Correlation between CXCR5 expression and prognosis in stage T1 non-small cell lung cancer

Yue Zhao
Chinese Academy of Medical Sciences Cancer Institute and Hospital
Ningning Ding
Chinese Academy of Medical Sciences Cancer Institute and Hospital
Ligong Yuan
Chinese Academy of Medical Sciences Cancer Institute and Hospital
Feng Li
Chinese Academy of Medical Sciences Cancer Institute and Hospital
Shuaibo Wang
Chinese Academy of Medical Sciences Cancer Institute and Hospital
Yousheng Mao (youshengmao@gmail.com)
Chinese Academy of Medical Sciences and Peking Union Medical College https://orcid.org/0000-0003-1120-241X

Research

Keywords: Non-small cell lung cancer, CXCR5, Prognosis, Pathologic subtype

Posted Date: April 20th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22636/v1

Abstract

Background: Lung cancer is the leading cause of cancer-related death. Even if early detection has been applied and proved to be effective, the survival outcomes are still poor. Thus, to find out novel prognostic factors is of great significance to identify high-risk patients and guide individualized precise treatment in order to reduce recurrence and improve survival outcomes.

Methods: Tissue samples and clinicopathologic data of 244 stage T1 NSCLC patients were collected. We investigated CXCR5 expression using immunochemical method and analyzed its correlation with pathologic subtypes and prognosis.

Results: Elevated level of positive CXCR5 expression was found in tumor tissues (p<0.0001) and patients with positive CXCR5 expression are more likely to have LVIs (p=0.030) and recurrences (p<0.043). Besides, CXCR5 expression is also correlated to histological type and differentiation. Lepidic predominant tumors have significantly lower expression of CXCR5 (p<0.001). Survival analyses showed that patients with positive CXCR5 had a significantly lower DFS (p=0.038) and patients with solid or micropapillary predominant NSCLC had a significantly worse prognosis. CXCR5 expression was proved to be an independent prognostic factor for DFS through multivariate analysis.

Conclusions: CXCR5 expression has been proved to be an independent prognostic factor for stage T1 NSCLC patients. In addition, CXCR5 was also found to be relevant to cancer recurrence, and this indicated that CXCR5 may play an important role in lymph node and distant metastasis.

1. Background

Lung cancer is now the most prevalent malignant tumor and has become the leading cause of cancer-related death both in China and worldwide.[1, 2] Histologically, lung cancer is classified into non-small cell lung cancer (NSCLC), which accounts for approximately 85%, and small cell lung cancer (SCLC) accounts for the remaining 15%.[3] Despite advances in screening, diagnosis and multidisciplinary treatments made in recent years, the survival outcomes of lung cancer patients are still not satisfactory. Since early symptoms are not specific and conspicuous, most patients are diagnosed at advanced stages with poor prognoses. Fortunately, early detection with low-dose computed tomography (LDCT) has been reported to be effective and reduce lung cancer mortality up to 20% among high risk individuals.[4, 5] However, the 5-year survival of all stages is only 18%. [6]Thus, apart from LDCT screening, following precise treatments also play a pivotal role in improving survival of lung cancer patients. Previous studies demonstrated that recurrence or metastasis occurs in about 20% of early stage NSCLC patients even though surgeries had been done, which indicated that other clinicopathological factors that would impact the prognosis of early NSCLC patients did exist other than TNM stage. Thus, to find out these factors is of great significance to identify high-risk patients and guide individualized precise treatment in order to reduce recurrence and improve survival outcomes.

Histologically speaking, NSCLC were classified into adenocarcinoma (AC), squamous cell carcinoma (SCC), large-cell carcinoma and etc. Among them, Adenocarcinoma is the most common histologic type. To address advances in oncology, molecular biology, pathology, radiology, and surgery of lung adenocarcinoma, an international multidisciplinary classification was sponsored by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society (IASLC/ATS/ERS) in 2011.[7] This new classification has been shown to provide prognoses-relevant information for patients with lung adenocarcinoma after curative surgeries[8, 9] and it has been incorporated into the 2015 World Health Organization classification of lung tumors for its reliability and practicability.[10]

Chemokines are a superfamily of small (8-10kDa) proteins which can cause the directed migration of certain subsets of leukocytes and are induced by inflammatory cytokines, growth factors and pathogenic stimuli.[11-13] Chemokines are the major regulators of cell trafficking and adhesion in the body.[14] Based on the configuration of the first two conserved cysteine residues adjacent to the N-terminal, chemokines are classified into four subfamilies (C, CC, CXC and CXXXC families).[11] Chemokine receptors are a superfamily of seven transmembrane spanning proteins coupled to G-protein-coupled receptors (GPCRs). Most of these receptors bind to more than one type of chemokine. Their binding leads to conformational changes, following activation of different signaling pathways which mediate different biological processes and finally results in tumor angiogenesis, growth, epithelial mesenchymal transition (EMT), and further metastasis.[15-17]

