An inter-correlation among chemokine (C-X-C motif) ligand (CXCL) 1, CXCL2 and CXCL8, and their diversified potential as biomarkers for tumor features and survival profiles in non-small cell lung cancer patients

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Background: The aim was to explore the interaction among chemokine (C-X-C motif) ligand (CXCL) 1/2/8 expressions, and their associations with clinicopathologic features and survival profiles in non-small cell lung cancer (NSCLC) patients.

Methods: The tumor tissue specimens from 232 primary NSCLC patients with TNM stage I-IIIA underwent resection were obtained and the expressions of CXCL1, CXCL2 and CXCL8 were measured by immunohistochemical assay. Disease-free survival (DFS) and overall survival (OS) were calculated according to survival data.

Results: There were 117(50.4%) CXCL1 low expression patients versus (*vs.*) 115 (49.6%) CXCL1 high expression patients, 107(46.1%) CXCL2 low expression patients *vs.* 125 (53.9%) CXCL2 high expression patients, 93 (40.1%) CXCL8 low expression patients *vs.* 139 (59.9%) CXCL8 high expression patients. Meanwhile, CXCL1 expression was positively correlated with CXCL2 expression and CXCL8 expression; CXCL2 expression was also positively correlated with CXCL8 expression. For tumor features, CXCL1, CXCL2 and CXCL8 were positively correlated with lymph node (LYN) metastasis and TNM stage, but not correlated with differentiation, tumor size or carcinoembryonic antigen (CEA) level. For prognosis, CXCL1 high expression was associated with worse DFS and OS, so did CXCL2 high expression, while there was no correlation of CXCL8 with DFS or OS; Multivariate Cox's regression disclosed that high expression of CXCL1, but not CXCL2 or CXCL8, was an independent factor predicting shorter DFS and OS.

Conclusions: An inter-correlation is observed among CXCL1, CXCL2 and CXCL8 expressions, and they show diversified potential as biomarkers for tumor features and survival profiles in NSCLC patients.

Keywords: Chemokine (C-X-C motif) ligand 1; chemokine (C-X-C motif) ligand 2; chemokine (C-X-C motif) ligand 8; non-small cell lung cancer (NSCLC); survival profiles

Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer deaths (1). According to the global cancer statistics 2018, it causes approximately 2,093,876 new cases (11.6% of the total new cancer cases) and 1,761,007 deaths (18.4% of the total cancer deaths) worldwide (1). Non-small cell lung cancer (NSCLC), as the most common histologic subtype of lung cancer, accounts on nearly 85% of lung cancer and contributes to high morbidity and mortality (2,3). Current balance of benefits and risks suggests that completed surgical resection is an optimum treatment for NSCLC patients in early stage, while most of NSCLC patients have been in advanced stage at the first diagnosis, and over 65% of them present with local or distant metastasis, who lose the best time of surgical resection (2,4). In addition, although obvious improvements have been achieved in various treatments for NSCLC (including chemotherapy, radiotherapy as well as immunological therapy) and these treatments have effects on release symptoms and delay disease progression, the 5-year overall survival (OS) rate of NSCLC patients still range from 15% to 25% (2,4). Thus, the exploration of potential biomarkers supervising disease progression may provide opportunities for disease management and improve prognosis in NSCLC patients.

Chemokines (C-X-C motif) (CXC) are a group of 17 α-chemokines that exert physiological and pathological functions, and many CXC chemokines [such as CXC ligand (CXCL) 1, CXCL2 and CXCL8] not only play critical roles in tumor microenvironment alone, but also interact with each other to promote neutrophils' secretion of several pro-inflammatory, angiogenic and immunoregulatory factors, thereby contributing to tumor progression (5-8). Regarding lung cancer, several previous studies have shown that CXCL1, CXCL2 and CXCL8 participant in the microenvironment of lung cancer through participating in multiple mechanisms, including recruitment of tumor-associated neutrophils (9), involving in anlotinib resistance (10), or affecting tumor cell proliferation and angiogenesis (11). Apart from their roles in cancer pathology, CXCL1, CXCL2 and CXCL8 have been considered as tumor promoters in cancer patients (12-14). For example, CXCL1 is positively related to tumor invasion, tumor-node-metastasis (TNM) stage, tumor size and lymph node metastasis in gastric cancer patients (12); CXCL2 is positively associated with infiltration extent of primary tumor and lymph node metastasis in colorectal cancer (CRC) (13); CXCL8 is positively correlated with depth of

tumor invasion and C-reactive protein (CRP) concentrations in esophageal cancer (OC) (14). More importantly, a previous report explores the clinicopathological significance of CXCR2 ligands (including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8) in gastric cancer patients, and reveals that among the CXCR2 ligands, CXCL7 and CXCL1 server as important roles in the malignant progression of gastric cancer by regulating CXCR2 signaling (6). Whereas little is known about the clinical implication of CXCL1/2/8 in NSCLC patients. To solve this problem, we conducted this study with aim to explore the interaction among CXCL1/2/8 expressions, and their associations with clinical characteristics and prognosis in NSCLC patients. We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-2539).

