·Basic Research·

Expression of Elf-1 and survivin in non-small cell lung cancer and their relationship to intratumoral microvessel density

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[Abstract] Background and Objective: The expression of transcription factor Elf-1 and inhibitor of apoptosis survivin in non-small cell lung cancer (NSCLC) is correlated with the angiogenic factor vascular endothelial growth factor (VEGF), and are both factors affecting the cell cycle. This study investigated the expression of Elf-1, survivin, and intratumoral microvessel density (iMVD) assessed by monoclonal antibody CD105 in NSCLC, and explored their correlations with clinicopathologic features and angiogenesis of NSCLC. **Methods:** PowerVision™-9000 immunohistochemistry was used to evaluate the expression of Elf-1, survivin, and CD105 in tissue microarrays containing 60 specimens of NSCLC and 9 specimens of normal tissue. Western blot analysis was used to evaluate the protein levels of Elf-1 and survivin in 17 specimens of NSCLC and 5 specimens of normal tissue. **Results:** Elf-1 and survivin were detected in 1 of the 9 normal tissues. The positive rates of Elf-1 and survivin in NSCLC were 70.0% and 65.0%, respectively. The expression levels of both Elf-1 and survivin were significantly related to tumor differentiation, lymphatic metastasis, clinical stage, and postoperative survival time ($P < 0.05$). Overexpression of both were related to poor prognosis: the survival rates were significantly lower in patients with positive expression than in those with negative expression ($P < 0.01$). Elf-1 expression was positively correlated with survivin expression ($r = 0.769$, $P < 0.01$). Elf-1 and survivin expressions were positively correlated with iMVD ($r = 0.446$, $P < 0.01$; $r = 0.435$, $P < 0.01$). **Conclusions:** The expression of Elf-1 and survivin in NSCLC is related to differentiation, lymphatic metastasis, clinical stage, and prognosis, and both are positively correlated with iMVD. Detection their combined expression can help to predict the malignant behavior of NSCLC. Blocking the activity of Elf-1 and survivin may be a new way to inhibit angiogenesis in NSCLC.

Key words: Lung neoplasm, Elf-1, survivin, iMVD

Elf-1, a member of the Ets family of transcription factors, may be an important regulatory factor and is expressed in specific cell cycles and correlated with developmental processes, mitotic activation, tumorigenesis, and virus gene activation[1]. At present, there are more studies of the Ets family on Ets-1 and Ets-2 than on Elf-1. Our preliminary study found that the expression of Elf-1 in non-small cell lung cancer (NSCLC) tissue was correlated with vascular endothelial growth factor (VEGF)[2]. VEGF is the most important angiogenic factor. Studies have shown that the expression of VEGF in colorectal cancer tissue is correlated with survivin, the strongest apoptosis inhibitor protein, which is currently found in the inhibitor of apoptosis protein (IAP) family[3]. Therefore, we used PowerVision™-9000 immunohistochemistry and Western blot analysis to detect the expression of Elf-1, survivin, and intratumoral microvessel density (iMVD) in NSCLC tissue and analyzed the relationship among them and its significance for exploring the roles of Elf-1 and survivin in NSCLC angiogenesis, providing a theoretical basis for inhibiting tumor angiogenesis in clinical practice, and determining the biologic behavior of malignant NSCLC.

Materials and Methods

Materials

A total of 60 resected NSCLC specimens, which were verified by pathologic histology (no patient received preoperative radiotherapy and chemotherapy), and 9 normal lung tissue specimens were obtained at the Affiliated Hospital of Binzhou Medical College between March 2002 and March 2004. Among them, 17 cancer specimens and 5 normal lung tissue specimens were randomly selected and placed in liquid nitrogen immediately to freeze, and then stored in a freezer at −70℃. The remaining specimens were fixed in 10% formalin, conventionally embedded

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This paper was translated from Chinese into English by *CJC* Medical Translation and edited by Hope J. Lafferty on 2010-01-30.

The Chinese version of this paper is avaiable at http://www.cjcsysu.cn/cn/article.asp?id=16608.