Many kinds of chemokine ligands and receptors have been reported to be overexpressed in tumors,[18-27] and CXCR5 together with its ligand, CXCL13 composes such a receptor/ligand pair axis. Emerging evidence revealed that elevated levels of CXCR5 activate downstream signals and contribute to tumorigenesis, cell growth, survival, and site-specific metastasis.[28-33] Previous study demonstrated that CXCR5 is overexpressed in NSCLC tissues and the interaction between CXCR5 and its ligand promote migration of NSCLC cells, which indicates that CXCR5 may play a role in the dissemination and/or metastasis of primary lung cancers.[34]

To further investigate the correlation between CXCR5 expression and prognosis in stage T1 NSCLCs, we collected clinical and pathological data of stage T1 NSCLC patients who underwent curative lobectomies with systematic lymph node dissection, detected expression of CXCR5 in tumor tissues and corresponding non-neoplastic tissues using immunohistochemical method and then analyzed its correlation with prognosis, in order to evaluate the potential of CXCR5 to be a prognostic biomarker or a therapeutic target. In addition, we analyzed the survival outcomes of stage T1 NSCLC patients based on their pathological subtypes, and validated their relationship with CXCR5 expression.

2. Methods

2.1 Patients

From January 2011 to January 2012, information of patients with stage T1 NSCLC who underwent curative lobectomy with systematic lymph node dissection at Department of Thoracic Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China was retrospectively collected. The inclusion criteria were as follows: ≤ 75 years old; no history of other malignancies; no history of severe cardiovascular or pulmonary diseases. Finally, 244 patients were enrolled in this study.

2.2 Tumor Specimens

Tumor and its surrounding tissues were fixed in 10% (v/v) formalin right after resection. After embedded in paraffin, the specimens were prepared in serial sections of 5 μm and reserved for hematoxylin-eosin (HE) staining. Then the sections would be examined by professional pathologists to determine the pathological type, subtype (if AC) and the existence of lymphovascular invasion (LVI) of the tumor.

2.3 Immunohistochemistry

We performed immunohistochemistry (IHC) for CXCR5 on 244 NSCLC samples and their corresponding non-neoplastic samples. The slides were de-paraffinized in xylene (I, II and III) for 10 min each, then rehydrated through ethanol of different gradient (100%, 85% and 75%) for 10 min each and washed in deionized water and phosphate-buffered saline (PBS). Antigen retrieval was implemented by incubating with 0.01 M sodium citrate buffer (pH=6.0) in boiling water for 20 min and then cool down to room temperature and the activity of endogenous peroxidase was blocked by incubating with 3% hydrogen peroxide (H_2O_2) for 10 min. PBS washing was applied after each step for 4 times. After incubation in a non-specific stain blocking agent, slides were incubated for 2 hours at 37°C with primary antibody: anti-human CXCR5 antibody (GR297692-6; Abcam, Cambridge, UK; 1:100). This was followed by PV-9000 Polymer Detection System (ZhongShanJinQiao, Beijing, China) which contains PV 9000 Kit Polymer Helper and PV 9000 Kit polyperoxidase-anti-mouse/rabbit IgG, incubating for 20 min at 37℃ and then washed by PBS for 3 times. After incubation, slides were stained with a 3, 3'-diaminobenzidine (DAB) (ZhongShanJinQiao, Beijing, China) as a chromogen. Counterstaining was implemented with hematoxylin (ZhongShanJinQiao, Beijing, China). Subsequently, slides were washed with water and ammonia, dehydrated in 75%, 85%, and absolute alcohol for 5 min each, and sealed with resin for following evaluation.

To analyze the immunohistochemical staining of CXCR5, slides were evaluated by 2 professional pathologists who were blinded to each other. The intensity of staining was evaluated and graded from 0 to 3, where 0 for no staining (negative), 1 for light yellow (weak), 2 for yellow (moderate) and 3 for brown (strong). The numbers of positively stained cells were scored as follows: 0, ≤25%; 1, 25%-50%; 2, 51%-100%. The two values obtained were multiplied to calculate a final score (maximum value, 6). Samples were classified into negative (score ≤ 2) or positive (score ≥ 2) for further analysis.

2.4 Statistical Analyses

The clinicopathologic characteristics were compared between the groups using Student's t tests for continuous variables with normal distributions, Mann-Whitney U tests for continuous variables with

abnormal distributions, and chi-square tests for categorical variables. OS was defined as the period between the date of surgery and death due to any cause or the last follow-up. DFS was defined as the period after successful treatment during which there were no signs or symptoms of the disease that was treated. The cumulative OS and DFS rates were estimated by the Kaplan-Meier method, and the differences were compared between groups by the log-rank test. To identify prognostic factors, univariate and multivariate analyses were performed using the Cox proportional hazards regression model. Baseline variables that were considered clinically relevant or that showed a univariate relationship with the outcome were entered into multivariate Cox proportional hazards regression model. Variables for inclusion were carefully chosen, given the number of events available, to ensure parsimony of the final model.

All statistical analyses were performed using IBM SPSS software version 24.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). The results were considered statistically significant when *p* value was less than 0.05.

