

CXCL14 and NOS1 expression in specimens from patients with stage I–IIIA nonsmall cell lung cancer after curative resection

Xiaoqin Ji, MM, Zetian Shen, MM, Benxin Zhao, MM, Xi Yuan, MM, Xixu Zhu, MM*

Abstract

Many studies show that CXC chemokine ligand 14 (CXCL14) is highly expressed in tumor-associated stromal cells, promoting tumor cell growth, and invasion. Because of its unclear receptors, CXCL14-initiated intracellular signal cascades remain largely unknown. However, CXCL14 can regulate nitric oxide synthase 1 (NOS1) as its intracellular molecular target. In this paper, we investigated the expression of CXCL14 and NOS1 in specimens from patients with stage I–IIIA nonsmall cell lung cancer (NSCLC) after curative resection, and evaluated the prognostic significance of this gene expression in stromal fibroblasts and cancer cells.

Immunohistochemistry was used to detect the expression of CXCL14 and NOS1 in 106 formalin fixed, paraffin-embedded specimens from patients with stage I–IIIA NSCLC. The chi-square test was performed to examine the correlation of CXCL14 and NOS1 expression level with clinicopathological features. The effects of the expression of CXCL14 or NOS1 on progression-free survival (PFS) and overall survival (OS) were determined by Kaplan–Meier and Cox hazard proportional model.

The percentages of high CXCL14 expression in stromal fibroblasts and that in cancer cells were 46.2% (49/106) and 23.6% (25/106), respectively. The positive expression rates of NOS1 in cancer cells were 42.5% (45/106). The result indicated that there was a significant positive correlation between CXCL14 expression level in stromal fibroblasts and that in cancer cells ($\chi^2 = 4.158$, $P = .041$). In addition, the expression of CXCL14 in stromal fibroblasts was significantly correlated with NOS1 expression in cancer cells ($\chi^2 = 16.156$, $P < .001$). The 5-year PFS rates with low and high CXCL14 expression in stromal fibroblasts were 66.7% and 14.3% ($\chi^2 = 44.008$, $P < .001$), respectively, and the 5-year OS rates with those were 87.1% and 43.5% ($\chi^2 = 21.531$, $P < .001$), respectively. The 5-year PFS rates with negative and positive expression of NOS1 in cancer cells were 62.3% and 15.6% ($\chi^2 = 33.756$, $P < .001$), respectively, and the 5-year OS rates with those were 86.4% and 40.1% ($\chi^2 = 24.430$, $P < 0.01$), respectively.

Both the high expression of CXCL14 in stromal fibroblasts and the positive expression of NOS1 in cancer cells are independent negative predictors of PFS and OS in patients with stage I–IIIA NSCLC after curative resection.

Abbreviations: CXCL14 = CXC chemokine ligand 14, NO = nitric oxide, NOS1 = nitric oxide synthase 1, NSCLC = nonsmall cell lung cancer, OS = overall survival, PFS = progression-free survival.

Keywords: CXCL14, NOS1, NSCLC, prognosis, stromal fibroblasts

1. Introduction

Lung cancer is the leading cause of cancer death worldwide. In 2016, the number of newly diagnosed lung cancer cases was estimated to be 224,390 in the United States, of which lung cancer deaths accounted for 27% of all cancer deaths.^[1] About 80% of

lung cancer is nonsmall cell lung cancer (NSCLC), mainly including adenocarcinoma and squamous cell carcinoma.^[2] In recent years, although multidisciplinary treatment has been widely used in NSCLC patients, the overall prognosis is still poor. Previous studies showed that the prognosis of NSCLC patients after surgical resection depends on following clinical factors: tumor size, histological grade, vascular invasion, and visceral pleural infiltration.^[3–5] In addition, several molecular biomarkers can predict the prognosis of patients with NSCLC after surgical resection, such as RAS, MSH2, ERCC1, P27, etc.^[6] In recent years, many studies have shown that malignant progression of the tumor depends on both the tumor cells themselves and the surrounding microenvironment.^[7,8] The tumor microenvironment is associated with the extracellular matrix, metabolites, blood vasculature, growth factors, cytokines, and mesenchymal cells. Stromal fibroblasts are major cellular components of the tumor microenvironment and promote tumor cell growth, invasion, and metastasis through complex biological interactions between them and tumor cells.^[9,10]