Received: 2009-09-23; Accepted: 2009-12-28

in paraffin, cut into 4-μm-thick serial sections, and stained with hematoxylin-eosin (HE). Of 60 NSCLC patients, 40 were men and 20 were women. The age of the patients ranged from 37 years to 71 years, with a median age of 53.5 years. There were 34 patients with squamous cell carcinoma (6 had Stage-I disease, 18 had Stage-II, and 10 had Stage-III) and 26 with adenocarcinoma (10 had Stage-I disease, 13 had Stage-II, and 3 had Stage-III). According to tumor size (D), they were divided into 3 groups: D ⩽ 3 cm (20 patients), 3 cm < D < 5 cm (16 patients) and D ⩾ 5 cm (24 patients). Regarding age (Y), they were divided into two groups: Y ⩽ 55 years (27 patients) and Y > 55 years (33 patients). Lymph node metastasis for all cases were recorded, including 30 patients with metastasis. According to the 1997 staging system of the International Union Against Cancer (UICC) for lung cancer, 10 were in T1N0M0, 9 were in T1N1M0, 2 were in T1N2M0, 10 were in T2N0M0, 5 were in T2N1M0, 1 was in T2N2M0, 10 were in T3N0M0, 3 were in T3N1M0, 2 were in T3N1M1, 1 was in T3N2M0, 1 was in T3N2M1, 2 were in T3N3M1, 1 was in T4N1M0, 1 was in T4N1M1, 1 was in T4N2M0, and 1 was in T4N2M1. All patients were followed for more than 5 years by phone (before September 2009). The survival time of the patients was defined to be from the date of surgery to either the last date of follow-up or the date of death caused by recurrence and metastasis. The median survival time was 42.6 months. Of 60 patients, 22 died within 1 year and 18 patients survived for more than 5 years.

Main reagents

Rabbit anti-human Elf-1 antibodies, goat anti-human survivin antibodies, and mouse anti-human CD105 antibodies were purchased from Santa Cruz Biotechnology, Inc (USA). The PowerVision™-9000 kit was purchased from Beijing Zhongshan Biotechnology Co., Ltd.

Methods

PowerVision™-9000 immunohistochemistry Tissue chips were made by a needle-punching device, with a needle diameter of 2 mm. PowerVision™-9000 immunohistochemistry was performed to stain. In each staining batch, phosphate buffered saline (PBS) instead of the primary antibody as the negative control and a section known to be positive as the positive control were used. Steps were as follows: paraffin-embedded tissue was deparaffinized, hydrated through a graded series of ethanol to distilled water, and then incubated in 3% H_2O_2 to inactivate endogenous peroxidases. After microwave antigen retrieval, the sections were incubated with the rabbit anti-human Elf-1 antibodies (1:100), goat anti-human survivin antibodies (1:100), and mouse anti-human CD105 antibodies (1:20), reagent II and regent III. The color reaction was developed by diaminobenzidine (DAB) and terminated by tap water after observation under the microscope. Afterward, the sections were counterstained with Mayer's hematoxylin and mounted with neutral gum.

Western blot analysis A total of 17 NSCLC tissues and 5 paraneoplastic tissues that were stored at −70℃ were added to a cooled protein lysis buffer, treated with homogenized ultrasound, centrifuged at high speed and low temperature, and then the supernatant fluid was collected as total cell proteins. After 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the protein was transferred to a membrane followed by incubation with 5% nonfat dried milk to block for 1 h, incubation with the primary antibody at 4℃ overnight, and incubation with the corresponding secondary antibody at room temperature for 2 h. Then DAB color reactions were performed. After rinsing with double-distilled water, the membrane was air-dried and stored in the dark. The results were collected by the automatic electrophoresis gel image analyzer (ChemiImager 5500, AlphaInnotech, USA) to measure the gray values.