Methods

Patients

We retrospectively reviewed 232 primary NSCLC patients who underwent resection in our hospital between January 2011 and December 2013. The inclusion criteria were: (I) histopathological proof of NSCLC; (II) age above 18 years; (III) received resection, and tumor tissues excised from resection were well preserved; (IV) clinical features were completely recorded; (V) follow-up data were complete and available. The exclusion criteria included: (I) TNM stage IIIB or IV; (II) relapsed NSCLC; (III) received chemotherapy, radiotherapy or other anti-cancer therapies before resection; (IV) history of hematologic malignancies or other solid tumors; (V) pregnant or lactating women. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Shanghai Chest Hospital, Shanghai Jiao Tong University (KSY1672). The written informed consents were acquired from the patients or their family members.

Data and sample collection

Clinicopathologic features including demographics, complications and tumor features were collected from electronic medical records. Preoperative carcinoembryonic antigen (CEA) level in the serum of patients was also collected, which was determined by electrochemiluminescence immunoassay (ECLIA) using RocheElecsys601 automatic immune analyzer (Roche diagnostics, Mannheim, Germany)

and corresponding test kits. And the Formalin-fixed and paraffin-embedded tumor tissue specimens were obtained from the store room of pathology department.

Immunohistochemical (IHC) assay

The expressions of CXCL1, CXCL2 and CXCL8 in tissue specimens were measured by IHC. All antibodies used in IHC were purchased from Thermo Fisher Scientific (Waltham, Massacsetts, USA), which included Rabbit CXCL1 Polyclonal Antibody (1:20), Rabbit CXCL2 Monoclonal Antibody (1:20), Rabbit CXCL8 Polyclonal Antibody (1:200) and Goat anti-Rabbit IgG (H+L) (HRP) Antibody (1:10,000). All procedures were carried out referring to a previous study (15). The IHC score was assessed by a semi-quantitative scoring method as previously described (16), and the total IHC score was ranging from 0 to 12. The IHC score >3 was defined as high expression, while the IHC score ≤3 was defined as low expression.

Follow-up

Survival data were extracted from follow-up records, and the last follow-up date was December 31, 2018. According to the survival data, the disease-free survival (DFS) and OS were calculated. The DFS was defined as the duration from resection to disease relapse, disease progression or death, and OS was defined as the duration from resection to death.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 software (IBM, Chicago, IL, USA), and figures were plotted using GraphPad Prism 7.01 software (GraphPad Software, San Diego, California, USA). Correlation analysis was determined by Chi-square test or Spearman's rank correlation test. DFS and OS were displayed using Kaplan-Meier (K-M) curve. Comparison of DFS and OS between two groups was determined by Log-rank test. Factors predicting DFS and OS were analyzed by univariate and forward stepwise multivariate Cox's proportional hazard regression model. P value <0.05 was considered significant.

Results

Clinicopathological characteristics in NSCLC patients

The mean age was 61.1±10.6 years of 232 NSCLC patients

[including 68 (29.3%) female and 164 (70.7%) males]. The number of patients with history of smoke, history of drink, hypertension, hyperlipidemia and diabetes was 128 (55.2%), 97 (41.8%), 89 (38.4%), 74 (31.9%) and 43 (18.5%) respectively. For tumor features, there were 34 (14.7%), 137 (59.0%) and 61 (26.3%) patients with well, moderate and poor differentiation; the mean value of tumor size was 5.2±2.0 cm, and there were 141 (60.8%) patients with tumor size ≤5 cm, but 91 (39.2%) patients with tumor size >5 cm; 88 (37.9%) patients had lymph node (LYN) metastasis. As to TNM stage, the number of patients with stage I, stage II and stage III was 76 (32.8%), 67 (28.9%) and 89 (38.3%), respectively. For carcinoembryonic antigen (CEA) level, the median value was 6.8 (3.1–27.3) ng/mL, and there were 137 (59.1%) patients with abnormal CEA level and 95 (40.9%) patients with normal CEA level (*Table 1*).