3. Results

3.1 Patient clinicopathological characteristics

A total of 244 qualified patients who were diagnosed with stage T1 NSCLC were enrolled in this study, and the clinicopathological characteristics are shown in *Table 1*. There were 138 male patients and 106 female patients, with a median age of 59 years old. 189 patients were diagnosed as AC, 50 patients as SCC and 5 patients as other type of lung cancer. The median tumor size is 2.0 cm. 181 patients were free of lymph node metastasis, 28 patients with N1 metastasis and 35 patients with N2 metastasis. 37 patients were with a tumor of well differentiation, 131 with moderate differentiation and 76 with poor differentiation. 96 patients were diagnosed with LVI during pathological examination.

3.2 Expression of CXCR5 in tumor and non-neoplastic tissues

Figure 1 showed the expression of CXCR5 in tumor and non-neoplastic tissues. Immunohistochemical analyses revealed that CXCR5 mainly expresses on the membrane of tumor cells. Positive CXCR5 expression was found in 155 (63.5%) tumor tissues and 15 (6.1%) non-neoplastic tissues. As it showed in *Figure 2*, the positive rate of CXCR5 expression in tumor tissues is significantly higher than that in non-neoplastic tissues ($p<0.0001$).

3.3 Correlation between CXCR5 expression and clinicopathological characteristics

We then evaluated the correlation between CXCR5 expression and clinicopathological characteristics and the results were listed in *Table 2*. Patients were classified into two groups depending on the CXCR5 expression in tumor tissues. From the table, we can see that CXCR5 expression was positive in 155 patients and negative in 89 patients. Sex, age, tumor size, tumor location and N stage were similar between the groups, while histological type ($p<0.001$), tumor differentiation ($p<0.001$) and LVI ($p=0.030$) differs significantly. In addition, recurrence was significantly higher in CXCR5 positive group ($p=0.043$), while survival was similar between the groups. Further analyses were conducted and showed in *Figure 2*. We

found that positive rate of CXCR5 is significantly higher in patients with LVI than those without LVI (71.9% vs. 58.1%, $p<0.05$) and in AC than SCC (69.8% vs. 40.0%, $p<0.001$). Moreover, positive CXCR5 expression was significantly higher in moderate and poor differentiated tumors when comparing to well differentiated ones (Moderate vs. Well = 71.0% vs. 32.4%, $p<0.0001$, Poor vs. Well = 65.8% vs. 32.4%, $p<0.01$). Nonetheless, no significant differences were found in the positive rate of CXCR5 in tumor location and N stage.

3.4 Correlation between CXCR5 expression and pathological subtypes of lung adenocarcinoma

CXCR5 expression and other variables were compared among different pathological subtypes (*Table 3*). It revealed in the table that CXCR5 expression ($p<0.001$) was significantly different among the groups, besides, the differences in N stage ($p<0.001$), recurrence ($p<0.001$) and survival ($p<0.001$) were also significant. In subgroup analyses (*Figure* 3), difference in CXCR5 expression ($p=0.0001$) was found to be between lepidic predominant (LP) group and the other four groups, and differences in N stage ($p<0.0001$), recurrence ($p<0.0001$) and survival ($p<0.0001$) were between solid predominant (SP), micropapillary predominant (MPP) and the other three groups.

3.5 Survival analyses based on CXCR5 expression

Survival outcomes based CXCR5 expression was compared between CXCR5 positive group and CXCR5 negative group using Kaplan-Meier method (*Figure 4*). Patients in CXCR5 positive group tended to have a lower 5-year OS than them in CXCR5 negative group, but the difference was not significant (CXCR5 positive vs. CXCR5 negative, 78.7% vs. 86.5%, $p=0.220$). On the contrary, the difference in 5-year DFS was significant between the groups (CXCR5 positive vs. CXCR5 negative, 65.2% vs. 79.8%, $p=0.038$).

3.6 Survival analyses based on pathological subtypes of lung adenocarcinoma

Survival analyses were completed based on pathological subtypes of lung adenocarcinoma (*Figure 5*). The 5-year OS and DFS were both significantly different among the groups (OS: $p<0.0001$, DFS: $p<0.0001$). Further analyses showed the details between each group (*Table 4*). Significant differences were found between SP group and LP group (DFS: $p<0.001$, OS: $p=0.005$), acinar predominant (AP) group (DFS: $p<0.001$, OS: $p=0.001$) and papillary predominant (PP) group (DFS: $p<0.001$, OS: $p=0.003$) in DFS and OS. Similar results were found between MPP group and these three groups (LP: DFS: $p<0.001$, OS: $p=0.001$; AP: DFS: $p<0.001$, OS: $p<0.001$; PP: DFS: $p<0.001$, OS: $p<0.001$). While DFS and OS between SP group and MPP group were similar to each other (DFS: $p=0.567$, OS: $p=0.434$). Besides, DFS between LP group and AP group was also significantly different ($p=0.048$).