Determining results

Determining immunohistochemical results of Elf-1 A total of 10 high power fields (HPF) were observed randomly in each section, with 100 cells counted per HPF. The extent of staining was observed and the percent of positively stained cells was recorded. As Elf-1 was a transcription factor and was mainly located in the nucleus[4], only the nuclear staining was counted. According to the extent of staining, positive scores were: 0 = no staining; 1 = light yellow; 2 = yellow; 3= brown or dark brown. According to the percent of positively stained cells, positive scores were: 0 ⩽ 5%; 1 = 6%−30%, 2 = 31%−70%, and 3 ⩾ 71%. The total score equals the sum of the positive range score and the positive extent score: 0 was defined as negative, 1−2 was defined as weak positive, 3−4 was defined as moderate positive, and 5−6 was defined as strong positive[5].

Determining immunohistochemical results of survivin Survivin was located mainly in the cytoplasm, presenting yellow to brown. According to Lu *et al.*[5], the extent of staining was scored as follows: 0 = no staining, 1 = weak positive, 2 = moderate positive, and 3= strong positive, and the percent of positively stained cells was scored as follows: 0 < 5%, 1 = 5%−25%, 2 = 26%−50%, 3 = 51%−75%, and 4 > 75%. The sum of any ⩾ 2 is defined as positive. Positive sections were divided into different groups according to their score: 2−3 was defined as (+), 4−5 was defined as (++), and 6−7 was defined as (+++).

Determining immunohistochemical results of CD105 and iMVD CD105 is a specific marker of vascular endothelial cells. After immunohistochemical staining, it shows brown or brown-yellow. Sections with 10% of the cytoplasm manifesting brown-yellow were defined as positive. Owing to the presence of heterogeneous iMVD in tumor sections, the assessment of iMVD was based on the criteria of Weidner *et al.*[6]: any brown-stained endothelial cells or endothelial cell clusters that were clearly separate from adjacent microvessels, tumor cells, and other connective tissue elements, were considered as a single countable microvessel (CD105 as the endothelial markers). The assessment of vascularity was performed by first scanning the sections at low power (×100) using a light microscope to identify the areas containing the greatest number of stained vessels (hotspots). Counting iMVD was performed at a higher power (× 200) using a light microscope. Five hotspot fields were counted and the average was regarded as the iMVD of that section. The sections with positive rates of vessels ⩾ 30% were defined as the high iMVD group, while the sections where < 30% were defined as the low iMVD group.

Statistical analysis

The statistical software SPSS11.0 was used for the statistical analysis. The relationship between each indicator and the clinicopathologic features were analyzed by rank-sum tests (Kruskal-Wallis and Mann-Whitney tests). The interrelationships between indicators used the Spearman rank correlation or the Kendell rank correlation for analysis. Survival analysis used the Kaplan-Meier method and a log-rank test was used to determine the differences. Statistical significance was assumed when $P < 0.05$.

Results

Expression of Elf and survivin

The positive rates of Elf and survivin expression in NSCLC were 70.0% and 65.0%, respectively. Both expressed weakly in only one case (11.11%) of normal lung tissue. Elf-1 positive staining granules were mainly located in the nucleus (Figure 1) and also discovered in the cytoplasm of some cancer cells. Positive staining for survivin was yellow-brown granules and mainly located in the cytoplasm (Figure 2). Microvasculature in NSCLC marked by CD105 appeared brown (Figure 3).

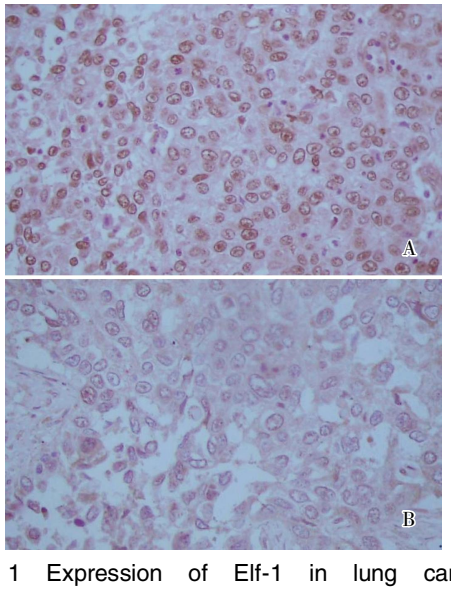

Figure 1 Expression of Elf-1 in lung carcinoma (PowerVision™-9000 ×400)

A, Elf-1 is intensely expressed in the nuclei of cancer cells in poorly differentiated lung squamous cell carcinoma.