CXCL1, CXCL2 and CXCL8 expressions in NSCLC patients

The examples of CXCL1, CXCL2 and CXCL8 staining results by IHC were shown in *Figure 1A*. For CXCL1, there were 117 (50.4%) patients with CXCL1 low expression and 115 (49.6%) patients with CXCL1 high expression (*Figure 1B*). For CXCL2, there were 107 (46.1%) patients with CXCL2 low expression and 125 (53.9%) patients with CXCL2 high expression. For CXCL8, there were 93 (40.1%) patients with CXCL8 low expression and 139 (59.9%) patients with CXCL8 high expression.

Association among CXCL1, CXCL2 and CXCL8 expressions in NSCLC patients

CXCL1 expression was positively correlated with CXCL2 expression (P<0.001) and CXCL8 expression (P<0.001) (*Table 2*). Meanwhile, CXCL2 expression was also positively correlated with CXCL8 expression (P<0.001).

Association of CXCL1, CXCL2 and CXCL8 expressions with tumor features in NSCLC patients

For CXCL1, it was positively correlated with LYN metastasis (P=0.002) and TNM stage (P=0.010), while there was no correlation of CXCL1 expression with differentiation (P=0.126), tumor size (P=0.295) or CEA level (P=0.275) (*Table 3*). For CXCL2, it was positively correlated with LYN metastasis (P=0.039) and TNM stage (P=0.008), while no correlation was found of CXCL2 expression with differentiation (P=0.638), tumor size

Table 1 Clinicopathologic characteristics

Items	NSCLC patients (N=232)
Age (years), mean ± SD	61.1±10.6
≤60 years, No. (%)	115 (49.6)
>60 years, No. (%)	117 (50.4)
Gender, No. (%)	
Female	68 (29.3)
Male	164 (70.7)
History of smoke, No. (%)	
No	104 (44.8)
Yes	128 (55.2)
History of drink, No. (%)	
No	135 (58.2)
Yes	97 (41.8)
Hypertension, No. (%)	
No	143 (61.6)
Yes	89 (38.4)
Hyperlipidemia, No. (%)	
No	158 (68.1)
Yes	74 (31.9)
Diabetes, No. (%)	
No	189 (81.5)
Yes	43 (18.5)
Differentiation, No. (%)	
Well	34 (14.7)
Moderate	137 (59.0)
Poor	61 (26.3)
Tumor size (cm), mean ± SD	5.2±2.0
≤5 cm, No. (%)	141 (60.8)
>5 cm, No. (%)	91 (39.2)
LYN metastasis, No. (%)	
No	144 (62.1)
Yes	88 (37.9)
TNM stage, No. (%)	
I	76 (32.8)
II	67 (28.9)
III	89 (38.3)
CEA (ng/mL) level, median (IQR)	6.8 (3.1–27.3)
Normal*, No. (%)	95 (40.9)
Abnormal*, No. (%)	137 (59.1)

*, normal CEA level ≤5 ng/mL and abnormal CEA level >5 ng/mL. NSCLC, non-small cell lung carcinoma; SD, standard deviation; LYN, lymph node; CEA, carcinoembryonic antigen; IQR, interquartile range.

(P=0.423) or CEA level (P=0.827). For CXCL8, it was also positively correlated with LYN metastasis (P=0.005) and TNM stage (P=0.004), while presented with a trend to be correlated with differentiation (P=0.064) but with no statistical significance; meanwhile, there was no correlation of CXCL8 expression with tumor size (P=0.219) or CEA level (P=0.180). In addition, we further explored the correlation of CXCL1/CXCL2/CXCL8 with lymphocytes, and we found no correlation of CXCL1 (P=0.963), CXCL2 (P=0.209), CXCL8 (P=0.434) with lymphocyte count in NSCLC patients (Table S1). The possible explanation might be that lymphocytes counts in blood sample reflect the whole-body conditions, thus, their associations with tumor CXCL1, 2, 8 might be weak.

Association of CXCL1, CXCL2 and CXCL8 with survival profiles in NSCLC patients

The high expressions of CXCL1 (P<0.001) (*Figure 2A*) and CXCL2 (P<0.001) (*Figure 2B*) were correlated with worse DFS, while no correlation was found of CXCL8 (P=0.256) (*Figure 2C*) with DFS in NSCLC patients. In addition, high expressions of CXCL1 (P<0.001) (*Figure 3A*) and CXCL2 (P=0.014) (*Figure 3B*) were correlated with poor OS, while there was no correlation of CXCL8 (P=0.160) (*Figure 3C*) with OS in NSCLC patients.

