CD4^+CXCR4^{high}CD69^+ T Cells Accumulate in Lung Adenocarcinoma

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CD4⁺CXCR4\textsuperscript{high}CD69⁺ T Cells Accumulate in Lung Adenocarcinoma

Ori Wald,¹* Uzi Izhar,¹*‡ Gail Amir,⁷ Shani Avniel,* Yochai Bar-Shavit,* Hanna Wald,* Ido D. Weiss,* Eithan Galun,* and Amnon Peled²*

The chemokine receptor CXCR4 is involved in the growth and metastasis of tumor cells. However, the expression of its ligand, the chemokine CXCL12, in tumors and its role in regulating the accumulation of immune cells within the tumors is not clear. Using ELISA and immunohistochemistry we found that CXCL12 is expressed in the majority of nonsmall cell lung cancer tissue sections obtained from stage IA to IB nonsmall cell lung cancer patients undergoing operation. Histopathologic examination of these sections indicated that high CXCL12 expression correlated with increased tumor inflammation. In addition, disease recurrence rates in a subgroup of adenocarcinoma patients showed a tendency to correlate with high CXCL12 expression in the tumor. Isolation of adenocarcinoma-infiltrating immune cells demonstrated an increase in the percentage of CD4⁺CD69⁺CXCR4⁺ T cells as compared with normal lung tissue. About 30% of these cells expressed the regulatory T cell markers CD25\textsuperscript{high} and FoxP3. The percentage of CD8 T cells within the tumor did not change, however; the percentage of NK and NK T cells was significantly reduced. In correlation with CXCR4 expression, CD4 T cells showed increased migration in response to CXCL12 compared with CD8 T cells and NK cells. Overall, these observations suggest that CXCL12 expression may influence tumor progression by shaping the immune cell population infiltrating lung adenocarcinoma tumors. *The Journal of Immunology, 2006, 177: 6983–6990.

Lung cancer is the leading cause of cancer death in the United States and throughout the world. The pathogenesis of nonsmall cell lung cancer (NSCLC) is critically linked to cigarette smoking and exposure to secondhand smoke (1). It is estimated that in 2006 ~174,000 new cases of lung cancer will be diagnosed and that ~162,000 cases of death will result from lung cancer. NSCLC accounts for nearly 80% of these cases (1).

Chemokines, a subfamily of chemotactic cytokines, play a central role in regulating immune cell trafficking in the body (2, 3). Furthermore, a complex network of chemokines/chemokine receptor interactions is involved in the development of primary malignant tumors and in the process of metastasis (2, 3). Several such interactions participate in the development, progression, and metastasis of NSCLC. Angiogenesis in NSCLC is mediated in part by the chemokines CXCL8 and CXCL5, and their expression correlates with tumor vascularity (4–6). Moreover, high CXCL8 mRNA levels in stage I NSCLC were associated with shortened survival (7). Chemokines also regulate the recruitment of subpopulations of immune cells into tumors (8). For example, CCL4, CCL5, and CXCL10 expression in lung adenocarcinoma was associated with the presence of an active lymphocytic response in the tumor (9). Similarly, CCL2 produced by NCSLC recruits macrophages into tumors (10). These cells in turn, support tumor growth by the secretion of various factors, among them the immune-modulating cytokine TGF-β and the proangiogenic chemokine CXCL8 (11–14). TGF-β, among other immune-modulating cytokines, is also produced by regulatory T cells. It was shown that the bone marrow is a reservoir of regulatory T cells that traffic to this site through CXCL12/CXCR4 interactions (15). The trafficking of regulatory T cells into NSCLC tissue has not been studied to date.

