

Analysis of the chemotactic factors for tumor-infiltrating fibrocytes and their prognostic significances in lung cancer

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Received July 5, 2022; Accepted September 8, 2022

DOI: 10.3892/ol.2022.13537

Abstract. Fibrocytes, which are bone marrow-derived collagen-producing cells, have been reported to be involved in pathogenesis of pulmonary fibrosis. Our previous study reported that tumor-infiltrating fibrocytes play a role in tumor progression and drug resistance in lung cancer. The present study therefore examined chemotactic factors for fibrocytes in tissues of non-small cell lung cancer (NSCLC) and their prognostic significance. Surgically resected tumor tissues were examined for the expression of chemotactic factors, including C-X-C motif chemokine 12 (CXCL12), CCL2, platelet-derived growth factor (PDGF)-AA and PDGF-BB, as well as tumor-infiltrating fibrocytes by immunostaining. The chemotactic ability of fibrocytes in response to each factor was evaluated using a migration assay by counting the migrated cells microscopically, and expression of receptors for chemotactic factors were analyzed by flow cytometry. The expression of CXCL12, but not CCL2, PDGF-AA, or PDGF-BB, was associated with the number of tumor-infiltrating fibrocytes in lung adenocarcinoma (LUAD), but not lung squamous cell carcinoma (LUSQ). In addition, patients with an increased expression of CXCL12 in LUAD but not LUSQ showed a significantly poorer prognosis compared with those with a decreased expression. However, the expression of CCL2,

PDGF-AA and PDGF-BB was not correlated with the prognosis of patients with NSCLC. The number of fibrocytes was associated with a poor prognosis in LUAD. Fibrocytes derived from the peripheral blood of healthy subjects as well as patients with lung cancer expressed higher levels of CXCR4 compared with CCR2, PDGF and receptor- α and receptor- β . Overall, these results suggested that targeting tumor-infiltrating fibrocytes via the CXCL12/CXCR4 axis may be a useful strategy for controlling the progression of NSCLC, particularly LUAD.

Introduction

Fibrocytes are bone marrow-derived collagen-expressing cells and constitute approximately 0.5% of the peripheral blood leukocyte population (1,2). They express markers of hematopoietic cells (CD34), leukocytes (CD11b, CD13, and CD45), and extracellular matrices (collagens I and III, fibronectin) (1-4). A number of studies have suggested their potential role in fibrotic diseases in various organs, including skin, lung, liver, and kidney (5-9). Previous studies have also identified fibrocytes in tumor tissues (10,11), but their role in tumor progression has not yet been discussed. We and others previously demonstrated that fibrocytes have the ability to produce several growth factors, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) (12-14). Furthermore, we recently found that fibrocytes had accumulated in the tumor tissues of lung adenocarcinoma (LUAD) patients after treatment with the anti-VEGF antibody bevacizumab, and the accumulated fibrocytes mediated the acquisition of resistance to anti-angiogenic agents by producing FGF-2 in murine models (15).

Based on these findings, we suspected that tumor-infiltrating fibrocytes might influence the growth and progression of tumor cells and that regulating the accumulation of such fibrocytes in the tumor microenvironment might be a novel therapeutic approach. In addition to our previous study, which demonstrated that fibrocytes accumulated in the tumor microenvironment via the CXCL12/CXCR4 axis (15), several chemotactic factors, such as CCL2, CCL5, CCL11, and CCL24, and PDGFs, have also been shown to induce the migration of fibrocytes in pulmonary fibrosis and/or asthma (16-18).

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Abbreviations: NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; LUAD, lung adenocarcinoma; LUSQ, lung squamous cell carcinoma; PBS, phosphate-buffered saline; FSP-1, fibroblast-specific protein 1

Key words: fibrocyte, C-X-C motif chemokine 12, C-X-C chemokine receptor type 4, NSCLC, LUAD

In the present study, to determine the key therapeutic target regulating the migration of fibrocytes into the tumor micro-environment, we examined the expression of representative chemotactic factors and the number of tumor-infiltrating fibrocytes in surgically resected tumor tissues from lung cancer patients. We also analyzed the significance of these factors for the prognosis of these patients.

Materials and methods

Patients' samples. Patients who underwent surgical resection from 2011 to 2012 in Tokushima University Hospital and whose resected tumor tissues were available for analyses were included in this study. Paraffin-embedded sections of tumor tissues surgically resected from 52 lung cancer patients were used (Table I). The sections were taken from 23 adenocarcinoma (LUAD) and 29 squamous cell carcinoma (LUSQ) patients. They were analyzed to evaluate the chemokine expression on cancer cells and number of fibrocytes. All patients received follow-up. The protocol was approved by the Institutional Review Board (IRB) of Tokushima University Hospital (no. 2471).

Isolation of human fibrocytes. Human fibrocytes were isolated from the peripheral blood of healthy volunteers and patients with lung cancer as previously reported (13,14,17). Mononuclear cells were isolated from the peripheral blood of healthy volunteers using Ficoll density centrifugation. The isolated cells were cultured in DMEM supplemented with 20% fetal bovine serum (FBS), penicillin, and streptomycin on bovine fibronectin (R&D Systems)-coated 150 mm cell culture dishes (BD Pharmingen). The medium was changed twice a week. After seven days, the medium was aspirated, and cells were washed with sterile phosphate-buffered saline (PBS) three times. The adherent cells were harvested using 0.125% trypsin. Greater than 90% of the adherent cells prepared using this method consisted of fibrocytes as determined by the expression of CD45, collagen I, and CXCR4. Informed consent was obtained from all volunteers, and the protocol was approved by the IRB of Tokushima University Hospital (no. 2838).