Predictive factors for DFS in NSCLC patients

Based on univariate Cox's regression analysis, CXCL1 high expression (P<0.001) and CXCL2 high expression (P<0.001) were associated with shorter DFS, and male (P=0.038), poor differentiation (P=0.038), larger tumor size (*P*=0.013), LYN metastasis (P<0.001), increased TNM stage (P<0.001) as well as abnormal CEA level (P=0.024) were also correlated with poor DFS in NSCLC patients (*Table 4*). Forward stepwise multivariate Cox's regression disclosed that CXCL1 high expression (P=0.003) was an independent factor predicting shorter DFS, and male (P=0.012) as well as LYN metastasis (P<0.001) also independently predicted worse DFS in NSCLC patients.

Predictive value of factors for OS in NSCLC patients

Univariate Cox's regression analysis displayed that CXCL1 high expression (P<0.001) and CXCL2 high expression (P=0.015) were associated with poor OS, meanwhile, poor differentiation (P=0.019), larger tumor size (P=0.001), LYN
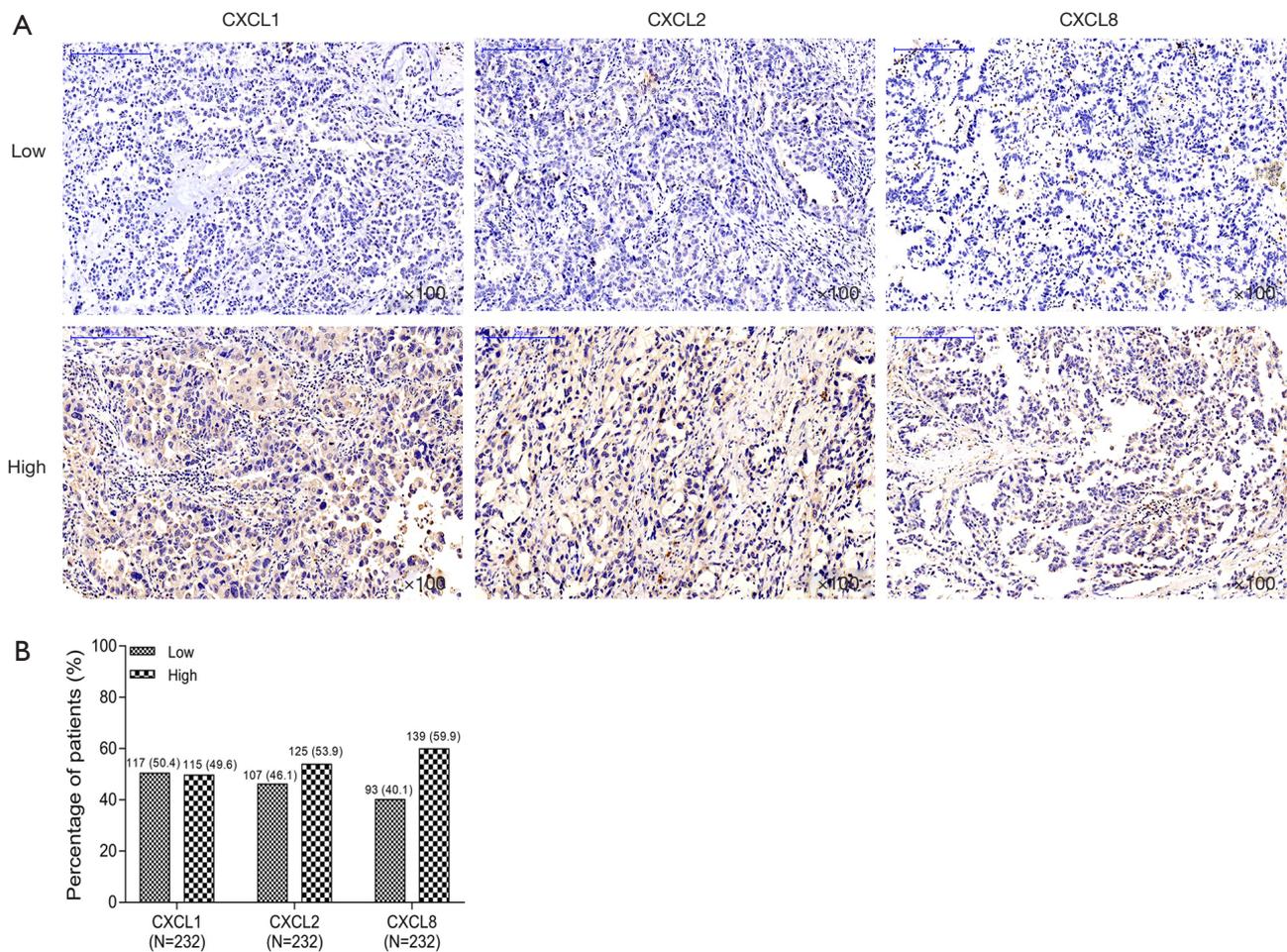
Figure 1 CXCL1, CXCL2 and CXCL8 expressions. (A) Example samples of CXCL1, CXCL2 and CXCL8 staining results by immunohistochemical assay in NSCLC patients; (B) the percentage of NSCLC patients with high expression and low expression of CXCL1, CXCL2 and CXCL8, respectively. NSCLC, non-small cell lung cancer; CXCL, chemokine (C-X-C motif) ligand.