Chemokine receptor expression is a critical step in tumor metastasis. The most studied chemokine receptor in this regard is CXCR4, which is expressed by 23 different cancerous tissues (2, 16, 17). Recent reports indicated that CXCR4, one of the two known receptors for CXCL12, is expressed in NSCLC cells (18, 19). It has been suggested that CXCL12/CXCR4 interactions facilitate the metastasis of the NSCLC tumor to the bone marrow and dissemination into the pleural cavity (18, 20). Nonetheless, CXCL12 is highly expressed in the normal lung and is associated with the establishment of metastasis of breast and colon carcinoma in the lung (21, 22). The expression of CXCL12 in NSCLC cells has not been clearly characterized to date. Phillips et al. (18) reported that NSCLC cell lines (A549 and Calu-1) do not express CXCL12 and suggested that CXCL12 is not expressed in lung adenocarcinoma tumors. Using immunohistochemistry and ELISA, we analyzed CXCL12 expression in patients with stage IA–IB NSCLC. CXCL12 staining was detected in 42 of 46 tumor samples tested and confirmed by ELISA. Isolated lung adenocarcinoma and normal lung infiltrating lymphocytes were immunostained for CD3, CD4, CD8, CD25, FoxP3, CD56, CD69, and the CXCL12 receptor CXCR4. In lung adenocarcinoma, relative to the normal lung decreased percentages of NK and NK T cells (12 vs 28.8%, p < 0.01 and 6.4 vs 13.8%, p < 0.05, respectively) and increased percentages of CD4⁺ T cells (48.8 vs 24.5%, p < 0.01) were found. Increased percentages of tumor infiltrating CD4⁺ T cells expressed the early activation marker CD69 (66.1 vs 41.8%, p < 0.01), the regulatory T cell marker CD25\textsuperscript{high} (18 vs 7.6%, p < 0.01).
and CXCR4 (62.8 vs 49.5%, p < 0.01). Furthermore, lung adenocarcinoma-derived CD4 T cells migrated better than CD8 T cells and NK cells in response to CXCL12. CXCL12/CXCR4 interactions may therefore play a role in the accumulation of CXCR4-positive T cells and a subset of CD25-high regulatory T cells within NSCLC tissue.

Materials and Methods

Tissue collection, histological evaluation, and patient-specific clinical data

Fresh human lung specimens, paraffin-embedded tissue sections, and fresh blood samples were obtained according to the guidelines approved by the Hadassah Hospital Ethics Committee (Jerusalem, Israel). Forty-nine patients with early stage NSCLC (clinical stage IA-IIIB) that underwent complete tumor resection in our institute between January 2000 to October 2003 participated in the study. No chemotherapy or radiotherapy was administered before the operation. Histological sections were prepared from formalin-fixed, paraffin-embedded tissues and stained with H&E. Histopathological diagnosis was confirmed for each specimen. Postoperative staging indicated that three of the 49 patients had a higher stage of disease; these patients were omitted from the study. Of the 46 lung tumor samples, 29 samples were of lung adenocarcinoma, and 17 samples were of squamous cell carcinoma. Analysis of CXCL12 expression by immunohistochemistry was performed on these tumors. Sections were blindly assessed by a pathologist and scored for the extent and intensity of CXCL12 staining; in addition, tumor inflammation was scored. Intensity of staining was scored between 0 and 3. The extent of staining, i.e. the percentage of tumor cells expressing CXCL12, was scored between 1 and 4 (score of 1: 0–25%; score of 2: 25–50%; score of 3: 50–75%; and score of 4: 75–100% of tumor cells that stained positively to CXCL12). Inflammation was scored between 0 and 3; lymphocytes were used as indicators of inflammation. A score of 0 was assigned to slides where no lymphocyte collection was identified; a score of 1 was assigned to slides with only few small lymphocyte collections; a score of 2 was assigned to slides where multiple small collections of lymphocytes were identified; and a score 3 was assigned to slides with multiple large lymphocyte collections. Scoring of inflammation was done blindly. The sum of the scores for the extent and intensity of CXCL12 staining was calculated and correlated to tumor inflammation. Furthermore, this score was examined in regard to long-term disease progression. Fourteen of 46 patients participating in the study had recurrence of disease. Parameters regarding patients participating in the study are presented in Table I.