B, Elf-1 is weakly expressed in the nuclei of cancer cells in well differentiated lung squamous cell carcinoma.

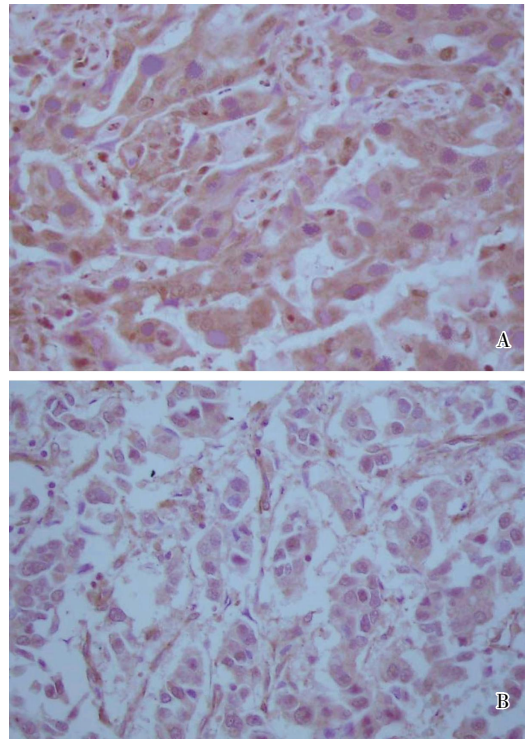

Figure 2 Expression survivin in lung carcinoma (PowerVision™-9000 ×400)

A, survivin is intensely expressed in the cytoplasm of cancer cells in lung adenocarcinoma with lymphatic metastasis.

B, survivin is weakly expressed in the cytoplasm of cancer cells in lung adenocarcinoma without lymphatic metastasis.

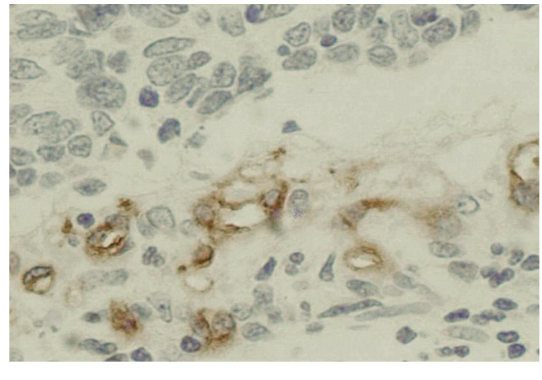

Figure 3 Microvessel in lung squamous cell carcinoma marked with CD105 antibodies (PowerVision™-9000 × 400)

Correlation between the expression of Elf-1 and the clinicopathologic features and prognosis in NSCLC

Results of immunohistochemistry The expression of Elf-1 was independent of tumor size, age, and sex of the patient ($P > 0.05$), while it was correlated with tumor differentiation ($P = 0.019$), lymph node metastasis ($P = 0.004$), clinical stage ($P = 0.001$), and postoperative survival ($P < 0.001$) (Table 1). The Kaplan-Meier survival curve showed that the 5-year survival rate for patients with NSCLC with Elf-1 expression was 19.0% and the average survival time was 35.76 months. The 5-year survival rate of patients without Elf-1 expression was 55.6% and the average survival time was 59.50 months (Figure 4). The log-rank test showed that $\chi^2 = 9.345$ and $P = 0.002$, suggesting that the expression of Elf-1 was correlated with the survival rate of patients and the survival rate of patients with Elf-1 expression was significantly lower than that of patients without Elf-1 expression.

