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ORIGINAL ARTICLE

CUG-binding protein 1 (CUGBP1) expression and prognosis of brain metastases from non-small cell lung cancer

Xiaofei Wang, Wenjie Jiao, Yandong Zhao, Liangdong Zhang, Ruyong Yao, Yongjie Wang, Mingzhao Wang, Yiren Luo & Jinpeng Zhao

Department of Thoracic Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China

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Correspondence
Wenjie Jiao, Department of Thoracic Surgery, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266000, China.
Tel: +86 53282912305
Fax: +86 53282911999
Email: xwkejwj@126.com

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Abstract

Background: The brain is a frequent site of metastases from non-small cell lung cancer (NSCLC). The purpose of this study was to detect the expression of CUG-binding protein 1 (CUGBP1) messenger ribonucleic acid (mRNA) and Ki-67 in metastasized brain tissue from NSCLC and determine the relationship between CUGBP1 and brain metastases.

Methods: The expression of CUGBP1 mRNA and Ki-67 in metastasized brain tissue from NSCLC was investigated by semiquantitative polymerase chain reaction and immunohistochemistry, respectively. The expression of CUGBP1 and Ki-67 in metastasized brain tissue from NSCLC was related to clinical characteristics, as assessed using the chi-square test. The prognostic significance was assessed by univariate and multivariate analyses using the Cox hazard model.

Results: The expression of CUGBP1 mRNA and Ki-67 was overexpressed in metastasized brain tissue from NSCLC and was correlated with differentiation. In addition, by both univariate and multivariate survival analyses, CUGBP1 expression, Ki-67 expression, and age were noted to be independent indicators of a shorter post-surgical survival.

Conclusion: The expression of CUGBP1 is an important factor in the development of brain metastases from NSCLC.

Introduction

Lung cancer, particularly non-small cell type, is the most frequent type of cancer worldwide.[1] Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths with brain metastasis being one of the direst complications.[2] Brain metastasis is an important prognostic factor of NSCLC.[3] More accurate assessment of brain metastasis is an important part in the management of lung cancer, as an early diagnosis would contribute to a better survival rate. Many investigations have been conducted to diagnose lung cancer and brain metastasis through the identification of molecular targets.[4–7] However, to date, a significant correlation between CUG-binding protein 1 (CUGBP1) expression and brain metastases in lung cancer has not been reported.

CUG-binding protein 1 is a member of the CEFE (CUGBP and embryonic lethal abnormal vision-like factor) family of human ribonucleic acid (RNA)-binding proteins.[8] RNA CUG repeats are expanded in the 3′-untranslated region (UTR) of the gene encoding the myotonic dystrophy protein kinase (DMPK) and cause myotonic dystrophy type 1 disease (DM1).[9,10] CUGBP1 is involved in the control of splicing, deadenylation, messenger (m)RNA decay, and translation.[10–12] In addition to its role in embryonic and cardiac development, skeletal muscle and adipose tissue differentiation, and germ cell formation, CUGBP1 plays an important role in genesis and deterioration of certain tumors. The overexpression of CUGBP1 has been reported in DM1 myoblasts, the heart, esophageal epithelial cells, skeletal muscle tissues, NSCLC, and some DM1 mouse models.[13] Although the overexpression of CUGBP1 has been evaluated in NSCLC, the correlation between this expression and brain metastasis remains unclear. The Ki-67 antigen has been developed to investigate the cell cycle, as well as cell proliferation. In lung cancer, Ki-67 has been reported to be a marker for evaluating cell proliferative activity and cancer metastasis.[4,14] We used this marker in our study to investigate the expression of CUGBP1 and Ki-67, and we assessed whether there was an association between CUGBP1 expression and brain metastasis and NSCLC prognosis.

Materials and methods

Patients

In total, 68 NSCLC patients with metachronous brain metastasis who underwent brain metastasis tumor resection in the Department of Neurosurgery at the Affiliated Hospital of Qingdao University from January 2009 to April 2014 were enrolled in our investigation. Written and informed consent was obtained from all patients and the ethical committee of our hospital approved the investigation. Brain metastasis tissue was obtained via surgery and immediately stored at −80°C until processing. The clinical and pathological data of the 68 patients were recorded according to the 7th edition of the tumor node metastasis (TNM) classification and staging system for lung cancer, published by the International Association for the Study of Lung Cancer (IASLC), the International Union Against Cancer (UICC), and the American Joint Committee on Cancer (AJCC), enacted on 1 January 2010.[15] All patients underwent radiotherapy after surgery in the Department of Oncology at the Affiliated Hospital of Qingdao University. The clinico-pathological data analyzed included gender, age, smoking, histology, T-stage, and differentiation. The characteristics of the patients are listed in Table 1.