Table 2 Correlations among CXCL1, CXCL2 and CXCL8 expressions

Items	CXCL1 expression			CXCL2 expression		
	Low	High	P value	Low	High	P value
CXCL2 expression, No. (%)			<0.001			–
Low	85 (36.6)	22 (9.5)		–	–	
High	32 (13.8)	93 (40.1)		–	–	
CXCL8 expression, No. (%)			<0.001			<0.001
Low	85 (36.6)	8 (3.4)		73 (31.5)	20 (8.6)	
High	32 (13.8)	107 (46.1)		34 (14.7)	105 (45.3)	

CXCL, chemokine (C-X-C motif) ligand.

Table 3 Correlation of CXCL1/CXCL2/CXCL8 with tumor features

Items	CXCL1			CXCL2			CXCL8		
	Low	High	P	Low	High	P	Low	High	P
Differentiation, No. (%)			0.126			0.638			0.064
Well	22 (18.8)	12 (10.4)		18 (16.8)	16 (12.8)		18 (19.4)	16 (11.5)	
Moderate	67 (57.3)	70 (60.9)		61 (57.0)	76 (60.8)		55 (59.1)	82 (59.0)	
Poor	28 (23.9)	33 (28.7)		28 (26.2)	33 (26.4)		20 (21.5)	41 (29.5)	
Tumor size, No. (%)			0.295			0.423			0.219
≤5 cm	75 (64.1)	66 (57.4)		68 (63.6)	73 (58.4)		61 (65.6)	80 (57.6)	
>5 cm	42 (35.9)	49 (42.6)		39 (36.4)	52 (41.6)		32 (34.4)	59 (42.4)	
LYN metastasis, No. (%)			0.002			0.039			0.005
No	84 (71.8)	60 (52.2)		74 (69.2)	70 (56.0)		68 (73.1)	76 (54.7)	
Yes	33 (28.2)	55 (47.8)		33 (30.8)	55 (44.0)		25 (26.9)	63 (45.3)	
TNM stage, No. (%)			0.010			0.008			0.004
I	47 (40.2)	29 (25.2)		45 (42.0)	31 (24.8)		39 (41.9)	37 (26.6)	
II	33 (28.2)	34 (29.6)		28 (26.2)	39 (31.2)		28 (30.1)	39 (28.1)	
III	37 (31.6)	52 (45.2)		34 (31.8)	55 (44.0)		26 (28.0)	63 (45.3)	
CEA level, No. (%)			0.275			0.827			0.180
Normal*	52 (44.4)	43 (37.4)		43 (40.2)	52 (41.6)		43 (46.2)	52 (37.4)	
Abnormal*	65 (55.6)	72 (62.6)		64 (59.8)	73 (58.4)		50 (53.8)	87 (62.6)	

*, normal CEA level ≤5 ng/mL and abnormal CEA level >5 ng/mL. CXCL, chemokine (C-X-C motif) ligand; LYN, lymph node; CEA, carcinoembryonic antigen.
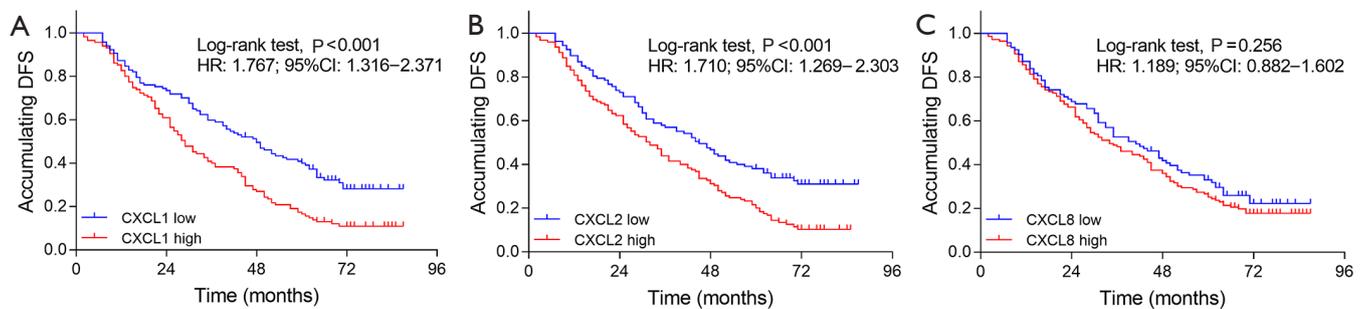