3.7 Prognostic factors for survival of 244 NSCLC patients

Univariate and multivariate analyses were conducted using the Cox proportional hazards regression model (*Table 5*). The results showed that CXCR5 expression (HR: 2.428, 95%CI: 1.157-5.095, $p=0.019$) as well as N stage (HR: 2.457, 95%CI: 1.717-3.516, $p<0.001$), tumor differentiation (HR: 3.216, 95%CI: 1.671-6.190, $p<0.001$), and LVI (HR: 8.791, 95%CI: 3.680-21.001, $p<0.001$) are the independent prognostic factors for

DFS. While N stage (HR: 1.627, 95%CI: 1.074-2.463, p=0.022) and LVI (HR: 6.366, 95%CI: 2.129-19.028, p=0.001) are the independent prognostic factors for OS.

4. Discussion

Lung cancer is now the leading cause of cancer-related death[1] and even for early stage patients, recurrence occurs in about 20% of them after surgery. Therefore, it is significant to find out the prognostic factors to guide individualized precise treatment to improve the survival outcomes. To our knowledge, this is the first study that analyzes the correlation with CXCR5 and prognosis.

Interactions between chemokines and their receptors have been reported to correlate to tumor dissemination, metastasis, tumor growth and cell survival.[35-37] Previous study showed that CXCR5 correlates with the stage/grade of NSCLC and promotes migration.[34] There were also studies revealed the significance of other chemokine/chemokine receptor expression in NSCLCs, and demonstrated that CXCL12/CXCR4,[38-40] CXCL1/CXCR2,[41] CCL2/CCR2,[42-45] CCL19/21/CCR7,[46] CCL25/CCR9,[47] CCL4/CCR5[48] and other atypical chemokine receptors[49, 50] were associate with tumor progression and prognosis.

CXCR5 was first isolated for Burkitt Lymphoma and named as Burkitt's lymphoma receptor 1 (BLR1),[51] and it is highly expressed on mature recirculating B-lymphocytes, a subpopulation of follicular helper T cells (T_{FH}) and skin-derived migratory dendritic cells (DCs), and controls their migration into secondary lymphoid organs towards the gradient of its ligand, CXCL13.[52-54] CXCL13/CXCR5 axis was involved in the progression of many hematological and solid malignancies like B-cell chronic lymphocytic leukemia,[55] different types of lymphoma,[56-60] prostate cancer,[29, 33] breast cancer,[61-63] oral squamous cell carcinoma[64, 65] and etc. However, the role of CXCR5 in lung cancer is still unclear.

This study examined the level of CXCR5 expression in tissues using immunohistochemical methods, analyzed the correlation among CXCR5 expression, pathological subtypes, patients' prognosis and other clinicopathological characteristics and investigated the prognostic factors in NSCLC patients. In this study, we demonstrated that CXCR5 expression is significantly higher in tumor tissues. Patients with positive CXCR5 expression are more likely to have LVIs and recurrences. We also found that patients with ACs had a significantly higher level of CXCR5 expression than those with SCCs (Patients with other types of NSCLC were not included in the calculation because of the small sample size) and the positive rate of CXCR5 expression in patients with moderate or poor differentiation was significantly higher than patients with well differentiation, which indicated that CXCR5 expression is correlated to histological type and differentiation of NSCLC. As for pathological subtypes, we found that CXCR5 expression, N stage, recurrence and survival all differed significantly among pathological subtypes. Further analyses showed that positive CXCR5 expression in LP tumors was significantly lower than the other four groups, which may be part of the reason for the pretty good survival outcomes. In addition, SP and MPP group were found to have a more advanced N stage, higher recurrence and worse survival, and these results are consistent with previous studies.[8, 9, 66]

Survival analyses showed that patients with positive CXCR5 expression had a significantly lower DFS while OS was similar, which supported our results that CXCR5 expression is related to cancer recurrence. These results indicate that CXCR5 may be involved in recurrence and metastasis of NSCLC but it may not impact survival. Survival analyses based on pathological subtypes demonstrated that DFS and OS both differed significantly among the groups and intergroup analyses showed that patients with SP and MPP had a significantly lower DFS and OS than those with LP AP and PP. In other words, LP AP and PP are the “good subtypes” while SP and MPP are the “bad subtypes” regarding survival outcomes.

Multivariate analyses showed that CXCR5 expression as well as N stage, tumor differentiation, and LVI are the independent prognostic factors for DFS. While N stage and LVI are the independent prognostic factors for OS.

Aberrantly activation of CXCL13/CXCR5 signaling has been proved to correlate with the development and progression of several human cancers. Wang et al show that benzo(a)pyrene (BaP), an important carcinogen, induces lung carcinogenesis. BaP interacts with aryl hydrocarbon receptor (AhR) and targets CXCL13, and then CXCL13/CXCR5 binding would induce the production of secreted phosphoprotein 1 (SPP1) by tumor-associated macrophages (TAMs). SPP1 may organize a positive feedback loop network via activation and nuclear localization of β-catenin in epithelial and cancer cells, thus promoting EMT and lung cancer progression.[67] Divergent signaling cascades such as extracellular regulated protein kinases (ERK), phosphatidylinositol 3-kinase (PI3K)/Akt, stress-activated protein kinase (SAPK)/c-jun kinase (JNK), and protein kinase C epsilon (PKCε)/nuclear factor-kappa B (NF-kB) were also proved to be involved in CXCL13/CXCR5 associated tumor progressions. Whether these signaling pathways are involved in the tumorigenesis and progression of lung cancer remains to be validated.