Immunohistochemical staining. Fibrocytes in the clinical specimens were identified using anti-CD45 and anti-fibroblast-specific protein 1 (FSP-1) antibody as previously described (15,19). Paraffin-embedded tissues (4 μ m thick) were stained with a rabbit anti-FSP-1 polyclonal antibody (1:2,000 dilution, D9F9D; Cell Signaling Technology) and mouse anti-human CD45 monoclonal antibody (1:100 dilution, 135-4C5; Novus Biotechnology). These sections were re-incubated with appropriate secondary antibodies conjugated with peroxidase or alkaline phosphatase (ready to use; Nichirei). Immunoreactivity was detected using the DAB Liquid System or new fuchsin (Nichirei), and samples were counterstained with hematoxylin. We evaluated fibrocytes as double-positive cells for both FSP-1 and CD45 by counting the average number in 6 high-power fields at x400 magnification among the tumor stromal cells (Fig. 1B). To evaluate the expression of CXCR4 on tumor-infiltrated fibrocytes, we performed double staining for FSP-1 and CXCR4 using a mouse anti-CXCR4 antibody (1:25 dilution, 44716; R&D Systems).

To detect the chemokines in lung cancer cells, immunohistochemistry was performed using Leica Bond-Max (Leica) and Bond Polymer Refine Detection (DS9800; Leica) with the following antibodies: CCL12/SDF-1 (P-159X) (1:50 dilution, sc-74271; Santa Cruz Biotechnology), anti-CCL2/MCP-1 antibody (1:200 dilution, ab9669; Abcam, Cambridge, UK), anti-PDGF-AA polyclonal antibody (1:50 dilution, PAB3678; Abnova), and anti-PDGF-BB antibody (1:500 dilution, ab23914; Abcam). The expression of chemokines in lung cancer was determined by evaluation at x200 magnification. Images were acquired by Keyence BZ-9000 microscopy (Keyence). The intensity of staining was scored as 0 (absent), 1+ (low intensity), 2+ (intermediate intensity), or 3+ (high intensity) (Fig. 1A).

The evaluation was performed individually by two researchers in a blinded fashion.

Migration assays. Migration of fibrocytes was assayed as previously described (13,17). Recombinant human CXCL12/SDF-1 (350-NS-010/CF; R&D Systems), CCL2/MCP-1 (279-MC-010; R&D Systems), PDGF-AA, or PDGF-BB (221-AA-010, 220-BB-010; R&D Systems) was used as a chemoattractant. These chemoattractants were added to the well of a BD Falcon TC companion Plate (24-well plate). Immediately afterwards, 1×10^5 fibrocytes/well were added to the chambers in the well with DMEM containing 0.1% FBS or chemokines through 8- μ m filters. We incubated the cells for 6 h in a cell culture incubator. After a 6-h incubation, the cells that had migrated to the bottom surface of the filter were stained using Diff-Quik reagents I and II (Baxter), and were counted in 6 randomly selected fields on each filter under a microscope at x200 magnification. All assays were performed in triplicate. Migration was assessed by counting the number of cells in four high-power fields with a light microscope.

FACS analyses. To examine the expression of cell surface receptors of fibrocytes, fibrocytes isolated from peripheral blood were stained with PE Mouse IgG1, κ Isotype Ctrl (FC) antibody (#400113; Biolegend), PE CD184 (CXCR4) antibody (#306505; Biolegend), PE anti-human CD192 (CCR2) antibody (#357205; Biolegend), PE anti-human CD140a (PDGFR α) antibody, (#323505; Biolegend), and PE anti-human CD140b (PDGFR β) antibody (#323605; Biolegend). The stained cells were analyzed by flow cytometry using BD LSRFortessa (BD Bioscience) for acquisition and the FlowJo software program (Treestar Inc.) for analyses (17).

Statistical analyses. The end of the follow-up period was defined as either the date of patient mortality or the patient's last date of contact. The overall survival was defined as the duration from the date of the diagnosis to the date of last contact or patient mortality. We performed Welch's t-test, a one-way analysis of variance, Tukey's multiple-comparison post-hoc test, and a linear regression analysis for the statistical analyses using the GraphPad PRISM software program (5.01; GraphPad Software, Inc.) and R Commander plug-in EZR (1.55; Saitama Medical Center, Jichi Medical University, Saitama, Japan). The survival was estimated using the Kaplan-Meier method, and the log-rank test was used to assess differences in survival distributions among the groups. The data were expressed as mean \pm standard

Table I. Baseline characteristics of patients with lung cancer.