Figure 2 Correlation of CXCL1, CXCL2 and CXCL8 with DFS. (A) Correlation of CXCL1 with DFS in NSCLC patients; (B) correlation of CXCL2 with DFS in NSCLC patients; C: Correlation of CXCL8 with DFS in NSCLC patients. CXCL, chemokine (C-X-C motif) ligand; NSCLC, non-small cell lung cancer; DFS, disease-free survival.

metastasis (P<0.001), increased TNM stage (P<0.001) and abnormal CEA level (P=0.002) were associated with worse OS in NSCLC patients as well (*Table 5*). According to forward stepwise multivariate Cox's regression, CXCL1 high expression (P=0.004) independently predicted shorter OS, and poor differentiation (P=0.029), LYN metastasis (P<0.001) as well as abnormal CEA level (P=0.006) were also independent factors predicting worse OS in NSCLC patients.
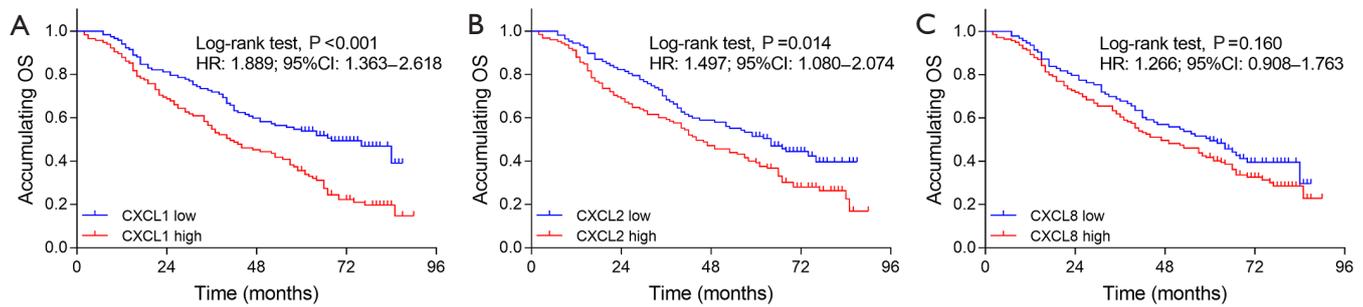
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Figure 3 Correlation of CXCL1, CXCL2 and CXCL8 with OS. (A) Correlation of CXCL1 with OS in NSCLC patients; (B) correlation of CXCL2 with OS in NSCLC patients; (C) correlation of CXCL8 with OS in NSCLC patients. CXCL, chemokine (C-X-C motif) ligand; NSCLC, non-small cell lung cancer; OS, overall survival.

Table 4 Analysis of factors predicting DFS

Items	Cox's regression model			
	P value	HR	95% CI	
			Lower	Higher
Univariate Cox's regression				
CXCL1 high	<0.001	1.767	1.316	2.371
CXCL2 high	<0.001	1.710	1.269	2.303
CXCL8 high	0.256	1.189	0.882	1.602
Age (>60 years)	0.086	1.291	0.965	1.728
Male	0.038	1.429	1.020	2.001
History smoke	0.527	0.910	0.680	1.218
History drink	0.297	1.168	0.872	1.565
Hypertension	0.628	0.929	0.689	1.253
Hyperlipidemia	0.406	0.874	0.637	1.200
Diabetes	0.222	0.786	0.534	1.157
Poor differentiation	0.038	1.250	1.013	1.542
Tumor size	0.013	1.453	1.083	1.951
LYN metastasis	<0.001	2.571	1.909	3.464
TNM stage	<0.001	1.499	1.257	1.788
Abnormal CEA level*	0.024	1.409	1.046	1.897
Forward stepwise multivariate Cox's regression				
CXCL1 high	0.003	1.582	1.174	2.133
Male	0.012	1.546	1.101	2.169
LYN metastasis	<0.001	2.536	1.873	3.433

Factors with P value less than 0.05 in univariate Cox's regression were included in forward stepwise multivariate Cox's regression. *, normal CEA level ≤5 ng/mL and abnormal CEA level >5 ng/mL. DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; CXCL, chemokine (C-X-C motif) ligand; LYN, lymph node; CEA, carcinoembryonic antigen.