In following researches, we would validate the expression of CXCL13, ligand of CXCR5, in tumor and metastatic tissue and peripheral blood samples, investigate how CXCL13/CXCR5 impact cell function and the exact signaling pathway behind.

5. Conclusions

In conclusion, the present study demonstrated that positive rate of CXCR5 is significantly higher in tumor tissues and positive CXCR5 expression was an independent prognostic factor for stage T1 NSCLC patients. CXCR5 was also proved to be relevant to cancer recurrence, and this indicated that CXCR5 may play an important role in lymph node and distant metastasis.

Abbreviations

NSCLC: Non-small cell lung cancer, *SCLC*: Small cell lung cancer, *AC*: Adenocarcinoma, *SCC*: Squamous cell carcinoma, *RUL*: Right upper lobe, *RML*: Right middle lobe, *RLL*: Right lower lobe, *LUL*: Left upper lobe, *LLL*: Left lower lobe, *LVI*: lymphovascular invasion, *LP*: Lepidic predominant, *AP*: Acinar predominant, *PP*: Papillary predominant, *SP*: Solid predominant, *MPP*: Micropapillary predominant, *DFS*: Disease-free survival, *OS*: Overall survival.

Declarations

Declarations

The authors declare no conflicts of interest.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Cancer Hospital, Chinese Academy of Medical Sciences (NCC2014ST-07).

Consent for publication

All patients' consent forms were signed and collected.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Beijing Municipal Science & Technology Commission (Capital specialty clinical research project, Grant number: Z141107002514047).

Authors' contributions

Zhao Yue analyzed and interpreted the data, drafted and modified this manuscript, Ding Ningning designed this study, did the immunological experiments, collected and analyzed the data, Yuan Ligong, Li Feng and Wang Shuaibo helped in data collection, Mao Yousheng designed the study, provided funds and modified the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Bray, F., et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 2018. 68(6):394-424.
2. Kexin Sun, et al., Report of Cancer Incidence and Mortality in Different Areas of China,2015. *China Cancer*, 2019(28):1-11.
3. Herbst, R.S., J.V. Heymach, and S.M. Lippman, Lung cancer. *N Engl J Med*, 2008. 359(13):1367-80.

4. National Lung Screening Trial Research, T., et al., Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med*, 2011. 365(5):395-409.
5. Marcus, P.M., et al., Did death certificates and a death review process agree on lung cancer cause of death in the National Lung Screening Trial? *Clin Trials*, 2016. 13(4):434-8.
6. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2018. *CA Cancer J Clin*, 2018. 68(1):7-30.
7. Travis, W.D., et al., International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol*, 2011. 6(2):244-85.
8. Hung, J.J., et al., Predictive value of the international association for the study of lung cancer/American Thoracic Society/European Respiratory Society classification of lung adenocarcinoma in tumor recurrence and patient survival. *J Clin Oncol*, 2014. 32(22):2357-64.
9. Hung, J.J., et al., Prognostic value of the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society lung adenocarcinoma classification on death and recurrence in completely resected stage I lung adenocarcinoma. *Ann Surg*, 2013. 258(6):1079-86.
10. Travis, W.D., et al., The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. *J Thorac Oncol*, 2015. 10(9):1243-1260.
11. Rossi, D. and A. Zlotnik, The biology of chemokines and their receptors. *Annu Rev Immunol*, 2000. 18:217-42.
12. Zlotnik, A. and O. Yoshie, Chemokines: a new classification system and their role in immunity. *Immunity*, 2000. 12(2):121-7.
13. Murphy, P.M., et al., International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev*, 2000. 52(1):145-76.
14. Schier, A.F., Chemokine signaling: rules of attraction. *Curr Biol*, 2003. 13(5):R192-4.
15. Mantovani, A., et al., The chemokine system in cancer biology and therapy. *Cytokine Growth Factor Rev*, 2010. 21(1):27-39.
16. Richmond, A., Nf-kappa B, chemokine gene transcription and tumour growth. *Nat Rev Immunol*, 2002. 2(9):664-74.
17. Sparmann, A. and D. Bar-Sagi, Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell*, 2004. 6(5):447-58.
18. Cheng, Z.H., et al., Chemokines and their receptors in lung cancer progression and metastasis. *J Zhejiang Univ Sci B*, 2016. 17(5):342-51.
19. Itatani, Y., et al., The Role of Chemokines in Promoting Colorectal Cancer Invasion/Metastasis. *Int J Mol Sci*, 2016. 17(5).
20. King, J., H. Mir, and S. Singh, Association of Cytokines and Chemokines in Pathogenesis of Breast Cancer. *Prog Mol Biol Transl Sci*, 2017. 151:113-136.