Factors	No. of patients	(%)
No. of patients	52	100
Sex (%)		
Male	35	67.3
Female	17	32.7
Age (%)		
<75	29	55.8
≥75	23	44.2
Smoking (%)		
Non-smoker	16	30.8
Smoker	36	69.2
pT factor (%)		
T1	28	53.9
T2	21	40.4
T3	2	3.8
T4	1	1.9
pN factor (%)		
N0	46	88.5
N1	5	9.6
N2	1	1.9
pStage (%)		
I	43	82.7
II	6	11.5
III	3	5.8
Histology (%)		
Ad	23	44.2
Sq	29	55.8

Ad, adenocarcinoma; Sq, Squamous cell carcinoma.

deviation, and P-values of <0.05 were considered to indicate significance.

Results

The number of fibrocytes was positively correlated with the expression level of CXCL12 in lung cancer specimens. We examined the expressions of chemotactic factors for fibrocytes with tumor tissues from 52 patients with lung cancers (LUAD: 23, LUSQ: 29) who underwent surgical resection between 2011 and 2012 at Tokushima University Hospital (Table I). These patients consisted of 35 males and 17 females, with a mean age of 71.2 years old (range 46-85) years old at the time of surgical resection. They were pathologically diagnosed as follows: Stage I in 43 patients (82.7%); Stage II in 6 patients (11.5%); and Stage III in 3 patients (5.8%).

To investigate correlations between the expression of chemokines and the accumulation of fibrocytes in lung cancer tissues, immunostaining for chemokines (CXCL12, CCL2, PDGF-AA, PDGF-BB) and fibrocytes was performed in serial sections (Fig. 1A and B). These chemokines were strongly detected in the cytoplasm of tumor cells, but not in interstitial

areas; however, fibrocytes were mainly observed in the peritumoral areas (Fig. 1B) (14). In LUAD, fibrocytes showed greater accumulation in the group with a high CXCL12 expression than in the groups with a low or intermediate CXCL12 expression ($P<0.05$) (Fig. 2A). No such association was seen in CCL2, PDGF-AA, and PDGF-BB.

Conversely, we did not find any correlations among the expression level of any chemotactic factor and the number of fibrocytes in LUSQ (Fig. 2B).

The survival of patients was negatively correlated with the expression level of CXCL12 in lung cancer specimens. We analyzed the impact of the expression of chemotactic factors for fibrocytes on the overall survival (OS) of patients. In this analysis, the patients were divided into two groups showing a high (score: 3+) and low (score: 0~2+) expression. A high expression of CXCL12 was observed in 34.8% of LUAD and 24.1% of LUSQ patients (Table II). Furthermore, a high expression of CCL2 was found in 52.2% of LUAD and 27.6% of LUSQ patients. However, a high expression of PDGF-AA was more common in LUSQ than in LUAD (31.0% vs. 17.4%, respectively) patients. As shown in Fig. 3A, the OS of NSCLC patients with a high expression of CXCL12 was significantly worse than that of patients with a low expression. However, there was no significant difference in the OS between patients with a high and low expression of CCL2, PDGF-AA, or PDGF-BB (Fig. 3A). Interestingly, a similar difference was found in patients with LUAD, but not in those with LUSQ (Fig. 3B and C). We also evaluated the impact of age on the expression of chemotactic factors, however, but no marked differences were seen between elderly patients (≥75 years old) and others (data not shown).

The number of fibrocytes was negatively correlated with the survival of LUAD patients. We also examined the correlations between the number of fibrocytes and the OS of patients with lung cancer. To increase the number of patients with LUAD, we combined the findings of our present study and our previous data (19). As a result, we further included a further 27 patients with stage I-III lung adenocarcinoma, resulting in 50 patients in total (Table SI). The patients with LUAD were divided into 2 groups by employing a cut-off value (12,000), yielding 0.724 as the area under the curve (AUC) (Fig. 3D). Under this condition, we found that the patient survival of the high-fibrocyte group was significantly worse than that of the low-fibrocyte group ($P\text{-value}=0.0274$) (Fig. 3E). There was no correlation between the number of fibrocytes and any clinical factors in LUAD (Table SI). In addition, there was no correlation between the number of fibrocytes and the OS of patients with NSCLC or LUSQ (data not shown).

Analysis of receptor expression for chemotactic factors and the migration capacity of fibrocytes in patients with lung cancer. To clarify the role of chemotactic factors in fibrocyte migration in patients with lung cancer, we examined the expression of receptors for chemotactic factors by flow cytometry. As shown in Fig. 4A, the expression of CXCR4, which is a receptor for CXCL12, was consistently detected in healthy donors as well as patients with LUAD. In contrast, the receptors for other chemokines (CCL2, PDGF-AA, and PDGF-BB) were not consistently detected and the level of expression was lower than that of CXCR4 (Fig. 4A). In the