Table I. Patient characteristics and tumor CXCL12 staining

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<td>Patients</td>
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<td>Omitted (postoperative stage, IIB)</td>
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<td>CXCL12 expression</td>
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<td>Squamous CA CXCL12 (scores of 0–3)</td>
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<td>Squamous CA CXCL12 (scores of 4–7)</td>
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*The characteristics of patients participating in the study are presented. The correlation between the scores of CXCL12 staining in patients with adenocarcinoma and squamous cell carcinoma (CA) and disease recurrence is shown.

The most common histologic type of lung cancer diagnosed in Israel and in the western world is of the adenocarcinoma type (23). Thirty samples of fresh lung adenocarcinoma tumor tissue and adjacent tumor-free lung tissue removed during operation were collected. In addition, fresh blood samples from these patients were obtained at the time of operation. Lymphocytes were extracted from adenocarcinoma tumor tissue and adjacent tumor-free normal lung tissue for phenotypic characterization and migration assays. In addition, ELISA was used to assess the expression of CXCL12 in homogenates derived from these tissues and in the peripheral blood. Protocols for these experiments are described below.

Immunohistochemistry

NSCLC tissue samples were routinely fixed with formalin and embedded in paraffin. Ag retrieval was performed in EDTA buffer for 15 min in a microwave, and sections were stained with the mAb 79018 (R&D Systems) for CXCL12 (1/100), using a standard indirect avidin-biotin HRP method according to the manufacturer’s instructions. 3-Amino-9-ethylcarbazole was used for color development and sections were counterstained with hematoxylin. As negative controls, sections were stained either with no primary Ab (PBS) or with an isotype-matched control Ab (24, 25).

ELISA

Tumor tissue homogenate and normal lung tissue homogenate were prepared on ice from weighed tissue samples. ELISA for CXCL12 in the patients plasma, normal lung tissue homogenate, and the NSCLC tissue homogenate were performed using the Quantikine kit (R&D Systems) according to the manufacturer’s instructions.

Isolation of normal lung and tumor tissue-derived lymphocytes

Normal lung and adenocarcinoma tumor tissue obtained during operations were washed twice in 10 ml of PBS to remove the remaining blood. Tissue was cut into ~1-mm pieces and incubated in 2 ml of RPMI 1640 (Invitrogen Life Technologies) for 20 min with collagenase type IV (Worthington Biochemical) and DNase I (Worthington Biochemical) at 37°C. Following incubation, the tissue was run through a 1-ml pipette and laid for separation over Ficoll/Histopaque gradients (Sigma-Aldrich). Cells were washed and centrifuged at 1,200 rpm for 5 min at 4°C. Pellets were resuspended in FACS buffer for flow cytometry analysis and in a migration medium for migration assays. Patients with early disease stages IA to IIB usually have small to moderate tumors. Furthermore, only a small section from the core of the tumor may be excised for scientific analysis, as the margins must be examined to assure complete resection. Often, small numbers of total tumor cells between 1 × 10⁶ to 1 × 10⁷ cells per biopsy were obtained, limiting the extent of experiments performed.

Phenotypic analysis of lymphocytes and flow cytometry analysis

Isolated lymphocytes were taken at a concentration of 1–5 × 10⁶ cells/ml in 0.1 ml of PBS containing 0.1% BSA (Biological Industries) and 0.01% sodium azide (FACS buffer). Fe receptors were blocked by incubation with 1% human plasma for 15 min on ice. Blocked cells were then mixed with FITC-, PE-, Cy3-, or allophycocyanin-conjugated CD3, CD4, CD8, (1/40; IQ Products), CXCR4 (1/40; R&D Systems), CD25 (Miltenyi Biotec), CD69, and CD56 (BD Biosciences) mAbs or isotype controls for 20 min on ice and washed with FACS buffer. A FoxP3 internal staining kit was purchased from R&D Systems, and staining was performed according to manufacturer protocol. Immunostained cells were analyzed by flow cytometry using the FACS Calibur flow cytometer (BD Biosciences); the data were analyzed using the CellQuest 3.3 software (BD Biosciences). For all analyses, lymphocyte gating was performed using the forward scatter vs side scatter plot. Population of T cells (CD3+CD56⁻), NK T cells (CD3+CD56⁺), and NK cells (CD3+CD56⁺) were defined. T cells were further classified according to CD4 and CD8 expression.