Semi-quantitative reverse transcriptase-polymerase chain reaction of CUG-binding protein 1 (CUGBP1) messenger ribonucleic acid

Each sample, including cancer and normal tissues from the same patient, was frozen in liquid nitrogen immediately after surgical resection before the extraction of RNA. Trizol was used to isolate the total RNA of the metastasized brain tissue according to the manufacturer's protocol. cDNA was prepared from each total RNA sample using 10 ng RNA for reverse transcription. The polymerase chain reaction (PCR) conditions were as follows: 95°C for five minutes; 40 cycles of 94°C for 15 seconds, 57°C for 20 seconds, and 72°C for 60 seconds; and a final extension at 72°C for 10 minutes. The sequences of CUGBP1 and glyceraldehyde 3-phosphate dehydrogenase primers were as follows: forward: 5′-GTC AGTGGTGGACCTGACCT-3′ and 5′-TGACTTCAACAGC GACACCCA-3′, reverse: 5′-AGGGGTCTACATGGCAAC TG-3′ and 5′-CACCCTGTTGCTGTAGCCAAA-3′. Samples were separated using 2% agarose gel electrophoresis. CUGBP1 mRNA samples were quantified using a FluoroImager scanner (Molecular Dynamics, Sunnyvale, CA, USA) and analyzed with ImageQuant software (Molecular Dynamics). We selected 0.6 as the cut-off value for CUGBP1 mRNA expression. If the value of CUGBP1 mRNA was above

Table 1 Correlation between CUGBP1 expression and clinicopathological characteristics

Clinicopathological characteristics	Cases (N)	CUGBP1 mRNA			Ki-67			
		Negative (N)	Positive (N)	P value	Negative (N)	Positive (N)	*P* value	*P* value
All patients	68	22	46		19	49		
Gender				0.536			0.284	
Male	46	16	30		11	35		
Female	22	6	16		8	14		
Age				0.577			0.232	
<60	28	8	20		10	18		
≥60	40	14	26		9	31		
Smoking				0.946			0.681	
Non-smoker	9	3	6		2	7		
Smoker	59	19	40		17	42		
Histology				0.257			0.903	
Squamous cell carcinoma	12	3	9		3	9		
Adenocarcinoma	40	16	24		12	28		
Other	16	3	13		4	12		
T-stage				0.678			0.062	
T1, T2	44	15	29		9	35		
T3	24	7	17		10	14		
Differentiation				0.024			0.007	
Well/moderate	36	16	20		15	21		
Poor	32	6	26		4	28		

CUGBP1, CUG-binding protein 1; mRNA, messenger ribonucleic acid.

the cut-off value, the patient was considered positive for CUGBP1 mRNA, otherwise, the patient was considered negative. Each assay was performed at least three times to verify the results.

Ki-67 immunohistochemistry staining

Tumor sections (4-mm-thick) were obtained from 68 formalin-fixed, paraffin-embedded archival brain metastasis tumor tissues. The slides were dewaxed in xylene and gradually rehydrated with alcohol. The slides were heated in a pressure cooker for 50 minutes in 10 mM citrate buffer (pH 6.0), treated with 0.3% H2O2 for five minutes, and finally incubated with MIB-1 monoclonal antibody (DAKO Corp., Carpinteria, CA, USA) for 10 minutes. After incubation with a secondary biotinylated antibody for 10 minutes and treatment with a streptavidin peroxidase reagent (DAKO Corp.), the slides were rinsed and then stained with diaminobenzidine chromogen solution (ResGen Invitrogen Corp., Carlsbad, CA, USA). After a light counterstaining with hematoxylin and dehydration, coverslips were mounted. To evaluate the percentage of cancer cells with Ki-67 nuclear immunoreactivity, at least 1000 tumor cells per slide were counted. The median value of this series (41% of positive cells) was used as the cut-off value to distinguish tumors with a low index of cell proliferation (0–40%) from those with a high index of cell proliferation (41–100%). If the extent of staining was above the cut-off value, the patient was considered positive for Ki-67, otherwise the patient was considered negative.

