Role of Serum C-C Motif Chemokine Ligand 2 Monocyte Chemotactic Activities in Patients with Non-Small Cell Lung Cancer

ZHEN GAO, CHENG QIAN AND LIANG ZHANG*

Department of Radiotherapy, The Second Affiliated Hospital of Nantong University, Nantong First People's Hospital, Nantong, Jiangsu 226001, China

To examine C-C motif chemokine ligand 2 chemotactic activities, expression and clinical relevance in the peripheral serum of non-small cell lung cancer patients is the objective of the study. The non-small cell lung cancer group consisted of 128 patients diagnosed with the disease in our institution between January 2018 and March 2022, while the healthy group consisted of 112 individuals. By using an enzyme-linked immunosorbent assay, the two group's peripheral serum was examined for C-C motif chemokine ligand 2 expression levels. The monocyte chemotactic activity of blood plasma from the two groups was assessed using the Transwell chamber technique after human monocytic leukaemia cell line THP-1 cells were grown. Analysis was done on C-C motif chemokine ligand 2 expression levels and the relationship between monocyte chemotactic activity and histopathological features in non-small cell lung cancer patients. Age and gender did not significantly connect with the expression level of C-C motif chemokine ligand 2 or its monocyte chemotactic activity ($p>0.05$); however, smoking, pathological categorization, tumor, nodes and metastases stage, tumor stage, nodes stage and tumor diameter did ($p<0.05$). The expression level of C-C motif chemokine ligand 2 was 235.4±25.7 pg/ml in the peripheral serum of non-small cell lung cancer patients and 45.7±10.5 pg/ml in the healthy population; the monocyte chemotactic activity of non-small cell lung cancer patients was 236.5 % and that of healthy people was 86.5 %. They differed from one other in a statistically significant way ($p<0.05$). In comparison to the normal control group ($r=0.87$), the lung cancer group's correlation coefficient between blood C-C motif chemokine ligand 2 and monocyte chemotactic activity was considerably lower ($r=0.39$). Patients with non-small cell lung cancer patients exhibit inadequate monocyte chemotactic activity, which may one day be targeted therapeutically.

Key words: Non-small cell lung cancer, C-C motif chemokine ligand 2, tumor, nodes and metastases staging, tumor size

With a high death rate, lung cancer ranks second in the world behind breast and prostate cancer in both men and women. The most prevalent malignant tumor in China is lung cancer, which also has the highest fatality rate for both men and women[1]. The Non-Small Cell Lung Cancer (NSCLC), accounts for more than 85 % of all cases. Squamous cell carcinoma and adenocarcinoma are the two primary histological subtypes. Additionally, there could be a few particular molecular gene mutations[2]. The average survival time of NSCLC patients with metastasis without treatment is 4-5 mo and only 10 % of patients achieve 1 y survival[3]. Because of the late detection and diagnosis of lung cancer, the prognosis of patients is often poor. Also the colorectal cancer, which is one of the most usual cancers, starts as a tissue overgrowth on the inner part of the colon or rectum and unfortunately, the early spread of tumor cells is usually not detectable. So, the prevention of metastasis is the main focus of clinical research[4,5]. While, talin-1 protein is a potential prognostic marker in renal cell carcinoma and based on the studies, investigation its expression levels leads to the renal cancer progression and prediction[6].

Most immunological and glial cells that have chemokines action and take part in the immune

***Address for correspondence**
E-mail: 578079160@qq.com

system's monitoring and removal of tumor cells release chemokines. Researchers have begun to pay more attention to the anti-tumor effects of Monocyte Chemoattractant Protein-1 (MCP-1), commonly known as C-C Motif Chemokine Ligand 2 (CCL2), which belongs to the CC chemokine subgroup (also known as the subfamily)[7]. CCL2 can not only directly affect the aggregation and migration of tumor cells and the proliferation of vascular endothelial cells, and stimulate the proliferation of tumor cells, but also chemoattract immune cells such as macrophages and mediate the differentiation and polarization of immune cells[8]. There are limited publications on alterations in CCL2 chemokines action in the peripheral blood of NSCLC patients, despite the fact that the process of CCL2 in the pathophysiology of tumors, prostate cancer, colorectal cancer and other cancers has been implicated in the present investigations. The purpose of this study was to investigate the function of CCL2 in the pathogenesis of NSCLC by comparing the levels of Monocyte Chemotactic Activity (MCA) and CCL2 expression in blood plasma between NSCLC patients and healthy individuals[9].