Transwell migration assays

Tumor-derived and normal lung-derived lymphocyte migration was assessed in 24-well chemotaxis chambers (6.5-mm diameter, 5-mm pore polycarbonate Transwell culture insert; Costar). Six hundred microliters of RPMI 1640 with 1% BSA (migration buffer) with or without 250 ng/ml CXCL12s (R&D Systems) was added to the lower wells. Cells (1 × 10⁶) suspended in 100 μl of RPMI 1640 with 1% BSA were added to the upper wells. Each experiment was performed in triplicate. After incubation for 3 h, the membrane was removed and migrating cells were collected from the lower well and immunostained for the cell markers CD3, CD4, CD8, and CD56. These cells were analyzed by flow cytometry. Each sample was acquired for 1 min, and the number of each subpopulation of migrating cells was determined. For comparison between different experiments, the...
percentage of CD4 and CD8 T cells and NK cells migrating in response to CXCL12/H9251 was calculated. The percentage of migrating cells for each subpopulation of immune cells was determined as follows. 1) The number of cells used at the beginning of the experiment and laid into the upper well, representing 100% of the cells, was determined by acquisition for 1 min and was termed the total. 2) The number of cells in the lower well, where no chemokine was added, was determined after 3 h of incubation and was termed spontaneous migration. 3) The number of cells in the lower well, where CXCL12/H9251 was present, was determined after 3 h of incubation and was termed migration. The percentage of chemokine-induced migration was then calculated as follows: percentage of migration = (migration - spontaneous migration)/total \times 100.

Results

**CD4⁺ T cells accumulate in lung adenocarcinoma**

Adenocarcinoma and normal lung resident lymphocytes were isolated and analyzed for expression of the cell markers for CD56⁻/CD3⁻ NK cells, CD3⁻/CD56⁻ NK T cells, CD3⁺/CD4⁺ T cells, and CD3⁺/CD8⁺ T cells (Fig. 1A). The relative percentage of CD4 T cells, CD8 T cells, NK cells, and NK T cells from total lymphocytes were calculated (Fig. 1B). We found that the percentage of NK cells and NK T cells was significantly reduced (12 vs 28.8%, p < 0.01 and 6.4 vs 13.8%, p < 0.05, respectively; n = 22) in tumor tissue relative to that for the normal lung (Fig. 1B). Furthermore, CD8 T cells failed to accumulate in tumor tissue whereas the percentage of CD4 T cell significantly increased (48.8 vs 24.5%, p < 0.01; n = 22) in tumor tissue (Fig. 1B). Our findings indicate that CD4 T cells specifically accumulate in adenocarcinoma relative to adjacent tumor-free lung tissue. Further characterization of the subtype of CD4 T cells accumulating in the tumors was done by staining these cells for the markers CD45RO, CD45RA, CD69, CD25, and the transcription factor FoxP3. Ninety-five percent of tumor CD4⁺ T cells express CD45RO, which is a marker for activated and memory T cells as compared with 75% in the normal lung (Fig. 1A). A significant increase in the percentage of CD4⁺ T cells expressing the early activation marker CD69 was also observed in the tumor (66.1 vs 41.8%, p < 0.01; n = 18) (Fig. 2B). Regulatory T cell may play a critical role in modulating the immune response against tumors. We found an increased percentage of CD4⁺ T cells expressing the regulatory markers CD25^high and FoxP3 in the tumor compared with the normal lung (18 vs 7.6%, p < 0.01; n = 17 and 29.1 vs 11.7%, p < 0.01; n = 8 respectively) (Fig. 2B and D). Interestingly we did not find CD40L expression on these cells (data not shown). Overexpression of CD69 was shown to induce the secretion of TGF-β from CD4 T cells (26, 27); furthermore, CD4⁺ CD25^highFoxP3⁺ regulatory T cells were also shown to secrete high levels of TGF-β (28). The accumulation of these cells in adenocarcinoma lung tumors may therefore contribute to suppression of the immune response against this tumor.