Table 1 Correlation of Elf-1 expression with clinicopathologic features of non-small cell lung cancer

Characteristic	Patient No.	Elf-1 expression (Patient No. (%))				χ^2 or Z	P
		–	+	++	+++		
Sex						–0.212	0.832
Men	40	12 (20.00)	13 (21.67)	9 (15.00)	6 (1.00)		
Women	20	6 (1.00)	7 (11.67)	5 (8.33)	2 (3.33)		
Age (years)						–0.449	0.653
⩽ 55	27	7 (11.67)	12 (20.00)	6 (1.00)	2 (3.33)		
> 55	33	11 (18.33)	8 (13.33)	8 (13.33)	6 (1.00)		
Histological type						–0.062	0.950
Squamous cell carcinoma	34	10 (16.67)	11 (18.33)	10 (16.67)	3 (5.00)		
Adenocarcinoma	26	8 (13.33)	9 (15.00)	4 (6.67)	5 (8.33)		
Histological grade						9.928	0.019[a]
I	16	8 (13.33)	6 (1.00)	1 (1.67)	1 (1.67)		
II	31	7 (11.67)	13 (21.67)	8 (13.33)	3 (5.00)		
III	13	3 (5.00)	1 (1.67)	5 (8.33)	4 (6.67)		
Lymph node metastasis						–2.850	0.004
Positive	30	4 (6.67)	11 (18.33)	9 (15.00)	6 (1.00)		
Negative	30	14 (23.33)	9 (15.00)	5 (8.33)	2 (3.33)		
Tumor size (cm)						0.065	0.968
⩽ 3	20	6 (1.00)	6 (1.00)	7 (11.67)	1 (1.67)		
> 3, < 5	16	5 (8.33)	6 (1.00)	2 (3.33)	3 (5.00)		
⩾ 5	24	7 (11.67)	8 (13.33)	5 (8.33)	4 (6.67)		
Clinical stage						–3.431	0.001
I–II	44	17 (28.33)	17 (28.33)	6 (1.00)	4 (6.67)		
III–IV	16	1 (1.67)	3 (5.00)	8 (13.33)	4 (6.67)		
Postoperative survival (years)						15.282	< 0.001[b]
⩾ 5	18	10 (16.67)	6 (1.00)	1 (1.67)	1 (1.67)		
> 1, < 5	20	6 (1.00)	9 (15.00)	3 (5.00)	2 (3.33)		
⩽ 1	22	2 (3.33)	5 (8.33)	10 (16.67)	5 (8.33)		

[a] Between grade I and grade II, $P = 0.042$; between grade I and grade III, $P = 0.014$. [b] Between > 1, < 5 group and ⩽ 1 group, $P = 0.010$; between ⩾ 5 group and ⩽ 1 group, $P < 0.001$.

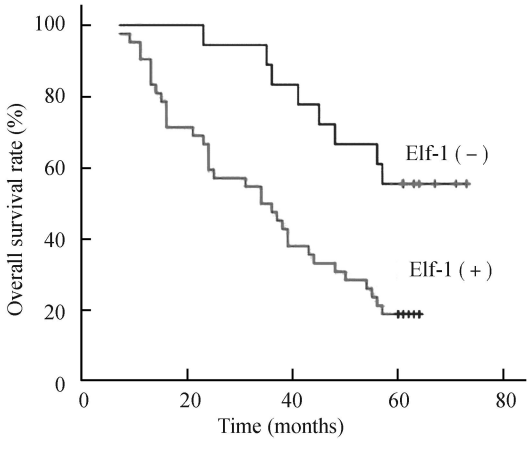

Figure 4 Relationship between Elf-1 expression and prognosis of patients with non-small cell lung cancer (NSCLC)

Results of Western blot analysis

The results of Western blot analysis confirmed the immunohistochemical analyses. A well-defined band with a molecular weight of 71 kDa was visible, suggesting the presence of Elf-1. However, Elf-1 was not seen in normal lung tissue. An analysis of absorbance showed lymph node metastasis and suggested that the lower the level of tumor cell differentiation, the higher the expression of the Elf-1 protein ($P < 0.05$) (Figure 5).