MATERIALS AND METHODS

Subjects used in the study:

128 patients with NSCLC confirmed by surgery and pathology in our hospital from July 2018 to June 2022 were randomly selected.

Inclusion criteria are as follows. All cases were diagnosed as NSCLC by pathology; all patients were treated for the first time and the clinical data was complete; there was no other tumor and metastasis; no preoperative chemoradiotherapy, biotherapy or targeted therapy. Tumor, Nodes and Metastases (TNM) staging was performed using the National Comprehensive Cancer Network (NCCN), 2011 criteria for NSCLC. Furthermore, 112 healthy non-smokers who took part in the outpatient clinic's health check were chosen as the healthy group. The Medical Ethics Board of our hospital gave its approval to the consent forms, which were signed by all participants in the study.

Research methods:

Determination of peripheral blood CCL2 level: Fasting venous blood (5 ml) was collected in the morning and centrifuged at 3000 rpm at 4° for 10 min. The serum was collected and stored at –80° for testing. Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect the expression level of CCL2 and the kit was human CCL2 ELISA kit (Thermo Scientific). The operation process was carried out according to the instructions.

Determination of peripheral blood MCA: MCA[10] was measured by Transwell chamber method in Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo Scientific) supplemented with 10 % fetal bovine serum and double antibodies (penicillin and streptomycin). Human Monocytic Leukaemia cell line (THP-1) cells (Type Culture Collection, Chinese Academy of Sciences) were cultured in 5 % Carbon dioxide (CO_2) at 37° and induced into macrophages with 1 mmol/l Phorbol-12-Myristate-13-Acetate (PMA) (Sigma) for 24 h. Chemotaxis experiment was performed by using Transwell chamber migration system with 8 μm aperture, 200 μl induced THP-1 cell suspension (1×10^5 cells) was added into the lower chamber and 200 μl peripheral serum of NSCLC group and healthy group was placed into the upper chamber, respectively. The cells were incubated at 37° and 5 % CO_2 for 90 min, fixed with 95 % ethanol for 15 min, then stained with 0.1 % crystal violet for 10 min and counted under high power microscope. MCA is expressed in chemotactic units (U). Chemotactic unit (U)=Number of monocytes in the lower compartment/number of monocytes in the upper compartment×100 %.

Statistical analysis:

The statistical program, Statistical Package for the Social Sciences (SPSS) 22.0 was used to examine the data. The enumeration data were represented as cases (%), the comparison between groups was carried out by Chi square (χ^2) test and multiple linear regressions was used to assess the effect of multiple independent factors on the dependent variable. The assessment data were expressed as mean±Standard Deviation (SD) (x±s) and the pairwise comparison between groups was carried out by t-test. The Pearson's linear correlation was used between the two indexes and the difference was statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

General information of the patients was shown in Table 1. Among 128 patients with NSCLC, 63 patients were <60 y old and 65 patients were ≥60 y old. There were 72 males and 56 females. There were 68 smokers and 60 non-smokers. Pathological type: Adenocarcinoma-70 cases, squamous cell carcinoma-58 cases; TNM staging: I-II 61 cases, III-IV 67 cases; Tumor (T) staging: T1-56 cases, T2-51 cases, T3-14 cases, T4-7 cases; Nodes (N) staging:

N0 59 cases, N1 15 cases, N2-N3 54 cases; Metastases (M) stage: M0 in 101 cases, M1 in 27 cases; Tumor diameter was ≤3 cm in 89 cases and >3 cm in 39 cases (Table 1). Among the 112 healthy people, 58 were younger than 60 y old and 54 were older than 60 y old. There were 62 males and 50 females. There were 59 smokers and 53 non-smokers. But between the two different groups, there were no discernible differences in terms of age, gender or smoking status ($p>0.05$) as shown in Table 1.

Comparison of serum CCL2 levels in NSCLC patients with different clinicopathological features was shown in Table 2. The expression of CCL2 and MCA had no significant correlation with age and gender ($p>0.05$). Smoking, pathological type, TNM, T, N stage and tumor diameter were significantly correlated with each other.