**CXCR4/CXCL12 interactions are involved in the recruitment/retention of CD4 T cells in lung adenocarcinoma**

CXCR4 was shown to play a role in the trafficking and tissue localization of CD4⁺ T cells and regulatory T cells (15, 29). To study whether CXCR4 is involved in the trafficking and tissue localization of CD4 T cells in the lung and in adenocarcinoma...
We analyzed the expression of CXCR4 by CD4 T cells, CD8 T cells, and NK cells derived from these tissues. In all samples tested, considerable percentages of tumor- and normal lung-derived CD4 T cells and CD8 T cells expressed CXCR4, whereas tumor- and normal lung-derived NK cells expressed only marginal levels of CXCR4 (Fig. 3A). Furthermore, we found that in both the lung and the tumor a higher percentage of CD4+ T cells stained positive for CXCR4 (49.5 and 62.8%, respectively; n = 22) as compared with CD8+ T cells (31.7 and 47.8%, respectively; n = 9) and NK cells (19.7 and 23.5%, respectively; n = 9) (Fig. 3A–D). In addition, in the tumor the level of CXCR4 expression significantly increased on both CD4 and CD8 T cells as compared with NK cells. Furthermore, in most cases tested the percentages and levels of CXCR4 on CD4 and CD8 T cells but not NK cells was increased in the tumor relative to tumor adjacent normal lung tissue. Moreover, most of the CD4+ T cells that express FoxP3 also express CXCR4 (Fig. 3E). We next tested the migratory response of tumor- and normal lung-derived CD4 and CD8 T cells and NK cells in response to CXCL12. Because the yield of tumor NK cell was very low, we compared the migration of CD4 and CD8 cells from a normal lung to that of normal lung NK cells. We found that both CD4 and CD8 T cells migrate better than NK cells in response to CXCL12 (Fig. 4A). CD4 and CD8 T cells derived from fresh adenocarcinoma samples also migrate well in response to CXCL12. In three of four cases studied, the ability of tumor-derived CD8 T cells to respond to CXCL12 was significantly reduced as compared with tumor-derived CD4 T cells (Fig. 4B). In normal lung tissue CXCL12 is expressed by epithelial cells and is involved in the recruitment of immune cell under normal and pathological conditions such as asthma (30). The expression of CXCL12 in NSCLC cells has not yet been tested. Using ELISA, we found that the levels of CXCL12 in a normal lung and in adenocarcinoma tumors is 5-fold higher compared with the levels of CXCL12 in the serum (Fig. 5A). Immunohistochemistry analysis confirmed that in NSCLC tumor cells expressed CXCL12 in 42 of 46 (91.3%) cases tested (Table I and Fig. 5, B and C). CXCL12 levels of expression by tumor cells correlated with the level of tumor inflammation in a subgroup of adenocarcinoma but not squamous carcinoma patients (Fig. 5D). Representative staining of a tumor that expresses a high level of CXCL12 and is highly inflamed is shown in Fig. 5B. Representative staining of a tumor that expresses a low level of CXCL12 and is not inflamed is shown in Fig. 5C. Overall, these results suggest a high degree of correlation between CXCL12 expression and degree of inflammation and the accumulation of CD4 T cells in adenocarcinoma. In this study group, adenocarcinoma patients with a low score of CXCL12 staining did not show disease recurrence (0 of 8). The recurrence of disease in adenocarcinoma patients with high score of CXCL12 staining was higher (7 of 21). In squamous cell carcinoma no difference between the two groups were found (Table I).
Taken together, these observations indicate a tendency of diseases recurrence to correlate with high CXCL12 expression in the tumor. However, the numbers of participants in this study is not sufficient for statistical significance to be obtained. Larger studies are therefore required; conducting such a study is beyond the scope of this report.