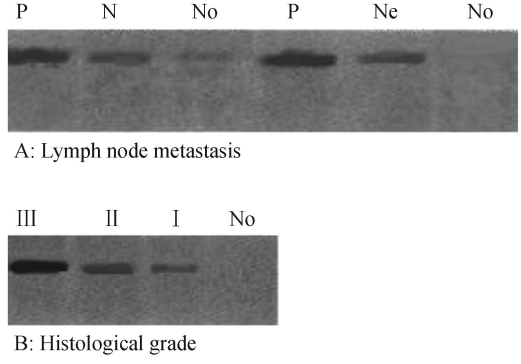

Figure 5 Results of Western blot analysis for Elf-1 in lung carcinoma and normal lung tissue
P, positive; Ne, negative; No, normal.

Correlation between the expression of survivin and the clinicopathologic features and prognosis in NSCLC

Results of immunochemistry The expression of survivin was independent of tumor size, age, and sex of the patient ($P >$

0.05), while it was correlated with tumor differentiation (P = 0.009), lymph node metastasis (P = 0.027), clinical stage (P = 0.001), and postoperative survival (P < 0.001) (Table 2). The Kaplan-Meier survival curve (Figure 6) showed that the 5-year survival rate of patients with NSCLC and survivin expression was 17.9% and the average survival time was 35.92 months. The 5-year survival rate of patients without survivin expression was 52.4% and the average survival time was 58.57 months. The log-rank test showed that χ^2 = 10.112 and P = 0.001, suggesting that the expression of survivin was correlated with the distribution of survival rate and the survival rate of patients with survivin expression was significantly lower than that of patients without Elf-1 expression.

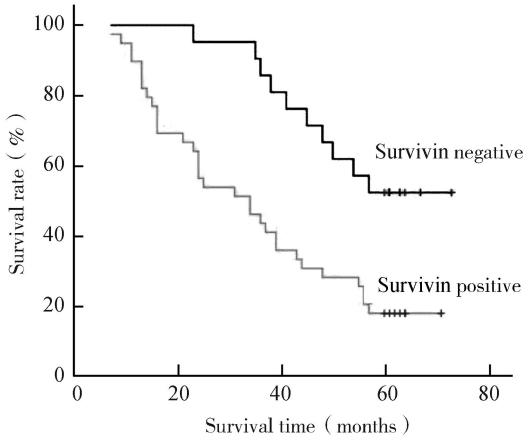

Figure 6 Relationship between survivin expression and prognosis of non-small cell NSCLC

Results of western blot analysis The results of Western blot analysis confirmed the immunohistochemical analyses. A well-defined band with a molecular weight of 16.5 kDa was visible, suggesting the presence of survivin. However, survivin was not seen in normal lung tissue. An analysis of absorbance showed lymph node metastasis and suggested that the lower the level of tumor cell differentiation, the higher the expression of the survivin protein (P < 0.05) (Figure 7).

Correlation between the expression of Elf-1, survivin, and iMVD

CD105 protein was mainly located in the membrane or the cytoplasm of microvascular endothelial cells, manifesting as brown granules. Elf-1 was positively correlated with iMVD (r = 0.446, P < 0.001) and survivin was also positively correlated with iMVD in NSCLC (r = 0.435, P = 0.001). There was a positive correlation between Elf-1 and survivin (r = 0.769, P < 0.001).