Statistical analysis

The association between the expression of CUGBP1 and clinicopathological characteristics were analyzed using the chi-square test. Cox univariate analysis was performed to compare time to progression (TTP) survival between patients, using the log-rank test. The value of the independent prognostic factors was assessed in multivariate analysis using the Cox hazard model. All statistical analyses were performed with the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at $P < 0.05$ in all tests.

Results

Patient characteristics

In total, 68 patients were enrolled in this study (Table 1). The age of the patients (46 men and 22 women) ranged from 39–72 years, with a mean age of 58 years. Smoking history was reported in 59 out of 68 patients (86.8%). The postsurgical pathological stage was determined using the current TNM classification. Histologically, 40 patients had adenocarcinoma, 12 had squamous cell carcinoma, and 16 had other cell carcinomas. Intraoperative therapy was not performed on any patient.

Relationship between the expression of CUGBP1 and, Ki-67 and brain metastasis in non-small cell lung cancer

Using immunohistochemistry and PCR analyses, we observed 67.6% CUGBP 1 mRNA expression ($x2 = 5.892$, $P = 0.015$) and 72.1% Ki-67 expression ($x^2 = 10.903$, $P = 0.001$) had positive significance (Table 1). CUGBP1 and Ki-67 expression was associated with the differentiation and brain metastasis (Tables 1, 2). The expression of CUGBP1 and Ki-67 is shown in Figure 1. The relationship between CUGBP1 and Ki-67 is shown in Table 3.

In the present study, we observed a significant correlation between the expression of CUGBP1 and Ki-67 and brain metastasis in NSCLC (Table 2). We observed a significant correlation between the levels of CUGBP1 and the Ki-67 expression ($x^2 = 7.86$, $P = 0.005$) (Table 3). No significant correlation was found between CUGBP1 mRNA expression and the histologic types of the tumors or the gender of the patients. The median TTP of all patients was five months (range: 1–13 months). Results of the log-rank test were marginally significant ($x^2 = 8.417$, $P = 0.004$): individuals without an elevated CUGBP1 had a TTP of 7.868 months, while those with an elevated CUGBP1 had a TTP of 5.076 months, as shown in Figure 2. In univariate analysis, our data indicated that survival rates were closely related to CUGBP1 ($P = 0.001$) and Ki-67 expression ($P = 0.004$) (Table 4). Cox regression multivariate analysis of all of these factors influencing TTP revealed that CUGBP1 expression in NSCLC patients with brain metastasis was an independent prognostic factor

Table 2 The expression of CUGBP1, Ki-67 in NSCLC with brain metastasis

		CUGBP1 mRNA				Ki-67			
Items	Cases	Negative N (%)	Positive N (%)	X^2	*P*	Negative N (%)	Positive N (%)	X^2	*P*
Cancer Group	68	22 (32.4)	46 (67.6)	5.892	0.015	19 (27.9)	49 (72.1)	10.903	0.001
Normal group	68	36 (52.9)	32 (47.1)			38 (55.9)	30 (44.1)		

CUGBP1, CUG-binding protein 1; mRNA, messenger ribonucleic acid; NSCLC, non-small cell lung cancer.

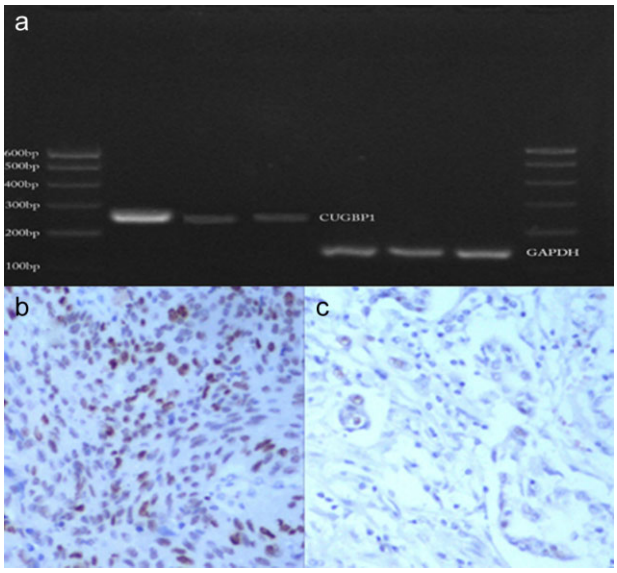

Figure 1 (**a**) Expression of CUG-binding protein 1 (CUGBP1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) messenger ribonucleic acid mRNA detected by reverse-transcription polymerase chain reaction. (**b**,**c**) Representative immunohistochemical staining for Ki-67. (**b**) Positive expression of Ki-67. (**c**) Negative expression of Ki-67.