Chemokines are 8 to 14kDa chemoattractant cytokines that mainly regulate cell migration during organogenesis and immune surveillance. Chemokines and their receptors are potentially important factors in oncogenesis, infection, allergy, and autoimmunity by modulating cellular attraction, proliferation, angiogenesis, as well as tumor cell growth and spreading.^[11] The chemokine (C-X-C Motif) ligand 14 (CXCL14) gene, also known as breast and kidney-expressed chemokine, is a member of the CXC chemokine family,

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Department of Radiation Oncology, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu Province, China.

* Correspondence: Xixu Zhu, Department of Radiation Oncology, Jinling Hospital, Medical School of Nanjing University, No. 305 Zhongshan East Road, Xuanwu District, Nanjing 210008, Jiangsu, China (e-mail: zhuxixu025@163.com).

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located on human chromosome 5q31.^[12] According to previous studies, enhanced expression of CXCL14 has been found in different types of cancers, including colon cancer,^[13] pancreatic cancer,^[14] prostate cancer,^[15] and breast cancer,^[16] where CXCL14 can be expressed by cancer cells or stromal cells or both cell types.^[17] Although the role of CXCL14 as a tumor suppressor seems well established,^[18,19] recent studies have shown that CXCL14 might actually promote tumor progression.^[20,21] Using a systems genetics approach, Williams et al^[22] found that the CXCL14, ITGAX, and LPCAT2 genes are identified as novel aggressive prostate cancer susceptibility genes. In another study conducted by Park et al,^[23] CXCL14 enhances the proliferation and migration of human lung cancer cell line NCI-H460 by interacting with heparan sulfate proteoglycans and sialic acids. However, previous studies have shown that the effects of CXCL14 on tumor growth depend on the cell type expressing this factor.^[24,25] When expressed by stromal fibroblasts, CXCL14 shows the tumor-promoting effect.^[24] However, it shows antitumor effect when expressed by malignant epithelial cells.^[25] In a study of Augsten et al,^[26] CXCL14-expressing fibroblasts promote the growth of prostate tumors by promoting the secretion of nitric oxide synthase 1 (NOS1) in fibroblasts. When the irreversible inhibitor L-NNA (Nw-nitro-L-arginine) of NOS1 was used or the NOS1 gene of fibroblasts was knocked out, the tumor-forming ability of CXCL14-expressing fibroblasts is inhibited, indicating that NOS1 may be an intracellular signaling pathway that CXCL14 promotes the tumor growth. NOS1, also known as neuronal nitric oxide synthase (NOS), is one of the 3 isomers of NOS. NOS1, NOS2 (iNOS), and NOS3 (eNOS), as well as their products, nitric oxide (NO), play an important role in carcinogenesis.^[27] Yang et al^[28] found that the expression of NOS1 in melanoma cells is higher than that in melanocytes. NOS1 enhances melanoma cell proliferation and invasion by inducing NO overproduction, leading to subsequent tumor metastasis. Similarly, NOS1 is also highly expressed in renal cell carcinoma and is associated with microvascular infiltration and lymph node metastasis.^[29]

However, there are few studies examining the correlation of CXCL14 and NOS1 expression level in cancer cells with their clinical significance. In this paper, we explored the immunohistochemical expression of CXCL14 and NOS1 in NSCLC. In addition, we examined the correlation of CXCL14 and NOS1 expression level with clinicopathological features, progression-free survival (PFS), as well as overall survival (OS). Our results show that both the high expression of CXCL14 in stromal fibroblasts and the positive expression of NOS1 in cancer cells are associated with poor PFS and OS for patients with stage I–III NSCLC. Thus, the CXCL14 and NOS1 can be identified as potential prognostic markers for patients with NSCLC.