Multiple linear regression of MCA expression and clinicopathological parameters of NSCLC patients were shown in Table 3.

Multiple linear regression between CCL2 expression and clinicopathological parameters of NSCLC patients were shown in Table 4.

Comparison of CCL2 expression level in peripheral blood and MCA was shown in Table 5. The expression levels of CCL2 in peripheral blood were 45.74±10.51 pg/ml and 235.43±25.75 pg/ml and MCA were 32.54 %±8.72 % and 26.44 %±9.33 %, respectively. There were significant differences in the expression of CCL2 and MCA between NSCLC group and healthy group ($p<0.01$). Both the normal group and the NSCLC group showed a positive connection between CCL2 and MCA ($r=0.87$, 0.39, $p<0.01$), and Table 5 demonstrates that the correlation in the NSCLC population was lower than that in the normal group.

Studies have revealed a link between inflammation and tumorigenesis. Tumor inflammatory microenvironment constitutes a complex inflammatory regulatory network through inflammatory cells, cytokines, chemokines and other mediators in the form of autocrine or paracrine, which jointly determines the occurrence and development of tumors[11]. Although only about 20 % of tumors are related to inflammation, the immune cells and mediators can be found in most human malignant tumors[12,13], one of the reasons may be that some cancerous changes *in vivo* induce or activate inflammatory pathways in precancerous or tumor cells[12]. Both tumor cells and host cells, including stromal cells, endothelial cells and immune cells, release certain cytokines and chemokines in the tumor inflammatory microenvironment such as Tumor Necrosis Factor alpha (TNF-α), Interleukin-(IL) 6, IL-1A, IL-8, the inflammatory chemokine-CCL2 and the C-X-C Motif Chemokine Ligand 12 (CXCL12)-C-X-C Chemokine Receptor type 4 (CXCR4) signaling cascade. Cytokines and chemokines have a broad spectrum of effects, generating inflammation-associated immune responses and recruiting inflammatory cells to promote tumor cell growth, survival, invasion and angiogenesis[14,15].

TABLE 1: GENERAL INFORMATION OF PATIENTS

Clinical features	Cases (%)	Clinical features	Cases (%)
Age (years)		T stage	
<60	63 (49.22)	T1	56 (43.75)
≥60	65 (50.78)	T2	51 (39.84)
Smoking		T3	14 (10.94)
Yes	68 (53.12)	T4	7 (5.47)
No	60 (46.88)	N stage	
Pathological type		N0	59 (46.09)
Adenocarcinoma	70 (54.69)	N1	15 (11.72)
Squamous cell carcinoma	58 (45.31)	N2-N3	54 (42.19)
TNM staging		M stage	
I-II	61 (47.66)	M0	101 (78.91)
III-IV	67 (52.34)	M1	27 (21.09)
Gender		Tumor diameter	
Male	72 (56.25)	≤3	89 (69.53)
Female	56 (43.75)	>3	39 (30.47)

TABLE 2: COMPARISON OF CCL2 AND MCA EXPRESSION LEVELS WITH CLINICOPATHOLOGICAL PARAMETERS OF NSCLC PATIENTS

Clinical features	n	CCL2 expression	p	MCA expression	p
Age (years)			0.129		0.123
<60	63	198.00±5.4		22.21±2.3	
≥60	65	209.89±9.1		24.11±8.9	
Smoking			0		0
Yes	68	129.06±10.1		23.09±3.9	
No	60	109.53±7.5		22.06±6.8	
Pathological type			0.044		0.015
Adenocarcinoma	70	254.18±6.9		21.08±8.5	
Squamous cell carcinoma	58	267.67±8.6		23.67±3.9	
TNM staging			0		0
I-II	61	322.88±10.11		27.04±3.5	
III-IV	67	309.37±9.12		25.15±5.4	
Gender			0.064		0.083
Male	72	277.34±10.1		29.14±4.1	
Female	56	269.09±7.3		30.1±8.1	
T stage			0		0
1	56	279.44±5.6		31.5±5.9	
2~4	51	287.04±8.9		29.6±4.2	
1~2	14	290.12±10.7		28.9±6.8	
3~4	7	308.34±7.9		27.3±7.7	
N stage			0		0
N0	59	311.43±9.0		33.8±6.3	
1~2	15	301.57±7.9		38.3±7.8	
Tumor diameter			0.01		0.014
≤3	89	323.76±11.2		39.5±5.8	
>3	39	343.56±10.7		33.3±9.2	