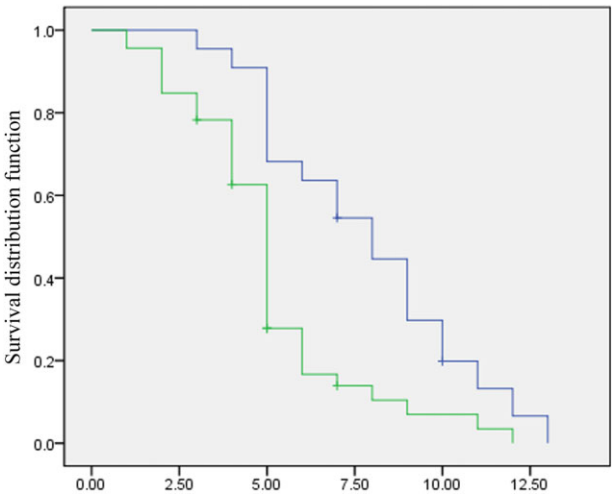

Figure 2 Cox model postsurgical survival according to CUG-binding protein 1 (CUGBP1) expression. The blue line represents patients with a low CUGBP1 expression (negative); the green line represents patients with a high CUGBP1 expression (positive).

(hazard ratio [HR] =2.411, 95% confidence interval [CI] 1.331–4.370), independent of Ki-67 expression (HR = 2.376, 95% CI 1.240–4.553) (Table 5).

Discussion

As NSCLC is the leading cause of cancer death, determining the molecular markers associated with progression and prognosis is of vital importance.[2,3,16] To the best of our knowledge, this is the first study in which the relationship between CUGBP1 expression and clinicopathological features, with special attention given to the prognostic significance of NSCLC with brain metastases, has been investigated. This study provides strong evidence that the overexpression of CUGBP1 is an independent indicator of poor prognosis, which provides us with new insights into the detection, treatment, and prognosis of NSCLC patients with brain metastases. On the basis of our statistical data, we suggest that CUGBP1 should be routinely detected by screening NSCLC patients with brain metastases in future clinical practice.

CUGBP1 is a human RNA-binding protein implicated in DM1, a neuromuscular disease associated with a CUG triplet expansion in the 3′-UTR of the DMPK gene. Various functions have been reported for CUGBP1, such as protein translation regulation, RNA stability, and splicing. In addition, CUGBP1 plays an important role in tumor genesis.[17] Arnal-Estape *et al*. reported the significance of CUGBP1 in the genesis and the deterioration of certain tumors.[18] Rattenbacher *et al*. reported that CUGBP1 and its binding target transcripts define a posttranscriptional regulatory network that functions to control cellular growth and homeostasis and suggested that disruptions in this network correlate with the development of cancer.[19] Wang *et al*. reported that silencing of CUGBP1 by RNA interference could be developed as a promising therapeutic approach for gastric cancer.[20] In addition, Jiao *et al*. found that CUGBP1 is overexpressed in NSCLC, and may be used as a biomarker in conjunction with other methods for clinical diagnosis.[21] The underlying biological mechanisms that might explain the relationship between CUGBP1 expression and brain metastasis in NSCLC are not known, but our data suggest that CUGBP1 plays an important role in the genesis and deterioration of tumors.

Table 3 Correlation between CUGBP1 mRNA and Ki-67 expression

	Ki-67				Consistency test	
CUGBP1 mRNA expression	Negative	Positive	X^2	*P*	Kappa	95% CI
Negative (N)	11	11	7.86	0.005	0.338	1.532–14.723
Positive (N)	8	38				

CI, confidence interval; CUGBP1, CUG-binding protein 1; mRNA, messenger ribonucleic acid.