2. Materials and methods

2.1. Patients and specimens

The postoperative paraffin-embedded tissue specimens were obtained from the archives of the Department of Pathology, Jinling Hospital Affiliated to Nanjing University Medical College, from March 2010 to May 2011. We retrospectively analyzed 106 tumor specimens from patients with stage I–III NSCLC. The study was approved by the Ethics Committee of Jinling Hospital, and written informed consents were obtained from all patients. The main characteristics of all patients are summarized in Table 1. The ages of the patients in this study were from 31 to 80 years (mean age was 60.5 years). According to the American Joint Committee on Cancer and the Union for International Cancer

Table 1

Patients' characteristics.

Characteristic	No of patients, %
Patients	106 (100%)
Mean age=60.5 y (range 31–80)	
Genders	
Male	77 (72.6%)
Female	29 (27.4%)
Smoke	
Yes	51 (48.1%)
No	55 (51.9%)
Pathologic type	
Squamous carcinoma	44 (41.5%)
Adenocarcinoma	58 (54.7%)
Adenosquamous carcinoma	4 (3.8%)
Pathologic differentiation	
High	20 (18.9%)
Median	56 (52.8%)
Low	30 (28.3%)
T category *	
T1	41 (38.7)
T2	38 (35.8)
T3	20 (18.9%)
T4	7 (6.6%)
N category *	
N0	62 (58.5%)
N1	23 (21.7%)
N2	21 (19.8%)
Pathologic stage *	
I	45 (42.4%)
II	27 (25.5%)
IIIA	34 (32.1%)
Treatment modality—curative resection	
Yes	106 (100%)
No	0 (0%)
Treatment modality—postoperative radiotherapy	
Yes	37 (34.9%)
No	69 (65.1%)
Treatment modality—postoperative chemotherapy	
Yes	67 (63.2%)
No	39 (36.8%)

* According to the American Joint Committee on Cancer and the Union for International Cancer Control stage system (7th edition).

Control stage system (7th edition), there were 45 patients with stage I NSCLC, 27 patients with stage II NSCLC, and 34 patients with stage IIIA NSCLC. All patients underwent radical resection. Thirty-seven patients received postoperative radiotherapy, and 67 patients received postoperative adjuvant chemotherapy. The chemotherapy regimens were cisplatin-based doublet.

2.2. Immunohistochemistry

A total of 106 specimens from patients were obtained for immunohistochemistry analyses. The formalin-fixed, paraffin-embedded tissues obtained from patients with stage I–III NSCLC were cut into consecutive 3- μ m-thick sections. One slide stained with hematoxylin eosin and the others immunohistochemically stained with a 2-step procedure. After deparaffinization and hydration, the tissue sections were subjected to antigen retrieval by pressurizing for 30 minutes. The sections were then incubated with 3% hydrogen peroxide solution at room temperature for 25 minutes to neutralize endogenous peroxidase activity. Next, the sections were incubated with antihuman CXCL14 rabbit polyclonal antibody (ab46010) (Abcam, Cambridge, UK; diluted

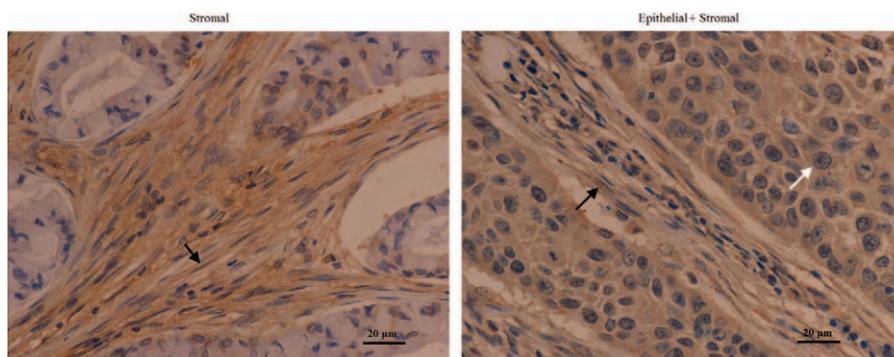


Figure 1. CXCL14 expression by immunohistochemistry in the specimens from patients with stage I–IIIa NSCLC after curative resection (magnification $\times 400$, scale bars = 20 μm). Examples of stromal as well as epithelial and stromal staining of CXCL14 in the specimen from patients with stage I–IIIa NSCLC. Black arrows show stromal CXCL14 expression and white arrows show epithelial CXCL14 expression. CXCL14=CXC chemokine ligand 14, NSCLC=non-small cell lung cancer.