Discussion

CXCR4 was found to play a critical role in the progression and development of various tumors including breast, prostate, and clear cell renal carcinoma (21, 31, 32). In clear cell renal carcinoma, CXCR4 expression correlates with increased tumor metastasis (32). In breast cancer tumors it was shown that CXCR4 expression is positively regulated by the human epithelial growth factor receptor 2 (HER2) (33) and that neutralizing the interaction between CXCL12 and CXCR4 by either anti-CXCR4 Abs or by silencing the expression of CXCR4 using RNA interference technology significantly impaired metastasis and progression of the tumor in vivo (21, 34). In regard to prostate cancerous tumors it was shown that overexpression of CXCR4 in the tumors is associated with increased tumor growth, angiogenesis, and metastasis (31). Schioppa et al. (35) have shown that CXCR4 is induced by hypoxic conditions via the activation and transcript stabilization of the transcriptional activator hypoxia-inducible factor (HIF) 1. This report has further suggested that hypoxia may increase CXCR4

![FIGURE 3.](image-url)
expression and thus promote the metastatic potential of cancer cells (35). Indeed, it was recently shown that HIF-1 regulates CXCR4 expression in NSCLC cells and may thus increase their metastatic potential (36, 37). Further support for this idea is provided by findings indicating that, in NSCLC tissue, HIF-1 is mainly expressed by squamous cell carcinoma at necrotic areas (38, 39) and that HIF-1 expression is associated with a worse prognosis (40, 41). Additional reports have also indicated that CXCR4 is expressed on NSCLC cells (18–20, 37). It was further shown that NSCLC cell lines express CXCR4 but not CXCL12 (18). By using NSCLC cell lines and anti-CXCR4 Abs it was demonstrated that CXCR4/CXCL12 interactions facilitate metastasis of NSCLC cell lines to the bone marrow (18). Oonakahara et al. (20) have shown that mesothelial cell produce high levels of CXCL12 and suggested that the dissemination of NSCLC cells into the pleural cavity is mediated by CXCR4. Taken together, these data may suggest that hypoxia-induced CXCR4 expression by NSCLC tumor cells may promote their metastatic potential and dissemination to the bone marrow and pleural cavity.

Interestingly HIF-1 was also shown to up-regulate the expression of CXCL12 in a manner similar to that of CXCR4 and to promote the recruitment of progenitor cells to hypoxic areas (42). Using immunohistochemical staining and ELISA, we found that the majority of NSCLC tumors in vivo expresses CXCL12. However, in our study the expression of CXCL12 was not restricted to necrotic areas; nonetheless, CXCL12 was also detected at necrotic areas of some tumors. Furthermore, high expression of CXCL12 in the tumors was found in 33% of the patients with recurrence but was not found in patients without recurrence. It is therefore possible that, similar to the correlation of HIF expression with NSCLC disease outcome, HIF may be involved in up-regulation of CXCL12 and thus support tumor progression.

The expression of CXCL12 by the majority of NSCLC tumors indicates that CXCR4-mediated metastasis of NSCLC tumor cells into the pleural cavity cannot be simply attributed to the migration of tumor cells into CXCL12-expressing tissues and that CXCR4 and CXCL12 may promote tumor growth and invasiveness (31). The RET/PTC1 oncogene is expressed in up to 30% of papillary thyroid carcinoma (PTC) tumors (43). It was recently reported that the RET/PTC1 oncogene induces the up-regulation of several inflammatory genes in primary human thyrocytes, and among these genes were both CXCR4 and CXCL12 (43, 44). The authors suggested that CXCL12 may act in an autocrine fashion on PTC cells to induce their proliferation and survival (43). Similarly it was shown that CXCR4/CXCL12 interactions promote breast carcinoma tumor growth by inducing tumor cell proliferation and angiogenesis (45). It is possible that CXCL12/CXCR4 interaction within the NSCLC microenvironment delivers antiapoptotic/proliferative signals to tumor cells and promotes disease progression. Moreover, CXCL12 in combination with additional proangiogenic factors produced at the tumor microenvironment may favor angiogenesis and increase tumor vascularity.