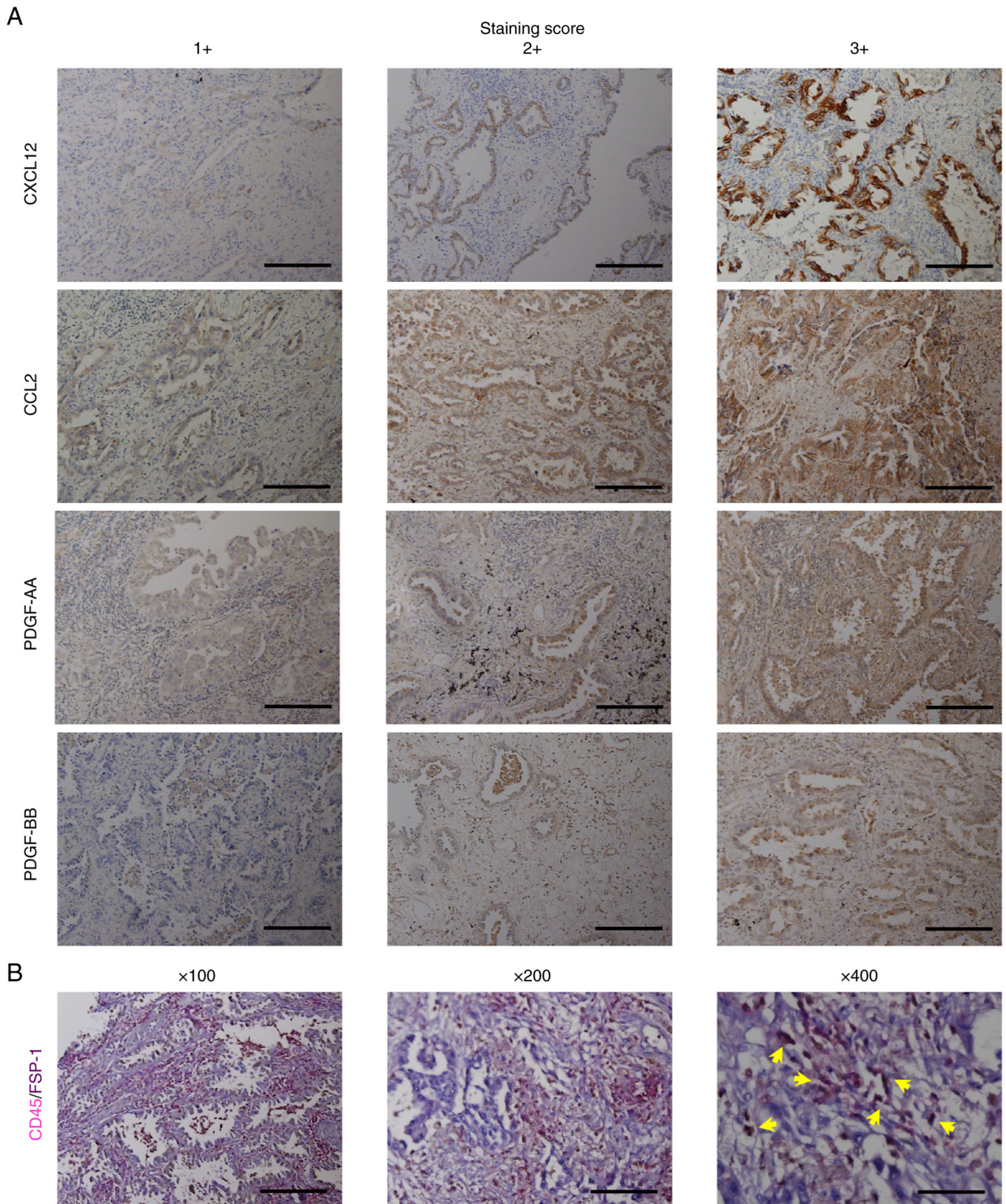
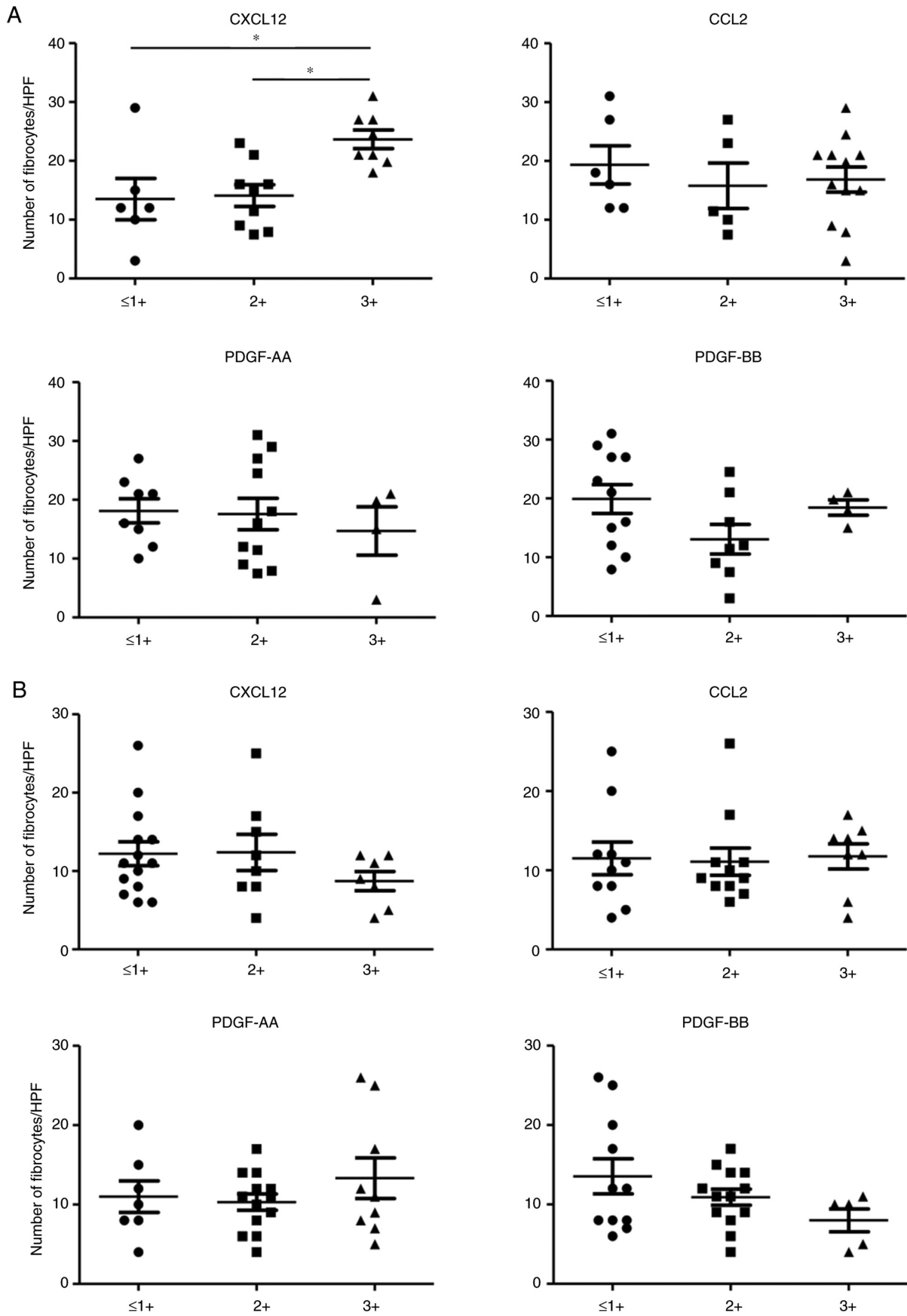