Table 5 Analysis of factors predicting OS

Items	Cox's regression model			
	P value	HR	95% CI	
			Lower	Higher
Univariate Cox's regression				
CXCL1 high	<0.001	1.889	1.363	2.618
CXCL2 high	0.015	1.497	1.080	2.074
CXCL8 high	0.164	1.266	0.908	1.763
Age (>60 years)	0.154	1.262	0.916	1.740
Male	0.346	1.189	0.830	1.704
History smoke	0.361	0.862	0.626	1.186
History drink	0.534	1.108	0.802	1.529
Hypertension	0.766	0.951	0.683	1.324
Hyperlipidemia	0.677	0.929	0.655	1.317
Diabetes	0.340	0.814	0.533	1.243
Poor differentiation	0.019	1.325	1.047	1.676
Tumor size	0.001	1.700	1.233	2.344
LYN metastasis	<0.001	3.219	2.323	4.458
TNM stage	<0.001	1.485	1.223	1.804
Abnormal CEA level*	0.002	1.685	1.205	2.355
Forward stepwise multivariate Cox's regression				
CXCL1 high	0.004	1.628	1.167	2.273
Poor differentiation	0.029	1.328	1.029	1.714
LYN metastasis	<0.001	2.983	2.142	4.154
Abnormal CEA level*	0.006	1.612	1.151	2.259

Factors with P value less than 0.05 in univariate Cox's regression were included in forward stepwise multivariate Cox's regression. *, normal CEA level ≤5 ng/mL and abnormal CEA level >5 ng/mL. OS, overall survival; HR, hazard ratio; CI, confidence interval; CXCL, chemokine (C-X-C motif) ligand; LYN, lymph node; CEA, carcinoembryonic antigen.

Discussion

As common members of the CXC chemokine family, CXCL1, CXCL2 and CXCL8 serve as critical roles in multiple malignancies. For example, one previous study discloses that CXCL1 actives the extracellular signal-regulated kinase (ERK)/matrix metalloproteinase (MMP) 2/9 signaling axis to stimulate cell migration and invasion in ER-negative breast cancer cells (17); In addition, CXCL1 and CXCL2 high expressions attract CD11b⁺Gr1⁺myeloid cells into the tumor to produce S100A8/9, thereby causing chemoresistance and metastasis in breast cancer (18); Furthermore, the Ras-dependent secretion of CXCL8 has been reported to induce neo-vascularization to accelerate tumor progression in ovarian cancer (19); Another recent study reveals that CXCL8 is interacted with ING4 (a tumor suppressor gene) to promote tumor growth and vascular density in gliomas (20). In lung cancer, CXCL1 has been reported to interact with miR141-CXCR2 pathway regulates migration of regulatory T cells (Treg) into malignant pleural effusion (MPE) (21). CXCL2 is discovered to obviously offset anlotinib-induced cell migration inhibition and promote invasion of anlotinib-treated cells in NSCLC, indicated that CXCL2 is involved in the resistance in anlotinib resistant NSCLC cells (10). CXCL8/interleukin-8 analogue CXCL8 [3–72] K11R/G31P interacts with CXCR1/2 to suppress tumor cell proliferation and inhibit angiogenesis, thereby restricting lung cancer growth (11). Except for the oncogenic effects of CXCL1, CXCL2 and CXCL8 in several malignancies alone, they also have the obvious interaction with each other in tumor microenvironment. For instance, CXCL1/8-CXCR2 axis is interacted with SMAD4 to attract neutrophils, subsequently promoting the neutrophils' secretion of matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) in colorectal cancer (7). In addition, CXCL1, CXCL2 and CXCL8 regulate multiple common genes [such as CXC type chemokine receptor (CXCR)2], suggesting that three of CXCL1, CXCL2 and CXCL8 are able to be interacted with each other via mediating common genes (22-24). Therefore, CXCL1, CXCL2 and CXCL8 not only play important roles in tumor microenvironments alone, but also they are interacted with each other to induce tumor progression.

Apart from the tumor promoter roles of CXCL1, CXCL2 and CXCL8 in cancer pathology, three of them also are discovered as an oncogene in patients with various carcinomas. For instance, one previous study CXCL1