TABLE 3: MULTIPLE LINEAR REGRESSION OF MCA EXPRESSION AND CLINICOPATHOLOGICAL PARAMETERS OF NSCLC PATIENTS

Indicators	Beta coefficient (β)	Parameter estimation	Standard Error (SE)	p
Smoking	0.624	0.074	0.031	0.000
Case classification	0.532	0.071	0.036	0.000
TNM staging	0.447	0.063	0.027	0.000
T stage	0.528	0.081	0.033	0.000
N stage	0.556	0.064	0.032	0.000
Tumor diameter	0.537	0.068	0.030	0.000

TABLE 4: MULTIPLE LINEAR REGRESSION BETWEEN CCL2 EXPRESSION AND CLINICOPATHOLOGICAL PARAMETERS OF NSCLC PATIENTS

Indicators	β	Parameter estimation	SE	p
Smoking	0.643	0.062	0.023	0.025
Case classification	0.511	0.061	0.033	0.000
TNM staging	0.443	0.059	0.029	0.000
T stage	0.513	0.074	0.026	0.000
N stage	0.544	0.069	0.031	0.000
Tumor diameter	0.517	0.072	0.036	0.000

TABLE 5: COMPARISON OF DIFFERENT EXPRESSION LEVELS OF CCL2 AND MCA IN NORMAL POPULATION AND NSCLC POPULATION (x±s)

Groups	n	CCL2 (pg/ml)	MCA (%)	r	p
Health group	112	45.74±10.51	32.54±8.72	0.87	<0.001
NSCLC group	128	235.43±25.75	26.44±9.33	0.39	<0.001
t		72.819	5.209		
p		<0.001	<0.001		

Lung cancer development, incidence and metastasis are all intimately correlated with CCL2. CCL2 expression is markedly up in the lung tissue of NSCLC patients and it is also elevated in cases of lymph node metastasis. It is also tightly positively linked with the microcirculation count of NSCLC[16,17]. CCL2 can also directly and indirectly induce tumor neovascularization, thereby promoting the metastasis of cancer tissue. If lung cancer tends to metastasize to bone tissue, chemokines play a central role[18].

CCL2 itself does not directly cause cytotoxic effects on tumor cells but CCL2 produced by tumors is considered to be an important cause of tumor-associated macrophage aggregation. The results showed that the tumor tissues producing high levels of CCL2 had a higher total number of infiltrating macrophages, a lower tumor inoculation index and a slower tumor invasion rate. CCL2 can also activate T cells, Natural Killer (NK) cells and other immune cells, promote the secretion of a variety of cytokines and mediators, directly and indirectly identify and attack tumors, and inhibit tumor growth and metastasis. In Huang *et al.*[19] research, when kidney cancer cells and fibroblasts that produce CCL2 were co-injected into mice, tumor development and metastasis were found to be suppressed. The Lipopolysaccharides (LPS) enhanced synergistically the killing activity of macrophages against the immunogenicity of mouse renal carcinoma cells, which could be blocked by CCL2 monoclonal Antibodies (mAb). After receiving Bacillus Calmette-Guerin (BCG) therapy, bladder cancer patient's cytokine expression was studied by Iantorno *et al.*[20], who discovered that enhanced CCL2 expression was linked to the preservation of tumor-free status. However, it is yet unknown how CCL2 changes in lung cancer patients and whether there is a connection between CCL2 and lung cancer incidence.

This study demonstrated a strong link between smoke, pathologic type, TNM, T stage, tumour diameter and CCL2 and MCA expression levels, but not between age and gender. Although the expression levels of CCL2 in peripheral serum of NSCLC patients increased, MCA did not increase, but decreased. This may be one of the important factors that cause lung cancer patients not to develop effective anti-tumor immunity. The possibility of CCL2 activity being inhibited by patient-specific mechanisms or CCL2 gene expression being altered is one explanation for the down-regulation of MCA, which can result in the onset and progression of lung cancer.

To summarize, our work originally demonstrated the degree of CCL2 expression and the MCA change rule in lung cancer patients. It is speculated that by changing the MCA of CCL2 in the inflammatory microenvironment of tumors in patients, effective cancer treatment interventions can be developed as new targets for drug development.