Figure 1. Immunohistochemical staining of CXCL12, CCL2, PDGF-AA and PDGF-BB, and fibrocytes in tumor tissues of lung cancer and their scoring. (A) Immunohistochemical staining of CXCL12, CCL2, PDGF-AA and PDGF-BB, and their scoring. Magnification, x100; scale bar, 200 μ m. (B) Immunohistochemical staining of tumor-infiltrating fibrocytes with antibodies for CD45 and FSP-1. Arrows indicate double-stained fibrocytes. Magnification left to right, x100, x200, x400; scale bar, 200 μ m (x100), 100 μ m (x200) and 50 μ m (x400). CXCL12, C-X-C motif chemokine 12; PDGF, platelet-derived growth factor; FSP-1, anti-fibroblast-specific protein 1.

analysis to determine the effects of chemokines on the migration of fibrocytes derived from healthy donor PBMCs, CXCL12 consistently induced their migration *in vitro*, but the effects of CCL2, PDGF-AA, and PDGF-BB were varied among donors (Fig. 4B). These results suggest the importance of the CXCL12-CCR4 axis

over the CCL2-CCR2 or PDGFs-PDGFRs axis in the migration of fibrocytes into the tumor microenvironment. We finally evaluated the expression of CXCR4 on tumor-infiltrated fibrocytes by double staining for FSP-1 and CXCR4 and confirmed that the FSP-1-positive fibrocytes definitely expressed CXCR4 (Fig. 4C).



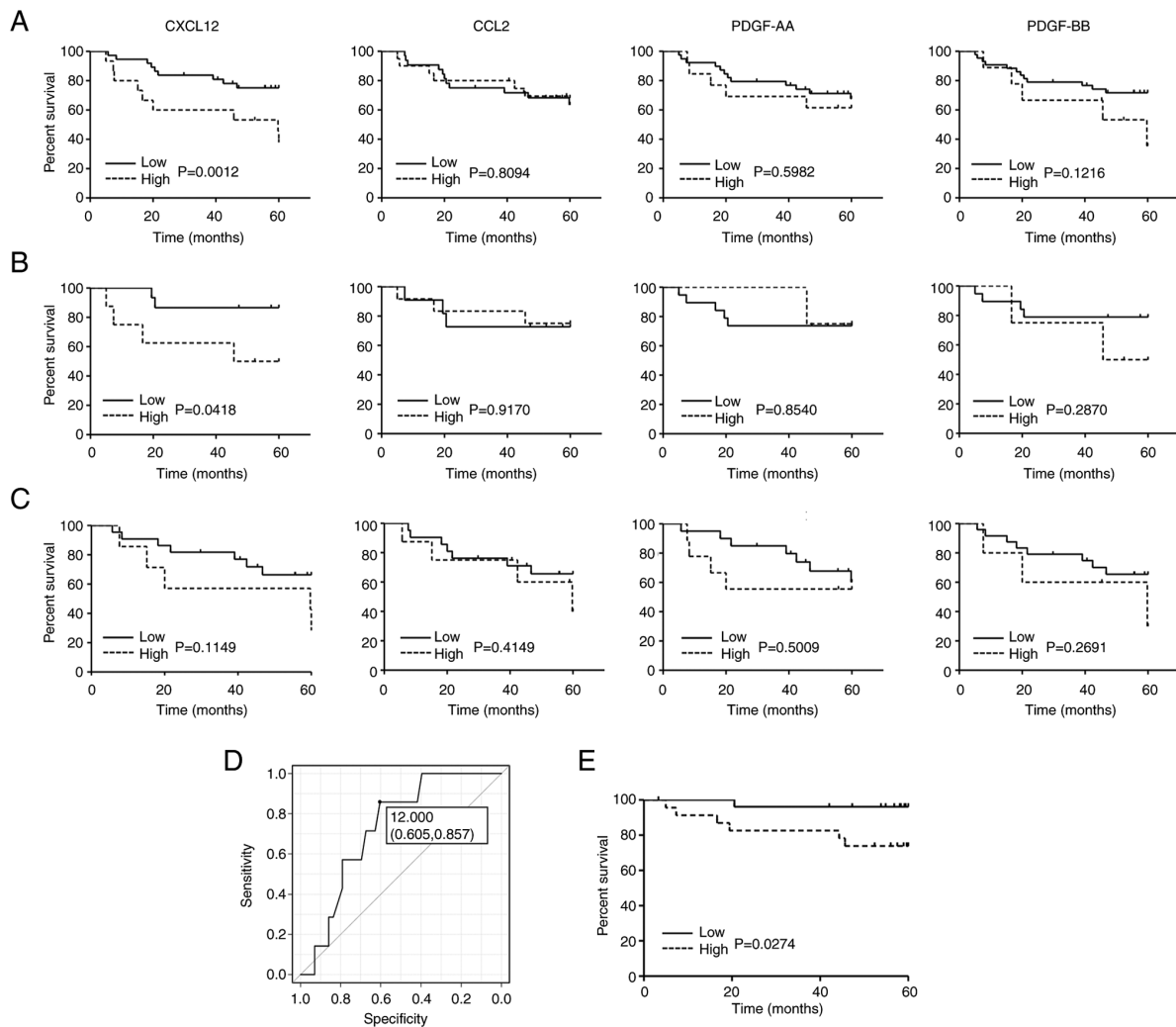
A: Adenocarcinoma, B: Squamous cell carcinoma * $p < 0.05$