1:100) and antihuman NOS1 rabbit monoclonal antibody (#4231) (Cell Signaling, Boston, MA; diluted 1:100) for overnight at 4°C. This was followed by incubation with the secondary antibody (ChemMate EnVision/HRP; Gene Tech, Shanghai, China) for 30 minutes at room temperature. Subsequently, the slides were treated with freshly prepared DAB liquid (DAB; Gene Tech, Shanghai, China) for 10 minutes, followed by counterstaining the nuclei with hematoxylin. The negative control used was the IgG2b isotype antibody (Dako), which was consistent with the same immunoglobulins concentration as for the anti-CXCL14 and anti-NOS1.

2.3. Immunohistochemical scoring

The immunohistochemical staining was scored by 2 pathologists. This is a single-blind study, and 2 pathologists read and scored the slides independently. The expression levels of each antibody in cancer cells and stromal fibroblasts were evaluated independently. The expression of CXCL14 and NOS1 was scored by assigning a percentage of positive cells and an intensity score. Immunohistochemical staining intensity was scored as 0, 1, 2, or 3, indicating absent, weak, moderate, or strong expression, respectively. The percentages of positive cells were scored as follows: 0=no staining, 1=1% to 5%, 2=5% to 25%, 3=25% to 50%, 4=50% to 75%, 5=75% to 100%. For each marker, the score of percentage and intensity was multiplied for final scores and to classify expression: 0 to 8, CXCL14 low expression; 9 to 15, CXCL14 high expression; 0, NOS1 negative expression; and 1 to 15, NOS1 positive expression.^[30]

2.4. Statistical analysis

The correlation of CXCL14 and NOS1 expression level in cancer cells or stromal fibroblasts with clinicopathological variables was examined using chi-square test. In addition, the Kaplan–Meier method and log-rank test were used for OS rate and PFS rate. Multivariate survival analysis was performed by the Cox proportional hazard model to assess the independent factors that may affect the prognosis of patients. Only variables with $P < .15$ from the univariate analyses or important ones were explored in the Cox regression analysis. PFS was calculated from the beginning date of surgery to the date of any new recurrence or death (without confirmed recurrence). OS was calculated from the date of surgery to the date of death from any cause or last follow-up. All the statistical analysis was analyzed by SPSS 19.0 statistical software, and a 2-sided $P < .05$ was considered significant.

3. Results

3.1. Expression of CXCL14 and NOS1 in stromal fibroblasts and cancer cells

Immunohistochemical staining showed that CXCL14 and NOS1 were mainly expressed in the cytoplasm. The percentages of high CXCL14 expression in stromal fibroblasts and cancer cells were 46.2% (49/106) and 23.6% (25/106), respectively (Fig. 1). There were 42.5% (45/106) of patients with positive NOS1 expression in cancer cells (Fig. 2). The expression of NOS1 was not observed in stromal fibroblasts (Fig. 2). The analysis revealed a significant

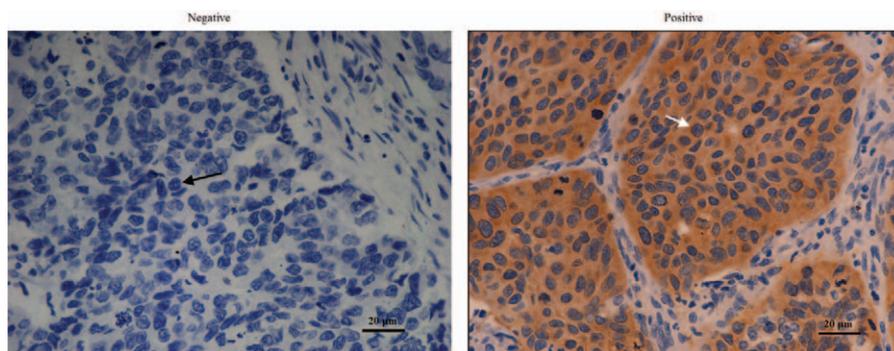


Figure 2. NOS1 expression by immunohistochemistry in the specimens from patients with stage I–IIIa NSCLC after curative resection (magnification $\times 400$, scale bars = 20 μm). Examples of negative and positive staining of NOS1 in cancer cells. Black arrows show negative NOS1 expression and white arrows show positive NOS1 expression. NOS1=nitric oxide synthase 1, NSCLC=non-small cell lung cancer.