21. Lee, H.J., et al., CXC chemokines and chemokine receptors in gastric cancer: from basic findings towards therapeutic targeting. *World J Gastroenterol*, 2014. 20(7):1681-93.
22. Lukaszewicz-Zajac, M., M. Gryko, and B. Mroczko, The role of selected chemokines and their specific receptors in pancreatic cancer. *Int J Biol Markers*, 2018. 33(2):141-147.
23. Lukaszewicz-Zajac, M., B. Mroczko, and M. Szmitkowski, Chemokines and their receptors in esophageal cancer--the systematic review and future perspectives. *Tumour Biol*, 2015. 36(8):5707-14.
24. Rivas-Fuentes, S., et al., Role of Chemokines in Non-Small Cell Lung Cancer: Angiogenesis and Inflammation. *J Cancer*, 2015. 6(10):938-52.
25. Rotondi, M., et al., Role of Chemokines in Thyroid Cancer Microenvironment: Is CXCL8 the Main Player? *Front Endocrinol (Lausanne)*, 2018. 9:314.
26. Vinader, V. and K. Afarinkia, The emerging role of CXC chemokines and their receptors in cancer. *Future Med Chem*, 2012. 4(7):853-67.
27. Zhou, Y.Q., et al., Chemokines and Their Receptors: Potential Therapeutic Targets for Bone Cancer Pain. *Curr Pharm Des*, 2015. 21(34):5029-33.
28. Airoldi, I., et al., CXCR5 may be involved in the attraction of human metastatic neuroblastoma cells to the bone marrow. *Cancer Immunol Immunother*, 2008. 57(4):541-8.
29. Ammirante, M., et al., Tissue injury and hypoxia promote malignant progression of prostate cancer by inducing CXCL13 expression in tumor myofibroblasts. *Proc Natl Acad Sci U S A*, 2014. 111(41):14776-81.
30. Biswas, S., et al., CXCL13-CXCR5 co-expression regulates epithelial to mesenchymal transition of breast cancer cells during lymph node metastasis. *Breast Cancer Res Treat*, 2014. 143(2):265-76.
31. El Haibi, C.P., et al., PI3Kp110-, Src-, FAK-dependent and DOCK2-independent migration and invasion of CXCL13-stimulated prostate cancer cells. *Mol Cancer*, 2010. 9:85.
32. Sambandam, Y., et al., CXCL13 activation of c-Myc induces RANK ligand expression in stromal/preosteoblast cells in the oral squamous cell carcinoma tumor-bone microenvironment. *Oncogene*, 2013. 32(1):97-105.
33. Singh, S., et al., Serum CXCL13 positively correlates with prostatic disease, prostate-specific antigen and mediates prostate cancer cell invasion, integrin clustering and cell adhesion. *Cancer Lett*, 2009. 283(1):29-35.
34. Singh, R., et al., Expression and clinical significance of CXCR5/CXCL13 in human nonsmall cell lung carcinoma. *Int J Oncol*, 2014. 45(6):2232-40.
35. Iwakiri, S., et al., Higher expression of chemokine receptor CXCR7 is linked to early and metastatic recurrence in pathological stage I nonsmall cell lung cancer. *Cancer*, 2009. 115(11):2580-93.
36. Rollins, B.J., Chemokines. *Blood*, 1997. 90(3):909-28.
37. Taub, D.D., Chemokine-leukocyte interactions. The voodoo that they do so well. *Cytokine Growth Factor Rev*, 1996. 7(4):355-76.
38. Phillips, R.J., et al., The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. *Am J Respir Crit Care Med*, 2003. 167(12):1676-86.

39. Wald, O., et al., Interaction between neoplastic cells and cancer-associated fibroblasts through the CXCL12/CXCR4 axis: role in non-small cell lung cancer tumor proliferation. *J Thorac Cardiovasc Surg*, 2011. 141(6):1503-12.
40. Wang, L., et al., High-level C-X-C chemokine receptor type 4 expression correlates with brain-specific metastasis following complete resection of non-small cell lung cancer. *Oncol Lett*, 2014. 7(6):1871-1876.
41. Lo, H.M., et al., Vascular endothelial growth factor induces CXCL1 chemokine release via JNK and PI-3K-dependent pathways in human lung carcinoma epithelial cells. *Int J Mol Sci*, 2013. 14(5):10090-106.
42. Maxwell, P.J., et al., HIF-1 and NF-kappaB-mediated upregulation of CXCR1 and CXCR2 expression promotes cell survival in hypoxic prostate cancer cells. *Oncogene*, 2007. 26(52):7333-45.
43. Yanagawa, J., et al., Snail promotes CXCR2 ligand-dependent tumor progression in non-small cell lung carcinoma. *Clin Cancer Res*, 2009. 15(22):6820-9.
44. Kudo-Saito, C., et al., CCL2 is critical for immunosuppression to promote cancer metastasis. *Clin Exp Metastasis*, 2013. 30(4):393-405.
45. Li, X. and H.H. Tai, Activation of thromboxane A2 receptor (TP) increases the expression of monocyte chemoattractant protein -1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2) and recruits macrophages to promote invasion of lung cancer cells. *PLoS One*, 2013. 8(1):e54073.
46. Zhang, Q., et al., CCL19/CCR7 upregulates heparanase via specificity protein-1 (Sp1) to promote invasion of cell in lung cancer. *Tumour Biol*, 2013. 34(5):2703-8.
47. Gupta, P., et al., CCR9/CCL25 expression in non-small cell lung cancer correlates with aggressive disease and mediates key steps of metastasis. *Oncotarget*, 2014. 5(20):10170-9.
48. Mango, R.L., et al., C-C chemokine receptor 5 on pulmonary mesenchymal cells promotes experimental metastasis via the induction of erythroid differentiation regulator 1. *Mol Cancer Res*, 2014. 12(2):274-82.
49. Addison, C.L., et al., Overexpression of the duffy antigen receptor for chemokines (DARC) by NSCLC tumor cells results in increased tumor necrosis. *BMC Cancer*, 2004. 4:28.
50. Wu, F.Y., et al., Atypical chemokine receptor D6 inhibits human non-small cell lung cancer growth by sequestration of chemokines. *Oncol Lett*, 2013. 6(1):91-95.
51. Forster, R., et al., A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell*, 1996. 87(6):1037-47.
52. Breitfeld, D., et al., Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med*, 2000. 192(11):1545-52.
53. Ohmatsu, H., et al., CXCL13 and CCL21 are expressed in ectopic lymphoid follicles in cutaneous lymphoproliferative disorders. *J Invest Dermatol*, 2007. 127(10):2466-8.
54. Schaerli, P., et al., CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med*, 2000. 192(11):1553-62.
55. Davids, M.S. and J.A. Burger, Cell Trafficking in Chronic Lymphocytic Leukemia. *Open J Hematol*, 2012. 3(S1).