Table 2 Correlation of survivin expression with clinicopathologic features of non-small cell lung cancer

Characteristic	Patient No.	Elf-1 expression (Patient No. (%))				χ^2 or Z	P
		−	+	++	+++		
Sex						−0.384	0.701
Men	40	14 (23.33)	11 (18.33)	8 (13.33)	7 (11.67)		
Women	20	7 (11.67)	6 (1.00)	6 (1.00)	1 (1.67)		
Age (years)						−0.473	0.636
⩽ 55	27	8 (13.33)	11 (18.33)	7 (11.67)	1 (1.67)		
> 55	33	13 (21.67)	6 (1.00)	7 (11.67)	7 (11.67)		
Histological type						−0.576	0.565
Squamous cell carcinoma	34	12 (20.00)	10 (16.67)	10 (16.67)	2 (3.33)		
Adenocarcinoma	26	9 (15.00)	7 (11.67)	4 (6.67)	6 (1.00)		
Histological grade						9.482	0.009a
I	16	9 (15.00)	5 (8.33)	1 (1.67)	1 (1.67)		
II	31	10 (16.67)	10 (16.67)	8 (13.33)	3 (5.00)		
III	13	2 (3.33)	2 (3.33)	5 (8.33)	4 (6.67)		
Lymph node metastasis						−2.205	0.027
Positive	30	6 (1.00)	10 (16.67)	9 (15.00)	5 (8.33)		
Negative	30	15 (25.00)	7 (11.67)	5 (8.33)	3 (5.00)		
Tumor size (cm)						−0.858	0.651
⩽ 3	20	5 (8.33)	6 (1.00)	7 (11.67)	2 (3.33)		
> 3, < 5	16	5 (8.33)	7 (11.67)	2 (3.33)	2 (3.33)		
⩾ 5	24	11 (18.33)	4 (6.67)	5 (8.33)	4 (6.67)		
Clinical stage						−3.347	0.001
I–II	44	20 (33.33)	14 (23.33)	5 (8.33)	5 (8.33)		
III–IV	16	1 (1.67)	3 (5.00)	9 (15.00)	3 (5.00)		
Postoperative survival (years)						20.834	< 0.001[b]
⩾ 5	18	11 (18.33)	5 (8.33)	1 (1.67)	1 (1.67)		
> 1, < 5	20	8 (13.33)	9 (15.00)	2 (3.33)	1 (1.67)		
⩽ 1	22	2 (3.33)	3 (5.00)	11 (18.33)	6 (1.00)		

[a] Between grade II and grade III, P = 0.041; between grade I and grade III, P = 0.005. [b] Between >1, < 5 group and ⩽ 1 group, P = 0.000; between ⩾ 5 group and ⩽ 1 group, P < 0.001.

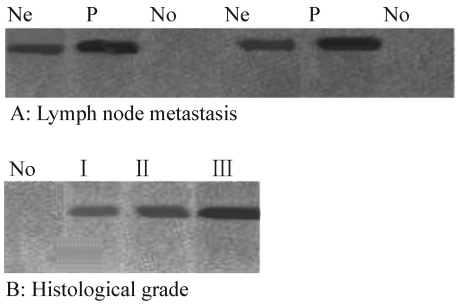

Figure 7 Results of Western blot analysis for survivin in lung carcinoma and normal lung tissue
P, positive; Ne, negative; No, normal.

Discussion

Lung cancer is the malignant tumor with the world's highest incidence and mortality rates, showing an upward trend. Studies confirmed that tumor foci existed as small and asymptomatic lesions before neovascularization. Tumor volumes increased rapidly and the capability of distant metastasis was enhanced after neovascularization [7-9]. Therefore, if we can find an effective means to regulate angiogenesis, we expect to control tumor growth and metastasis and extend the lives of patients. The number of factors of tumor angiogenesis can be evaluated by measuring iMVD[10]. At present, CD105 is an accurate index for measuring endothelial proliferation[11], with a higher specificity than CD34[4]. It is strongly expressed in tumor-associated neovascular endothelial cells. Therefore, CD105-iMVD was regarded as a quantitative indicator of tumorgenesis in this study.