Table 2

Association between CXCL14 expression in the specimens from patients with stage I–IIIA NSCLC after curative resection and clinicopathologic features of patients.

Variables	No of patients	CXCL14 expression in stromal fibroblasts			CXCL14 expression in cancer cells		
		Low, %	High, %	P*	Low, %	High, %	P*
Age, y							
≤60.5	52	33 (63.5)	19 (36.5)	.050	39 (75)	13 (25)	.736
>60.5	54	24 (44.4)	30 (55.6)		42 (77.8)	12 (22.2)	
Gender							
Male	77	41 (53.2)	36 (46.8)	.859	59 (76.6)	18 (23.4)	.934
Female	29	16 (55.2)	13 (44.8)		22 (75.9)	7 (24.1)	
Smoke							
Yes	51	29 (56.9)	22 (43.1)	.539	41 (80.4)	10 (19.6)	.353
No	55	28 (50.9)	27 (49.1)		40 (72.7)	15 (27.3)	
Pathologic type							
Squamous carcinoma	44	22 (50)	22 (50)	.413	35 (79.5)	9 (20.5)	.814
Adenocarcinoma	58	33 (56.9)	25 (43.1)		43 (74.1)	15 (25.9)	
Adenosquamous carcinoma	4	1 (25)	3 (75)		3 (75)	1 (25)	
Pathologic differentiation							
High	20	13 (65)	7 (35)	.26	16 (80)	4 (20)	.615
Median	56	26 (46.4)	30 (53.6)		44 (78.6)	12 (21.4)	
Low	30	18 (60)	12 (40)		21 (70)	9 (30)	
T category [†]							
T1	41	27 (65.9)	14 (34.1)	.059	31 (75.6)	10 (24.4)	.946
T2	38	20 (52.6)	18 (47.4)		29 (76.3)	9 (23.7)	
T3	20	9 (45)	11 (55)		15 (75)	5 (25)	
T4	7	1 (14.3)	6 (85.7)		6 (85.7)	1 (14.3)	
N category [†]							
N0	62	38 (61.3)	24 (38.7)	.084	48 (77.4)	14 (22.6)	.646
N1	23	12 (52.2)	11 (47.8)		16 (69.6)	7 (30.4)	
N2	21	7 (33.3)	14 (66.7)		17 (81.0)	4 (19.0)	
Pathologic stage [†]							
I	45	31 (68.9)	14 (31.1)		36 (80)	9 (20)	.157
II	27	16 (59.3)	11 (40.7)		17 (63.0)	10 (37.0)	
IIIA	34	10 (29.4)	24 (70.6)		28 (82.4)	6 (17.6)	
CXCL14 expression in cancer cells							
Low	81	48	33	.041			
High	25	9	16				
NOS1 expression in cancer cells							
Negative	61	43	18	<.001			
Positive	45	14	31				

* P-values were calculated using the χ^2 -test. CXCL14=CXC chemokine ligand 14, NOS1= nitric oxide synthase 1, NSCLC=non-small cell lung cancer.

[†] According to the American Joint Committee on Cancer and the Union for International Cancer Control stage system (7th edition).

positive correlation between CXCL14 expression in stromal fibroblasts and that in cancer cells ($\chi^2=4.158, P=.041$). In addition, the expression of CXCL14 in stromal fibroblasts was significantly correlated with NOS1 expression in cancer cells ($\chi^2=16.156, P<.001$).