In addition to its role in stimulating tumor growth and angiogenesis, CXCL12 may also participate in the recruitment and retention of immune cells in the tumor (2, 16, 17). This possibility may be supported by the fact that CXCL12 expression in NSCLC cells is significantly higher than in the serum. We have found that CD4+ T cells specifically accumulate within lung adenocarcinomas, whereas effector NK cells are depleted from the tumor microenvironment and CD8+ T cells are not enriched. Tumor-resident CD4+ T cells were shown to express high levels of CXCR4 relative to NK and CD8 cells and to migrate better than these subtypes of cells in response to CXCL12. Lung adenocarcinoma tissue homogenates had high levels of CXCL12 relative to the peripheral blood. Furthermore, high CXCL12 expression in lung adenocarcinomas is associated with increased tumor inflammation. The accumulation of CD4+ T cells expressing high CXCR4 levels was shown in RA and in fibrotic liver where the levels of CXCL12 expression are high (24, 29). Furthermore, overexpression of CXCR4 in CD4+ T cells induced their accumulation in tissues that express high levels of CXCL12 such as the bone marrow (46). We therefore suggest that CXCR4/CXCL12 interactions are involved in increasing...
the recruitment/retention of CD4⁺ T cells in lung adenocarcinomas. Nevertheless, it should be noted that NSCLC tumors were shown to express a wide array of chemokines such as CCL2, CCL4, CCL5, CXCL1, CXCL3, CXCL5, CXCL8, and CXCL10 and that these chemokines may also influence the recruitment/retention of distinct subpopulations of immune cells to the tumor (9, 10, 47).

The roles of CD4⁺ T cells in adenocarcinoma pathogenesis are not clear. We have found that tumor resident CD4⁺ T cells express high levels of the early activation marker CD69 relative to normal lung tissue resident cells. Nevertheless, other activation markers such as CD40L were not detected (data not shown). CD69 was recently characterized as an immune-modulating molecule that is up-regulated following cell activation and mediates the secretion of TGF-β. CD69-deficient mice showed reduced autoimmune reactivity and increased anti-tumor activity (26, 27). It is therefore possible that the presence of CD4⁺ T cells that express high levels of CD69 is linked to TGF-β secretion and mediates tumor evasion of the immune system. Indeed, immunostaining for TGF-β in NSCLC tissue has shown that this cytokine is mainly produced by tumor-infiltrating immune cells and is associated with a more aggressive pattern of disease (14). We found that CD4⁺CD25highFoxP3⁺ regulatory T cells are accumulating in adenocarcinoma tumors. Similar findings regarding the accumulation of CD4⁺CD25high T cells in NSCLC tissue were reported by Woo et al. (48, 49). CXCL12/CXCR4 interactions were shown to regulate regulatory T cells as well as T cells trafficking to the bone marrow and may also be involved in their recruitment into adenocarcinomas (15). Indeed, CD4⁺FoxP3⁺ regulatory T cells in the adenocarcinomas expressed CXCR4.

Further immune modulation in adenocarcinoma may be therefore mediated by the migration of CD4⁺CD25high regulatory T cell into the tumor and by the secretion of TGF-β. In addition to modulation of the immune response, TGF-β has been shown to up-regulate the expression of CXCR4 on naive T cells and to increase their migration in response to CXCL12 (50). We therefore suggest that a cycle of TGF-β secretion leading to CXCR4 up-regulation and the retention of additional CD3⁺CD4⁺CD69⁺ cells capable of releasing TGF-β in the tumor microenvironment may contribute to adenocarcinoma pathogenesis.

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Disclosures
The authors have no financial conflict of interest.

References