Figure 2. Number of tumor-infiltrating fibrocytes in tumor tissues expressing various levels of CXCL12, CCL2, PDGF-AA and PDGF-BB in LUAD and LUSQ. The dot indicates each patient. The staining levels of each factor were divided into three groups, $\le 1+$, 2+ and 3+. (A) Adenocarcinoma, (B) squamous cell carcinoma. All the data are expressed as mean \pm standard deviation. * $P < 0.05$. CXCL12, C-X-C motif chemokine 12; PDGF, platelet-derived growth factor; LUAD, lung adenocarcinoma; LUSQ, lung squamous cell carcinoma.

Table II. Expression of CXCL12, CCL2, PDGF-AA and PDGF-BB in lung cancer tissues.

Chemotactic factor	NSCLC		Ad		Sq	
	Low (0-2+)	High (3+)	Low (0-2+)	High (3+)	Low (0-2+)	High (3+)
CXCL12	37 (71.1)	15 (28.9)	15 (65.2)	8 (34.8)	22 (75.9)	7 (24.1)
CCL2	32 (61.5)	20 (38.5)	11 (47.8)	12 (52.2)	21 (72.4)	8 (27.6)
PDGF-AA	39 (75.0)	13 (25.0)	19 (82.6)	4 (17.4)	20 (69.0)	9 (31.0)
PDGF-BB	43 (82.7)	9 (17.3)	19 (82.6)	4 (17.4)	24 (82.8)	5 (17.2)

Data are expressed as number (%). The expression of chemotactic factors was analyzed in low-(score: 0-2+) and high-expression (score: 3+) groups. Ad, adenocarcinoma; Sq, Squamous cell carcinoma; CXCL12, C-X-C motif chemokine 12; PDGF, platelet-derived growth factor.



A: Non-small cell carcinoma, B: Adenocarcinoma, C: Squamous cell carcinoma, D: Fibrocytes

Figure 3. Association between expression of CXCL12, CCL2, PDGF-AA or PDGF-BB and the number of fibrocytes with the survival of patients. The staining level was divided into high (3+) and low (≤ 2) groups. (A) NSCLC, (B) LUAD and (C) LUSQ. (D) The patients with LUAD were divided into two groups based on the number of fibrocytes in their tumors (high- and low-fibrocyte groups) by employing a cut-off value (12,000) yielding 0.724 as the AUC. (E) The association between the number of tumor-infiltrating fibrocytes with the survival of patients. CXCL12, C-X-C motif chemokine 12; PDGF, platelet-derived growth factor; LUAD, lung adenocarcinoma; LUSQ, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; AUC, area under the curve.

Discussion

In the present study, we demonstrated that the expression of CXCL12 in tumor cells was associated with

the number of tumor-infiltrating fibrocytes in LUAD patients. However, CCL2, PDGF-AA, and PDGF-BB were not correlated with the accumulation of fibrocytes. These results suggest that inhibiting the CXCL12/CXCR4 axis