3.2. Associations of CXCL14 or NOS1 expression with clinicopathologic characteristics

To explore the clinical relevance of CXCL14 and NOS1, associations between clinicopathological characteristics (age, gender, smoke, pathologic type, pathologic differentiation, tumor category, node category, and pathologic stage) and epithelial or stromal CXCL14 expression, as well as that and NOS1 expression were analyzed in Tables 2 and 3, respectively.

Stromal CXCL14 expression was only significantly associated with advanced pathologic stage ($P=.002$) (Table 2). However, epithelial CXCL14 expression was not correlated with any clinicopathological characteristics of patients (Table 2). NOS1

expression in cancer cells was correlated with tumor stage in tumor category ($P=.014$), node category ($P=.031$), and pathologic stage ($P=.022$) (Table 3).

3.3. Associations of CXCL14 or NOS1 expression with PFS and OS

The mean follow-up time for these patients was 60 months (range 8–68 months). The Kaplan–Meier plots showed that the 5-year PFS rates and the 5-year OS rates were 42.5% and 67.7% of all patients, respectively. The 5-year PFS rates of patients with stage I, II, and IIIA were 62.2%, 44.4%, and 17.6%, respectively ($\chi^2=33.463, P<.001$). The 5-year OS rates of that were 88.5%, 63%, and 41.4%, respectively ($\chi^2=19.972, P<.001$). The 5-year PFS rates for patients with low and high CXCL14 expression in stromal fibroblasts were 66.7% and 14.3% ($\chi^2=44.008, P<.001$), respectively, and the 5-year OS rates of that were 87.1% and 43.5% ($\chi^2=21.531, P<.001$), respectively (Fig. 3). The 5-year PFS rates for patients of negative and positive expression of NOS1 in cancer cells were 62.3% and 15.6% ($\chi^2=$

Table 3

Association between NOS1 expression in the specimens from patients with stage I–IIIA NSCLC after curative resection and clinicopathologic features of patients.

Variables	No of patients	NOS1 expression in cancer cells		P*
		Negative, %	Positive, %	
Age, y				
≤60.5	52	28 (53.8)	24 (46.2)	.449
>60.5	54	33 (61.1)	21 (38.9)	
Gender				
Male	77	44 (57.1)	33 (42.9)	.891
Female	29	17 (58.6)	12 (41.4)	
Smoke				
Yes	51	32 (62.7)	19 (37.3)	.297
No	55	29 (52.7)	26 (47.3)	
Pathologic type				
Squamous carcinoma	44	24 (54.5)	20 (45.5)	.159
Adenocarcinoma	58	35 (60.3)	23 (39.7)	
Adenosquamous carcinoma	4	2 (50)	2 (50)	
Pathologic differentiation				
High	20	14 (70)	6 (30)	.450
Median	56	31 (55.4)	25 (44.6)	
Low	30	16 (53.3)	14 (46.7)	
T category†				
T1	41	31 (75.6)	10 (24.4)	.014
T2	38	15 (39.5)	23 (60.5)	
T3	20	11 (55)	9 (45)	
T4	7	4 (57.1)	3 (42.9)	
N category†				
N0	62	41 (66.1)	21 (33.9)	.031
N1	23	13 (56.5)	10 (43.5)	
N2	21	7 (33.3)	14 (66.7)	
Pathologic stage†				
I	45	30 (66.7)	15 (33.3)	.022
II	27	18 (66.7)	9 (33.3)	
IIIA	34	13 (38.2)	21 (61.8)	

* P-values were calculated using the χ^2 -test. NOS1 = nitric oxide synthase 1, NSCLC = nonsmall cell lung cancer.

† According to the American Joint Committee on Cancer and the Union for International Cancer Control stage system (7th edition).