expression is increased and it is positively correlated with T invasion (T2–T4), lymph node metastasis, lymphatic invasion, venous invasion, peritoneal cytology, peritoneal metastasis in gastric cancer patients (24); another study determines that CXCL2 high expression is associated with the multiple tumor numbers in hepatocellular carcinoma patients (25); Meanwhile, CXCL8 is highly expressed in cervical cancer tissues compared with normal cervical tissues, and it is associated with advanced clinical stage, distant metastasis, histological type and histological grade in cervical cancer patients (26). Although CXCL1, CXCL2 and CXCL8 have been reported to be oncogenic roles in patients with several cancers, the clinical implication of CXCL1, CXCL2 and CXCL8 in NSCLC patients is still unclear. To solve this issue, we performed this study that enrolled 232 primary NSCLC patients who underwent resection, and we observed that CXCL1, CXCL2 and CXCL8 had a positive correlation with each other, which might be that there were various common genes regulated by all of CXCL1, CXCL2 and CXCL8 (such as CXCR2), thus, they could be interacted with each other and had a positive association with each other in NSCLC patients (22-24). In order to further explore whether CXCL1, CXCL2 and CXCL8 have clinical significance in NSCLC patients, we assessed the correlation of CXCL1, CXCL2 and CXCL8 with tumor features in NSCLC patients, and we discovered that all CXCL1, CXCL2 and CXCL8 were positively correlated with LYN metastasis and TNM stage in NSCLC patients. The possible explanations were as follows: (I) for CXCL1, it might regulate multiple pathways (including ERK/MMP2/9 signaling axis) to accelerate cell migration and invasion, and then devoted into tumor metastasis and correlated with LYN metastasis in NSCLC patients; (II) for CXCL2, it might be interacted with receptor activator of NF-kappaB ligand to stimulate the cell adhesion and migration, thereby contributed to tumor metastasis in NSCLC patients (27); (III) for CXCL8, it has been considered as a pro-inflammatory chemokine [referred to as interleukin-8 (IL-8)] involving in tumor angiogenesis to promote tumorigenesis and metastasis, thus CXCL8 high expression was related to LYN metastasis in NSCLC patients (19,20). (IV) CXCL1, CXCL2 and CXCL8 were interacted with each other to promote the neutrophils' secretion of several pro-inflammatory, angiogenic and immunoregulatory factors (including MMPs and VEGF), thereby promoting tumor metastasis in NSCLC patients (7,8,28).

A number of recent studies have been performed to

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investigate the prognostic value of CXCL1, CXCL2 and CXCL8 in cancer patients. For example, CXCL1 is an independent factor predicting poor OS in gastric cancer patients (24); furthermore, CXCL8 overexpression is reported to be obviously associated with poor DFS and OS in colorectal cancer patients (29) as well as in lung adenocarcinoma patients (30). The prognostic significance of CXCL1, CXCL2 and CXCL8 is still unknown in NSCLC patients. In this study, we discovered that (I) CXCL1 high expression were correlated with shorter DFS as well as OS, and it was an independent factor predicting worse survival profiles, which might be resulted in that CXCL1 not only correlated with deteriorative disease conditions (above-mentioned), thereby indirectly related to worse prognosis in NSCLC patients, but also was interacted with S100A8/9 to increase chemoresistance and then caused worse treatment outcomes, thereby directly independently predicted worse prognosis in NSCLC patients; (II) CXCL2 high expression were associated with shorter DFS and OS, but it could not independently predict DFS and OS, which might be caused by that CXCL2 might be interacted with LYN metastasis to indirectly affect survival profiles in NSCLC patients; (III) CXCL8 was not related to DFS and OS in NSCLC patients, whose discrepancy with other previous data (29,30) would most likely to due to the heterogenicity in human cancers.

Despite of interesting results in this study, the main limitation of the relatively small sample size in this study still existed, further study enrolled more NSCLC patients was necessary. Moreover, the enrolled NSCLC patients in this study were at TNM stage I-IIIA, but not TNM stage IIIB or IV. Thus, the clinical value of CXCL1, CXCL2 and CXCL8 in all NSCLC patients was confused. Furthermore, although the clinical implication of CXCL1, CXCL2 and CXCL8 in NSCLC patients was explored, their detailed mechanism underlying NSCLC pathology was not investigated in this study, further *in vitro* and *in vivo* experiments were needed. Besides, the association between CXCL1, CXCL2, CXCL8 and inflammatory factors was not explored, further study should be performed.

In conclusion, an inter-correlation is observed among CXCL1, CXCL2 and CXCL8 expressions, and they show diversified potential as biomarkers for tumor features and survival profiles in NSCLC patients.

Acknowledgments

Footnote

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Table S1 Correlation of CXCL1/CXCL2/CXCL8 with lymphocytes

Items	Lymphocyte count ($\times 10^9$)	P value
CXCL1		0.963
Low	0.8 (0.6–1.0)	
High	0.8 (0.6–1.0)	
CXCL2		0.209
Low	0.7 (0.5–1.0)	
High	0.8 (0.6–1.1)	
CXCL8		0.434
Low	0.8 (0.5–1.0)	
High	0.8 (0.6–1.0)	

CXCL, chemokine (C-X-C motif) ligand.