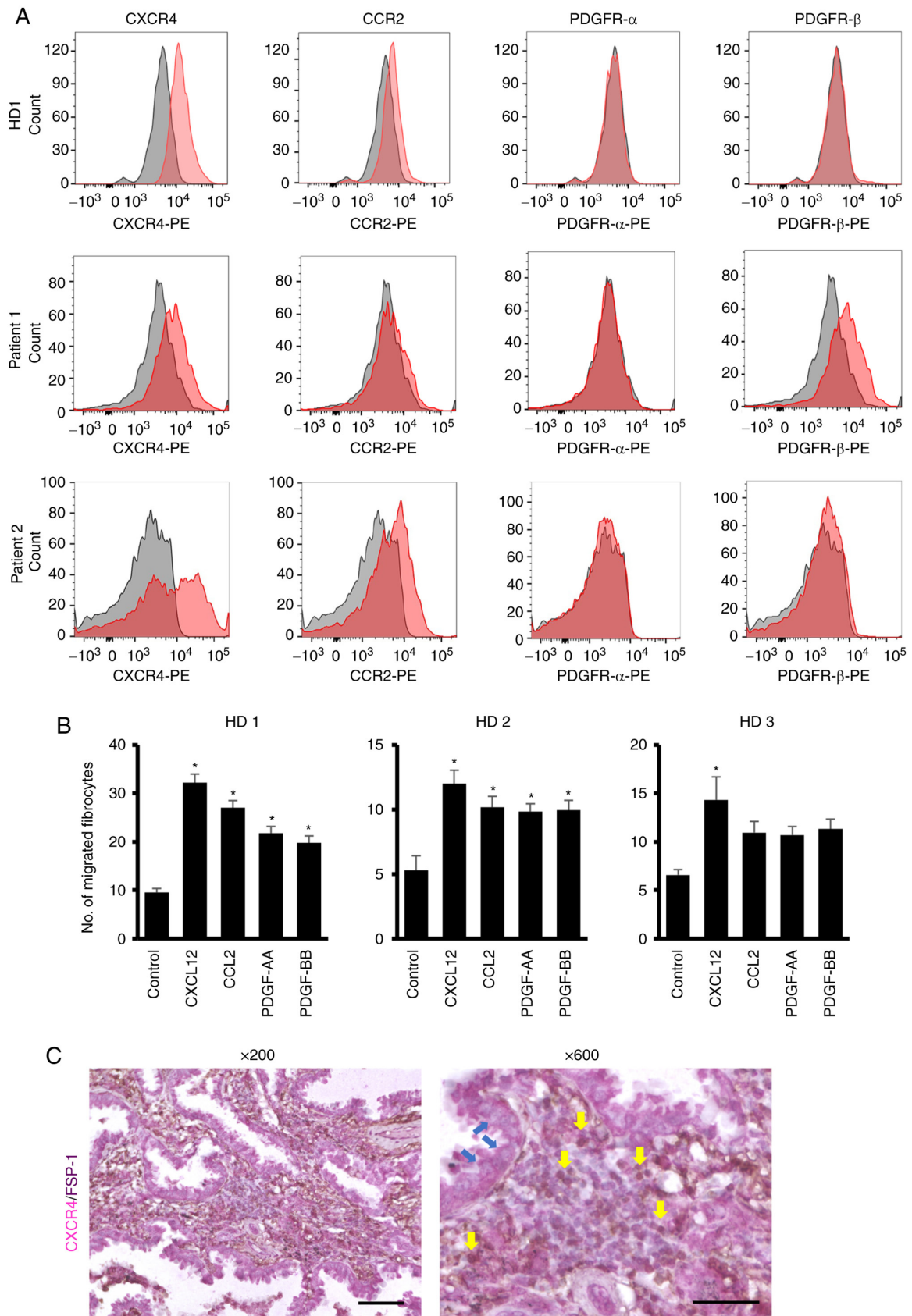


Figure 4. Expression of CXCR4, CCR2 and PDGF receptors in fibrocytes and their migration in response to their ligands. (A) Expression levels of CXCR4, CCR2 and PDGF receptors in fibrocytes generated from PBMC from HD and patients with LUAD. The expression of each receptor was examined by flow cytometry. (B) Migration of fibrocytes in response to CXCL12, CCL2, PDGF-AA and PDGF-BB. The fibrocytes were generated from three HD. The migration assay was performed with the optimal dose (100 ng/ml) of CXCL12, CCL2, PDGF-AA and PDGF-BB. (C) Expression of CXCR4 on tumor-infiltrated fibrocytes. The FSP-1-positive fibrocytes definitely expressed CXCR4 in the tumor tissues from a patient with LUAD. Yellow arrows indicate double-stained fibrocytes. Blue arrows indicate cancer cells. Magnification left to right: $\times 200$ and $\times 600$; scale bar $100 \mu\text{m}$ ($\times 200$) and $50 \mu\text{m}$ ($\times 600$). * $P < 0.05$ vs. control. HD, healthy donors; CXCR4, C-X-C chemokine receptor type 4; PDGF, platelet-derived growth factor; PBMC, peripheral blood mononuclear cells; LUAD, lung adenocarcinoma; FSP-1, anti-fibroblast-specific protein 1.

may be an effective strategy for regulating the number of fibrocytes in LUAD.