33.756, $P < .001$), respectively, and the 5-year OS rates for that were 86.4% and 40.1% ($\chi^2 = 24.430$, $P < .001$), respectively (Fig. 4). However, no significant association was observed between CXCL14 expression in cancer cells with respect to 5-

year PFS ($\chi^2 = 1.699$, $P = .192$) and 5-year OS ($\chi^2 = 0.076$, $P = .783$), respectively. Multivariate analysis using the Cox hazard proportional model showed that variables associated with OS and PFS included pathologic stage, CXCL14 expression level

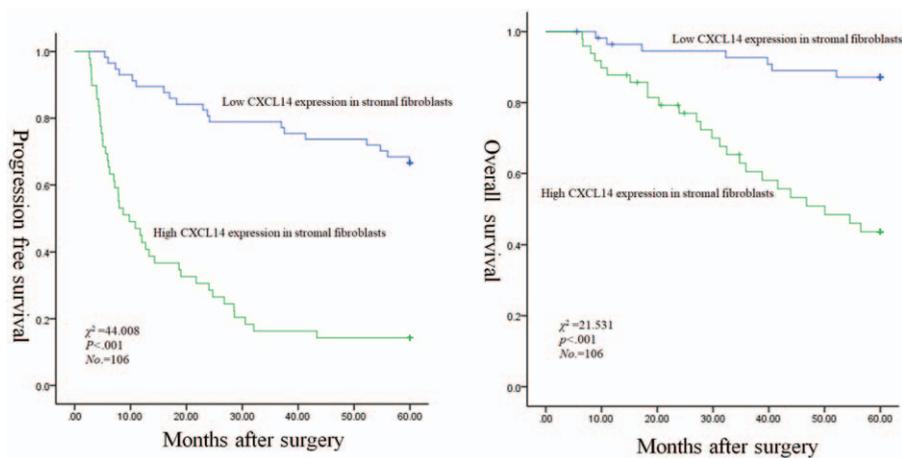


Figure 3. Correlations of stromal CXCL14 expression with worse PFS and OS for patients with stage I–IIIA NSCLC after curative resection: Kaplan–Meier curves for PFS (left panel) and OS (right panel). CXCL14 = CXC chemokine ligand 14, NSCLC = nonsmall cell lung cancer, OS = overall survival, PFS = progression-free survival.

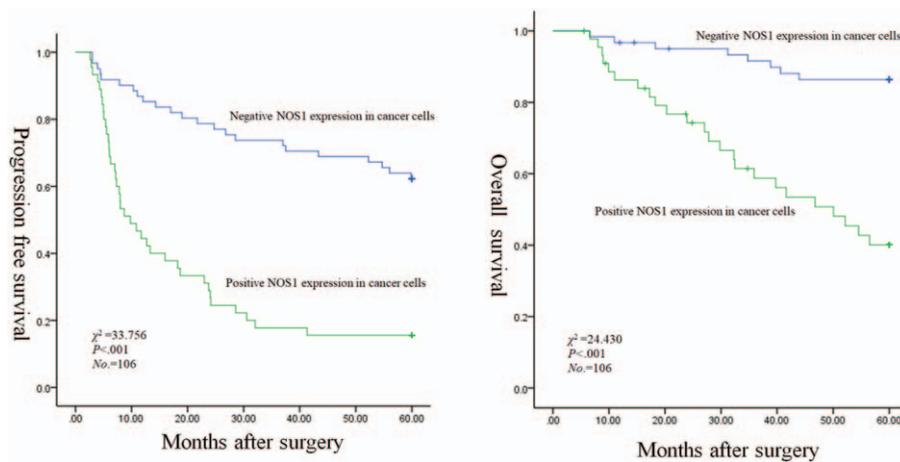


Figure 4. Correlations of NOS1 expression in cancer cells with worse PFS and OS for patients with stage I–IIIA NSCLC after curative resection: Kaplan–Meier curves for PFS (left panel) and OS (right panel). NOS1 = nitric oxide synthase 1, NSCLC = nonsmall cell lung cancer, OS = overall survival, PFS = progression-free survival.

in stromal fibroblasts, and NOS1 expression level in cancer cells (Table 4).

4. Discussion

CXCL14 is a novel non-ELR chemokine with nonspecific receptors associated with tumor progression and metastasis. This study found that the high expression of CXCL14 in stromal fibroblasts is identified as an independent novel predictive factor of poor prognosis for patients with stage I–IIIA NSCLC after curative resection. In addition, CXCL14 expression in cancer cells is not associated with clinical outcome. These are consistent with previous studies, indicating that marker proteins expressed by stromal fibroblasts show a prognostic significance. For example, the high expression of podoplanin,^[31] FAP,^[32] TGF-β, and α-SMA^[33] in cancer-associated fibroblasts indicates a short-term survival rate of patients with NSCLC.

