region of the murine cyclooxygenase-2 mRNA*. Journal of Biological Chemistry, 2004. 279(9): p. 8196–8205.
- 21.Jögi, A., et al., *Nuclear expression of the RNA-binding protein RBM3 is associated with an improved clinical outcome in breast cancer*. Modern Pathology, 2009. 22(12): p. 1564.

22.Ma, R., et al., *RNA binding motif protein 3 (RBM3) drives radioresistance in nasopharyngeal carcinoma by reducing apoptosis via the PI3K/AKT/Bcl-2 signaling pathway*. American journal of translational research, 2018. 10(12): p. 4130.

23.Zeng, D., J. Hua, and Y. An, *Expression and clinical significance of RBM3 in epithelial ovarian cancer*. Nanchang University, 2016. 22(31): p. 14–15.

Tables

Table 1. Relationship between RBM3 and clinical pathological parameters

	n=128 RBM3 (n=128)		χ^2	P
	poor (%) rich (%)			
Age			0.110	0.858
>55	63	35	28	
≤55	65	38	27	
Gender			0.316	0.687
Male	34	18	16	
Female	94	55	39	
Smoking			0.045	0.845
Yes	92	53	39	
No	36	20	16	
Degree of tumor differentiation			6.934	0.012
Medium + high differentiation	59	41	18	
Poor differentiation	69	32	37	
Lymphatic metastasis			5.723	0.02
Presence (N+)	73	35	38	
Absence (N0)	55	38	17	
T staging			6.386	0.041
T (1)	54	30	24	
T (2)	39	28	11	
T (3)	35	15	20	
AKT			5.466	0.021
Low expression	71	47	24	
High expression	57	26	31	

Table 2. Univariate analysis of RBM3 and OS, PFS (n=128)

	OS	PFS	
	hr (95% CI)	<i>P</i>	hr (95% CI) <i>P</i>
Age			
≤55	0.902 (0.588-1.384)	0.636	0.841 (0.552-1.280) 0.418
>55	1.000 (Ref.)		1.000 (Ref.)
Gender			
Male	0.642 (0.381-1.082)	0.096	0.658 (0.396-1.096) 0.108
Female	1.000 (Ref.)		1.000 (Ref.)
Smoking			
Yes	0.960 (0.590-1.563)	0.871	0.919 (0.566-1.491) 0.732
No	1.000 (Ref.)		1.000 (Ref.)
Degree of tumor differentiation			
Medium + high differentiation	0.662 (0.426-1.029)	0.067	0.639 (0.414-0.986) 0.043
Poor differentiation	1.000 (Ref.)		1.000 (Ref.)
Lymphatic metastasis			
Absence (N0)	0.217 (0.131-0.361)	0.000	0.233 (0.143-0.378) 0.000
Presence (N1+N2)	1.000 (Ref.)		1.000 (Ref.)
T staging			
T3	1.594 (0.959-2.649)	0.072	1.454 (0.885-2.390) 0.139
T2	1.806 (1.058-3.083)	0.030	1.191 (0.703-2.016) 0.052
T1	1.000 (Ref.)		1.000 (Ref.)
T1	0.554 (0.324-0.945)	0.030	0.594 (0.315-1.005) 0.052
T2	0.882 (0.513-1.517)	0.349	0.864 (0.503-1.486) 0.597
T3	1.000 (Ref.)		1.000 (Ref.)
AKT			
Low expression	0.522 (0.340-0.803)	0.003	0.543 (0.356-0.829) 0.005

High expression	1.000 (Ref.)	1.000 (Ref.)
RBM3		
Low expression	0.293 (0.187-0.458)0.000	0.310 (0.200-0.481) 0.000
High expression	1.000 (Ref.)	1.000 (Ref.)

Table 3. Multivariate analysis of RBM3 and OS, PFS (n=128)

	OS		PFS	
	hr (95% CI)	<i>P</i>	hr (95% CI)	<i>P</i>
Age				
≥55	0.658 (0.411-1.055)	0.083	0.631 (0.394-1.010)	0.055
≤55	1.000 (Ref.)		1.000 (Ref.)	
Gender				
Male	0.559 (0.302-1.033)	0.063	0.596 (0.323-1.100)	0.098
Female	1.000 (Ref.)		1.000 (Ref.)	
Smoking				
Yes	0.632 (0.308-1.298)	0.211	0.682 (0.338-1.374)	0.284
No	1.000 (Ref.)		1.000 (Ref.)	
Degree of tumor differentiation				
Medium + high differentiation	1.056 (0.604-1.849)	0.847	0.963 (0.560-1.656)	0.891
Poor differentiation	1.000 (Ref.)		1.000 (Ref.)	
Lymphatic metastasis				
Absence (N0)	0.162 (0.090-0.290)	0.000	0.174 (0.099-0.306)	0.000
Presence (N1+N2)	1.000 (Ref.)		1.000 (Ref.)	
T staging				
T3	1.583 (0.908-2.761)	0.106	1.388 (0.808-2.384)	0.234
T2	1.737 (0.901-3.351)	0.099	1.536 (0.813-2.903)	0.186
T1	1.000 (Ref.)		1.000 (Ref.)	
T1	0.576 (0.298-1.110)	0.099	0.651(0.344-1.230)	0.186

T2	0.911 (0.438-1.897)0.804	0.904 (0.443-1.842)0.780
T3	1.000 (Ref.)	1.000 (Ref.)
AKT		
Low expression	0.312 (0.193-0.503)0.000	0.333 (0.207-0.535) 0.000
High expression	1.000 (Ref.)	1.000 (Ref.)
RBM3		
Low expression	0.430 (0.256-0.721)0.001	0.450 (0.270-0.747) 0.002
High expression	1.000 (Ref.)	1.000(Ref.)

Figures

Figure 1

Immunohistochemical staining of RBM3 and AKT in lung cancer tissues.