The results of our preliminary study showed that the transcription factor Elf-1 was highly expressed in patients with NSCLC and correlated with VEGF. This study further found that the expression Elf-1 in NSCLC tissue was positively correlated with iMVD, suggesting that it played an important role in angiogenesis. Elf-1 is a less-studied transcription factor of the Ets family, which includes a group of transcription factors that contains a winged helix-turn-helix DNA-binding domain (known as ETS area) consisting of 85 amino acids and can recognize and bind to a purine-rich GGAA/T core sequence. The core sequence exists in 5'-flanking regulatory regions of a number of genes that are associated with extracellular matrix degradation and angiogenesis, such as the MMMP-3 and urokinase-type plasminogen activator (uPA). That led to the regulation of the transcription of these genes. Huang *et al.*[12] proposed that Elf-1 played a crucial role in angiogenesis. In the Ets family, Ets-1 and Ets-2 regulated the promoter gene of Tie1 and Tie2 via Elf-1 to play an import role in angiogenesis and its growth. Konno *et al.*[13] believed that Ets-1 was the intermediate link in the VEGF pathway of promoting angiogenesis, and blocking the expression of Ets-1 was likely to block tumor angiogenesis. Therefore, we thought that blocking the expression of Ets-1 could possibly become a new approach to block tumor angiogenesis. In addition, Khatun *et al.*[14] thought that Elf-1 was related to tumor invasion, metastasis, and poor prognosis by inducing angiogenesis. We also found that the expression of Elf-1 in NSCLC was correlated with lymph node metastasis, tumor cell differentiation, and clinical stage. The Kaplan-Meier survival analysis also showed that the expression of Elf-1 was correlated with the distribution of the survival rate of patients. Therefore, detecting the expression of Elf-1 can be used as a prognostic index in determining the malignant biology of NSCLC.

This study also detected that the expression of survivin, which was discovered to be strongest IAP member at present, increased significantly along with the increased malignancy of tumor cells, lymph node metastasis, and progression of TNM staging. The Kaplan-Meier survival analysis showed that the expression of survivin was correlated with the distribution of the survival rates of patients. This is consistent with the opinions found in most studies: survivin expressed widely in a variety of human malignant tumors and was closely related to the degree of malignancy and prognosis [15,16]. Besides, the expression of survivin in NSCLC was correlated with iMVD. In experiments of VEGF-mediated angiogenesis, Mesri *et al.*[17] showed that treatment with survivin antisense oligonucleotides resulted in a significant reduction in capillary networks. All of these indicate that survivin plays an important role in angiogenesis.

Therefore, the expressions of Elf and survivin were both correlated with iMVD and there was a positive correlation between them. On the one hand, they could cooperate with each other in the carcinogenesis and development of lung cancer, or they could cooperate with each other in angiogenesis. In our opinion, Elf-1 plays an important role in tumor angiogenesis by regulating promoter genes Tie1 and Tie2[12]. It is most important that the expression of VEGF is positively correlated with the expression of Elf-1 in NSCLC. VEGF can upregulate the expression of survivin[17], playing an important role synergistically in angiogenesis. Different from other IAP molecules, survivin is the only member of IAP family that was closely correlated with the cell cycle, being involved in regulating the cell cycle, regulating mitosis, and promoting cell proliferation. One of the mechanisms of survivin being involved in cell proliferation attributes to its overexpression, resulting in increased nuclear transport speed in the S phase and activation of the CDK2/cyclinE complex, leading to the phosphorylation of the Rb gene[18]. The studies of the Rb gene found that it contained an A/B pocket structure that can combine to proteins having an LXCXE sequence. Elf-1 contains an LXCXE sequence next to the amino-terminal and still has the ability to combine with variant Rb proteins that lack phosphorylation domains. Consequently, survivin and Elf-1 both have the ability to combine with Rb genes. The specific regulating mechanism remains to be studied.

In conclusion, this study demonstrated that Elf-1 and survivin were highly expressed in NSCLC and closely related to tumor cell differentiation, lymph node metastasis, clinical stage, and prognosis. There was a positive correlation between Elf-1 and survivin. Both correlated with iMVD and played an important role in invasion, metastasis, and angiogenesis of tumors. Combined detection of the expression of Elf-1 and survivin can be regarded as a reference indicator in determining the malignant biologic

behavior of NSCLC. Blocking their expression may be an effective antitumor approach.

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