This study suggests that the associations between stromal CXCL14 expression and a poor survival are consistent with previous findings.^[13,17,34] By analyzing CXCL14 protein expression, Shaykhiev et al^[34] found the chemokine to be upregulated in lung adenocarcinoma and lung squamous carcinoma by comparing healthy smokers with smokers having chronic obstructive pulmonary disease. Furthermore, the high expression of CXCL14 indicates worse survival in lung adenocarcinoma. Similarly, Zeng et al^[13] demonstrated that CXCL14 is highly expressed in colorectal cancer tissues, promotes colorectal cancer cell proliferation and invasion, and is associated with impaired

OS. Other researchers reported correlations between high CXCL14 expression and favorable prognosis in Ewing sarcoma^[35] and colorectal cancer.^[36] However, none of these studies considered the cell type expressing CXCL14.

It has been identified that activated fibroblasts in the stroma of breast and prostate cancer are the source for CXCL14.^[24,37] In a study of Takiguchi et al,^[38] CXCL14 can promote bone metastasis from lung cancer by enhancing cancer cell tropism to the bone and/or recruitment of bone marrow cells around metastatic cancer cells in vitro and in vivo. Besides, this study has shown that CXCL14 was not only expressed in tumor cells infiltrating bone marrow but also in the bone marrow stromal cells. In breast cancer, by analyzing CXCL14 mRNA expression, it was found that high stromal CXCL14 expression is an independent marker of worse prognosis.^[17] In the present study, we assessed the prognostic significance of stromal CXCL14 expression for patients with stage I–IIIA NSCLC after curative resection. The results showed that the expression level of CXCL14 in stromal fibroblasts was significantly correlated with TNM stage ($P = .002$). The more advanced pathologic stage had significantly higher CXCL14 expression. In addition, high expression of CXCL14 in stromal fibroblasts was associated with shorter PFS ($p < .001$) and shorter OS ($P < .001$).

However, the receptor for CXCL14 has not yet been identified. One study showed that in the prostate cancer and breast cancer animal models, NOS1 is an important determinant for stromal CXCL14 promoting tumorigenesis.^[26] Thus, whether CXCL14 affects the prognosis of patients with NSCLC through the NOS1

Table 4
Prognostic value of CXCL14 and NOS1 expression in the specimens from patients with stage I–IIIA NSCLC after curative resection from multivariate analysis using Cox hazard proportional model (n = 106).

Variables	Progression-free survival			Overall survival		
	HR	95% CI	P*	HR	95% CI	P*
Pathologic stage	2.085	1.448–3.003	<.001	1.784	1.114–2.855	.016
CXCL14 expression in stromal fibroblasts	3.225	1.708–6.089	<.001	2.627	1.012–6.816	.047
NOS1 expression in cancer cells	0.442	0.238–0.822	.010	0.323	0.132–0.789	.013

* P-values were calculated with a Cox proportional hazards model. CI = confidence interval, CXCL14 = CXCL chemokine ligand 14, HR = hazard ratio, NOS1 = nitric oxide synthase 1, NSCLC = nonsmall cell lung cancer.

signaling pathway? This study indicates that NOS1 was only expressed in cancer cells. In addition, the expression of CXCL14 in stromal fibroblasts and NOS1 expression in cancer cells was statistically significant ($P < .001$). Furthermore, the positive NOS1 expression in cancer cells predicted poor PFS ($P < .001$) and poor OS ($P < .001$). Therefore, we need further studies on the molecular mechanisms that CXCL14 and NOS1 promote the tumor malignant progression.

In summary, more experiments should be conducted to identify the potential of CXCL14 and NOS1 as lung cancer biomarkers. In addition, researches on the role of NOS1 in the intracellular signaling of CXCL14 is important. Understanding these mechanisms may lay a solid foundation for developing new therapeutic strategies.

Acknowledgments

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