56. Cha, Z., et al., Circulating CXCR5+CD4+ T cells assist in the survival and growth of primary diffuse large B cell lymphoma cells through interleukin 10 pathway. *Exp Cell Res*, 2017. 350(1):154-160.
57. Husson, H., et al., CXCL13 (BCA-1) is produced by follicular lymphoma cells: role in the accumulation of malignant B cells. *Br J Haematol*, 2002. 119(2):492-5.
58. Tang, X.F., et al., [Expressions of CXCL13, CD10 and bcl-6 in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, not otherwise specified]. *Zhonghua Bing Li Xue Za Zhi*, 2009. 38(4):224-30.
59. Ohtani, H., et al., Follicular Dendritic Cell Meshwork in Angioimmunoblastic T-Cell Lymphoma Is Characterized by Accumulation of CXCL13(+) Cells. *J Clin Exp Hematop*, 2015. 55(2):61-9.
60. Yu, H., A. Shahsafaei, and D.M. Dorfman, Germinal-center T-helper-cell markers PD-1 and CXCL13 are both expressed by neoplastic cells in angioimmunoblastic T-cell lymphoma. *Am J Clin Pathol*, 2009. 131(1):33-41.
61. Panse, J., et al., Chemokine CXCL13 is overexpressed in the tumour tissue and in the peripheral blood of breast cancer patients. *Br J Cancer*, 2008. 99(6):930-8.
62. Razis, E., et al., Improved outcome of high-risk early HER2 positive breast cancer with high CXCL13-CXCR5 messenger RNA expression. *Clin Breast Cancer*, 2012. 12(3):183-93.
63. Razmkhah, M., et al., Chemokine and chemokine receptors: a comparative study between metastatic and nonmetastatic lymph nodes in breast cancer patients. *Eur Cytokine Netw*, 2012. 23(3):72-7.
64. Yuvaraj, S., et al., A novel function of CXCL13 to stimulate RANK ligand expression in oral squamous cell carcinoma cells. *Mol Cancer Res*, 2009. 7(8):1399-407.
65. Ziober, A.F., et al., Identification of a gene signature for rapid screening of oral squamous cell carcinoma. *Clin Cancer Res*, 2006. 12(20 Pt 1):5960-71.
66. Cha, M.J., et al., Micropapillary and solid subtypes of invasive lung adenocarcinoma: clinical predictors of histopathology and outcome. *J Thorac Cardiovasc Surg*, 2014. 147(3):921-928 e2.
67. Wang, G.Z., et al., The chemokine CXCL13 in lung cancers associated with environmental polycyclic aromatic hydrocarbons pollution. *Elife*, 2015. 4.

Tables

Table 1. Clinicopathological characteristics of 244 NSCLC patients.

Variable	All Patients (n=244)
Sex, n (%)	
Male	138 (56.6%)
Female	106 (43.4%)
Median Age (range),yr	59 (37-74)
Median Tumor Size (range),cm	2.0 (1.1-3.0)
Tumor Location, n (%)	
RUL	81 (33.2%)
RML	11 (4.5%)
RLL	42 (17.3%)
LUL	73 (29.9%)
LLL	35 (14.3%)
Main Bronchus	2 (0.8%)
N Stage, n (%)	
N_0	181 (74.2%)
N_1	28 (11.5%)
N_2	35 (14.3%)
Histological Type, n (%)	
Adenocarcinoma	189 (77.5%)
Squamous cell carcinoma	50 (20.5%)
Others	5 (2.0%)
Pathological Subtype, n (%)*	
Lepidic Predominant	18 (9.5%)
Acinar Predominant	71 (37.5%)
Papillary Predominant	48 (25.4%)
Solid Predominant	25 (13.3%)
Micropapillary Predominant	27 (14.3%)
Tumor Differentiation, n (%)	
Well	37 (15.2%)

Moderate	131 (53.7%)
Poor	76 (31.1%)
Lymphovascular Invasion, n (%)	
Yes	96 (39.3%)
No	148 (60.7%)

*189 patients with adenocarcinoma were diagnosed with pathological subtypes.