Table 4 Cox univariate analysis of initial variables

Variables	Number of patients N (%)	X^2	*P* value
Gender		0.593	0.441
Male	46 (67.6)		
Female	22 (32.4)		
Age		1.512	0.216
<60	28 (41.2)		
≥60	40 (58.8)		
Smoking		0.056	0.813
Non-smoker	9 (13.2)		
Smoker	59 (86.8)		
Histology		2.237	0.312
Squamous cell carcinoma	12 (17.6)		
Adenocarcinoma	40 (58.8)		
Other	16 (23.6)		
T-stage		1.474	0.225
T1, T2	44 (64.7)		
T3	24 (32.3)		
Differentiation		1.392	0.238
Well/moderate	36 (52.9)		
Poor	32 (47.1)		
CUGBP1		10.834	0.001
Negative	22 (32.4)		
Positive	46 (67.6)		
Ki-67			
Negative	19 (27.9)	8.134	0.004
Positive	49 (72.1)		

CUGBP1, CUG-binding protein 1.

Ki-67 has also been previously defined as an important biomarker for metastasis in NSCLC.[22] Ki-67 is a nuclear antigen in proliferating human cells. It is expressed in mid G1, S, and G2; it reaches its peak in the M phase, and is very rapidly degraded at the end of the M phase.[22,23] Ki-67 expression is an important biomarker for proliferation and metastasis and has been reported in all types of cancer, including lung, breast, and prostate cancers and colorectal liver metastasis. Li *et al*. and Verhoven *et al*. reported that Ki-67 is an important prognostic factor for metastasis.[24,14] Bubb *et al*. reported that the expression of Ki-67 has a positive correlation with brain metastasis in NSCLC.[25] Furthermore, Yamayoshi *et al*. reported that the overexpression of Ki-67 is significantly associated with metastasis in lung cancer.[26] In our study, the expressions of CUGBP1 ($P = 0.015$) and Ki-67 ($P = 0.001$) were similar to previous studies. There is a significant correlation between the level of CUGBP1 mRNA and Ki-67 antigen expression ($x^2 = 7.86$, $P = 0.005$). Although a relationship between the expression of CUGBP1 and brain metastasis has not previously been reported in the literature, our data indicate that the level of expression of CUGBP1 is an important factor related to brain metastasis. Using Cox univariate survival analysis, the TTP of NSCLC patients with brain metastasis is associated with the variables Ki-67 ($x^2 = 8.134$, $P = 0.004$) and CUGBP1 ($x^2 = 10.834$, $P = 0.001$). Cox multivariate survival analysis revealed that overexpression of CUGBP1 predicted a poor survival (HR = 2.411), independent of other powerful predictors, such as Ki-67 (HR = 2.376), similar to conclusions found in previous studies.[27] Therefore, CUGBP1 is an excellent tumor marker for detecting brain metastasis in patients.

Table 5 Cox multivariate analysis of prognostic factors of NSCLC with brain metastasis

Characteristics	X^2	*P* value	HR	95% CI
CUGBP1	8.417	0.004	2.411	1.331–4.370
Ki-67	6.804	0.009	2.376	1.240–4.553

CI, confidence interval; CUGBP1, CUG-binding protein 1; HR, hazard ratio; NSCLC, non-small cell lung cancer.

Although the extent to which administered therapy determines survival for a brain metastasis patient with elevated CUGBP1 expression is unclear, our data indicate that CUGBP1 expression is a strong and consistent determinant of superior survival, regardless of other independent predictors.[28] No direct evidence from previous studies or formal clinical trials exists to guide treatment decisions for the individual patient with brain metastasis on the basis of CUGBP1 expression; however, our study provides a direction for future clinical research.[29] A combination of age, and Ki-67 and CUGBP1 expression may be used to classify patients as having a low, intermediate, or high risk of death, which represents a new direction for treatment of NSCLC patients with brain metastasis because inhibition of the molecular markers of expression can be applied in future clinical trials.

Several limitations of our study must be acknowledged. First, as a prospective study, the evaluation of clinical response and TTP is often imprecise. Second, the number of NSCLC patients with brain metastases in our study was small, thereby reducing the statistical power of our results. A larger study is necessary to validate our data reporting the influence of CUGBP1 levels on brain metastasis in NSCLC.

Conclusion

In summary, CUGBP1 may play a major role in the development of brain metastasis in NSCLC. Our study may have an impact on future developments for the prevention, diagnosis, and therapy of NSCLC patients with brain metastasis.

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Disclosure

No authors report any conflict of interest.

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