Author's contributions:

Zhen Gao and Cheng Qian contributed equally to this work.

Conflict of interests:

The authors declared no conflict of interest.

REFERENCES

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, *et al.* Cancer statistics in China, 2015. CA Cancer J Clin 2016;66(2):115-32.
2. Herbst RS, Heymach JV, Lippman SM. Lung Cancer. N Engl J Med 2008;359(13):1367-80.
3. Rapp E, Pater JL, Willan A, Cormier Y, Murray N, Evans WK, *et al.* Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer-Report of a Canadian multicenter randomized trial. J Clin Oncol 1988;6(4):633-41.
4. Vafaei S, Zanjani LS, Shams ZH, Naseri M, Fattahi F, Gheytanchi E, *et al.* Low expression of talin1 is associated with advanced pathological features in colorectal cancer patients. Sci Rep 2020;10(1):1-8.
5. Vafaei S, Taheri H, Hajimomeni Y, Yaseri FA, Zadeh FA. The role of NLRP3 inflammasome in colorectal cancer: Potential therapeutic target. Clin Transl Oncol 2022;24(10):1881-9.
6. Zanjani LS, Vafaei S, Abolhasani M, Fattahi F, Madjd Z. Prognostic value of talin-1 in renal cell carcinoma and its association with B7-H3. Cancer Biomark 2022;35(3):269-92.
7. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, *et al.* CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 2011;475(7355):222-5.
8. Roblek M, Strutzmann E, Zankl C, Adage T, Heikenwalder M, Atlic A, *et al.* Targeting of CCL2-CCR2-glycosaminoglycan axis using a CCL2 decoy protein attenuates metastasis through inhibition of tumor cell seeding. Neoplasia 2016;18(1):49-59.
9. Li X, Yao W, Yuan Y, Chen P, Li B, Li J, et al. Targeting of tumour-infiltrating macrophages *via* CCL2/CCR2 signalling as a therapeutic strategy against hepatocellular carcinoma. Gut 2017;66(1):157-67.
10. Marshall J. Transwell® invasion assays. Methods Mol Biol 2011;769:97-110.
11. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454(7203):436-44.
12. Crusz SM, Balkwill FR. Inflammation and cancer: Advances and new agents. Nat Rev Clin Oncol 2015;12(10):584-96.
13. Kundu JK, Surh YJ. Inflammation: Gearing the journey to cancer. Mutat Res Rev Mutat Res 2008;659(1-2):15-30.
14. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation and cancer. Cell 2010;140(6):883-99.
15. Josephs DH, Bax HJ, Dodev T, Georgouli M, Nakamura M, Pellizzari G, *et al.* Anti-folate receptor-α IgE but not IgG recruits macrophages to attack tumors *via* TNFα/MCP-1 signaling. Cancer Res 2017;77(5):1127-41.
16. Jin D, Yang JP, Hu JH, Wang LN, Zuo JL. MCP-1 stimulates spinal microglia *via* PI3K/Akt pathway in bone cancer pain. Brain Res 2015;1599:158-67.
17. Zou W, Hu TH. Effect of chemokine interleukin-8, monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 on the angiogenesis of non-small cell lung cancer. J Cent South Univ Med Sci 2007;32(4):665-70.
18. Cai Z, Chen Q, Chen J, Lu Y, Xiao G, Wu Z, *et al.* Monocyte chemotactic protein 1 promotes lung cancer-induced bone resorptive lesions *in vivo*. Neoplasia 2009;11(3):228-36.
19. Huang S, Xie K, Singh RK, Gutman M, Bar-Eli M. Suppression of tumor growth and metastasis of murine renal adenocarcinoma by syngeneic fibroblasts genetically engineered to secrete the JE/MCP-1 cytokine. J Interferon Cytokine Res 1995;15(7):655-65.
20. Iantorno R, Nicolai M, Cipollone G, di Federico G, Tenaglia R. Role of MCP-1 chemokine in the response of monocytes of patients with superficial tumors of the bladder treated with BCG immunotherapy. Arch Ital Urol Androl 1997;69(1):71-2.

This article was originally published in a special issue, "Advanced Targeted Therapies in Biomedical and Pharmaceutical Sciences" Indian J Pharm Sci 2023:85(1) Spl Issue "167-172"