Recently, substantial attention has been paid to the roles of fibrocytes in tumor progression (20,21). As fibrocytes can produce multiple factors that affect the function of cancer cells, tumor-infiltrating fibrocytes have been thought to play a role in tumor progression (1,3,13,14,20). However, few studies have examined the relationships between fibrocytes and tumors. We recently demonstrated that fibrocytes have the ability to promote the stem cell-like property of tumor cells by secreting the soluble factors, such as osteopontin, and aid in the tumorigenesis of lung cancer cells (19). Based on these results, regulating tumor progression by controlling the accumulation of fibrocytes into tumors may be possible. Thus, it is necessary to clarify the chemotactic factors of fibrocytes active in tumor tissues. While several chemotactic factors, such as CXCL12, CCL2, CCL5, CCL11, CCL24, and PDGFs, have been reported to play roles in the recruitment of circulating fibrocytes, we focused on the expressions of CXCL12, CCL2, PDGF-AA, and PDGF-BB in the present study, as those factors have also been reported to be expressed in lung cancer tumor tissues and associated with the prognosis of patients (22-30).

The present study found that the expression of CXCL12 in tumor tissues was associated with a poor prognosis of patients with lung cancer. Similar results have been reported in several studies of lung cancer (22-25). Furthermore, it was also reported that the intra-tumoral expression of CXCL12 was much higher than its expression in the serum (31), suggesting the importance of its expression in the tumor microenvironment. In addition, we found that the number of tumor-infiltrating fibrocytes was associated with the expression level of CXCL12 in tumors, but not CCL2, PDGF-AA, or PDGF-BB in tumor, indicating that fibrocytes were migrated into the tumor microenvironment via the CXCL12/CXCR4 axis. We also demonstrated that the density of tumor-infiltrating fibrocytes was correlated with the prognosis of LUAD patients. To our knowledge, only one report has demonstrated the correlation of circulating fibrocytes and the prognosis of the patients with lung cancer (32), so our finding supports the novel rationale in the utility of fibrocytes as a prognostic factor in the patients with LUAD. However, additional studies are required to explore the detailed relationship of CXCL12 expression and the survival of patients with lung cancer and to determine the correlation of serum CXCL12 levels with the density of tumor-infiltrating fibrocytes or the patient prognosis. In the present study and two previous reports, the correlation was mainly found in LUAD patients, but not LUSQ patients (22,24); however, this point should also be explored further for clarification.

Conversely, no correlation was noted among the expression of CCL2 or PDGFs and the survival of patients with lung cancer or the number of tumor-infiltrating fibrocytes. In previous studies, the association of the expression of CCL2 or PDGFs in tumor tissues and the prognosis of patients was considered controversial in lung cancer (26-30). On examining the expression of receptors for these chemotactic factors in fibrocytes, the high and consistent expression of CXCR4, which is the receptor for CXCL12, but not that of CCR2 and PDGFRs, was observed in specimens from healthy subjects and patients with LUAD. The pattern of receptor expression may affect the migration of fibrocytes

in vivo, as the migration of fibrocytes was strongly induced by CXCL12.

Several limitations associated with the present study warrant mention. First, the patients analyzed in this study were biased toward having early-stage disease, given that surgically resected tumor specimens and not trans-bronchial biopsy specimens were used. Therefore, additional analyses will be needed to determine whether or not similar findings are observed in patients with stage III or IV disease. Second, the expression of chemotactic factors in tumor tissues was examined based on immunohistochemistry, a semi-quantitative method. Third, the expression was evaluated in tumor cells but not in stromal cells, as the expression was mainly found in tumor cells (although PDGF-AA was to a lesser extent expressed in stromal cells in some patients). Fourth, the influence of the CXCL12/CXCR4 autocrine pathway in cancer cells was not evaluated, although the importance of this pathway in the pathogenesis of lung cancer was already reported in a previous study (33). In the immunohistochemical analyses described above, we observed a clear positive signal on the cancer cells themselves (Fig. 4C). Based on these findings, we suspect that the impact of CXCL12 on fibrocytes would be more significant than that on cancer cells. However, we cannot deny the impacts of the CXCR4/CXCL12 autocrine pathway on the correlation of the CXCL12 expression and the prognosis of the patients with LUAD.

In summary, our findings suggest that the CXCL12 may be an important chemotactic factor for tumor-infiltrating fibrocytes in LUAD, regardless of the patient age. Thus, blockade of the CXCL12/CXCR4 axis may be a therapeutic strategy for LUAD through inhibition of the migration of fibrocytes into tumors.

Acknowledgements

We thank Ms. Tomoko Oka and Ms. Akie Tanabe (Department of Respiratory Medicine and Rheumatology, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan) for their technical assistance.

Funding

This work was partly supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (grant no. JP16H0530910) and a grant from the Ministry of Health, Labour and Welfare, the Study Group on Diffuse Pulmonary Disorders and Scientific Research/Research on Intractable Diseases (grant no. 0000025921).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YN conceptualized and designed the study. MT, AM, AS, HOgi, TA, HG, SS, AA, KH and RO performed the flow cytometry, migration assay and statistical analyses. MT, HOga and HT performed the histological analyses with the surgically resected tumor specimens. MT, AM, HOgi and YN confirmed the authenticity of all the raw data, and participated

in writing and editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Institutional Review Board of Tokushima University Hospital approved this study (approval nos. 2471 and 2838). Regarding the lung cancer patients who provided the tumor specimens for the analyses, the need for written informed consent was waived by the Ethics Committee of Tokushima University, because of its retrospective nature.

Patient consent for publication

Written informed consent was obtained from both patients and healthy donors who provided their peripheral blood.

Competing interests

The authors declare that they have no competing interests.

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