RUL: Right upper lobe. *RML*: Right middle lobe. *RLL*: Right lower lobe. *LUL*: Left upper lobe. *LLL*: Left lower lobe.

Table 2. Patients' clinicopathological characteristics based on CXCR5 expression.

Variables	CXCR5		*p* Value
	Positive (n=155)	Negative (n=89)	
Sex, n (%)			0.129
Male	82 (52.9%)	56 (62.9%)	
Female	73 (47.1%)	33(37.1%)	
Median Age (range),yr	59 (37-74)	59 (39-74)	0.921
Median Tumor Size (range),cm	2.0 (1.1-3.0)	2.1 (1.2-3.0)	0.311
Tumor Location, n (%)			0.287
RUL	48 (31.2%)	33 (37.5%)	
RML	6 (3.9%)	5 (5.7%)	
RLL	29 (18.8%)	13 (14.8%)	
LUL	47 (30.5%)	26 (29.5%)	
LLL	24 (15.6%)	11 (12.5%)	
Main Bronchus	1 (0.6%)	1 (1.1%)	
N Stage, n (%)			0.277
N_0	110 (71.0%)	71 (79.8%)	
N_1	19 (12.2%)	9 (10.1%)	
N_2	26 (16.8%)	9 (10.1%)	
Histological Type, n (%)			<0.001
Adenocarcinoma	132 (85.2%)	57 (64.1%)	
Squamous cell carcinoma	20 (12.9%)	30 (33.7%)	
Others	3 (1.9%)	2 (2.2%)	
Tumor Differentiation, n (%)			<0.001
Well	12 (7.7%)	25 (28.1%)	
Moderate	93 (60.0%)	38 (42.7%)	
Poor	50 (32.3%)	26 (29.2%)	
Lymphovascular Invasion, n (%)			0.030
Yes	69 (44.5%)	27 (30.3%)	
No	86 (55.5%)	62 (69.7%)	

Recurrence, n (%)			0.043
Yes	54 (34.8%)	20 (22.5%)	
No	101 (65.2%)	69 (77.5%)	
Survival, n (%)			0.241
Yes	121 (78.1%)	75 (84.3%)	
No	34 (21.9%)	14 (15.7%)	

RUL: Right upper lobe. *RML*: Right middle lobe. *RLL*: Right lower lobe. *LUL*: Left upper lobe. *LLL*: Left lower lobe.

Table 3. Patients' clinicopathological characteristics based on pathological subtypes.

Variable	Lepidic Predominant	Acinar Predominant	Papillary Predominant	Solid Predominant	Micropapillary Predominant	*p* value
CXCR5 expression						<0.001
Negative	13 (72.2%)	19 (26.8%)	14 (29.2%)	5 (20.0%)	6 (22.2%)	
Positive	5 (27.8%)	52 (73.2%)	34 (70.8%)	20 (80.0%)	21 (77.8%)	
N Stage						<0.001
N_0	18 (100.0%)	59 (83.1%)	45 (93.8%)	11 (44.0%)	15 (55.6%)	
N_1	0 (0.0%)	8 (11.3%)	1 (2.1%)	5 (20.0%)	4 (14.8%)	
N_2	0 (0.0%)	4 (5.6%)	2 (4.2%)	9 (36.0%)	8 (11.3%)	
Recurrence						<0.001
Yes	0 (0.0%)	14 (19.7%)	6 (12.5%)	16 (64.0%)	18 (66.7%)	
No	18 (100.0%)	57 (80.3%)	42 (87.5%)	9 (36.0%)	9 (33.3%)	
Survival						<0.001
Yes	18 (100.0%)	64 (90.1%)	44 (91.7%)	16 (64.0%)	14 (51.9%)	
No	0 (0.0%)	7 (9.9%)	4 (8.3%)	9 (36.0%)	13 (48.1%)	

Table 4a. Subgroup analyses on DFS based on pathological subtypes of lung adenocarcinoma.

p value	Lepidic Predominant	Acinar Predominant	Papillary Predominant	Solid Predominant	Micropapillary Predominant
Lepidic Predominant	-	0.048	0.123	<0.001	<0.001
Acinar Predominant	0.048	-	0.310	<0.001	<0.001
Papillary Predominant	0.123	0.310	-	<0.001	<0.001
Solid Predominant	<0.001	<0.001	<0.001	-	0.567
Micropapillary Predominant	<0.001	<0.001	<0.001	0.567	-

Table 4b. Subgroup analyses on OS based on pathological subtypes of lung adenocarcinoma.

p value	Lepidic Predominant	Acinar Predominant	Papillary Predominant	Solid Predominant	Micropapillary Predominant
Lepidic Predominant	-	0.173	0.213	0.005	0.001
Acinar Predominant	0.173	-	0.804	0.001	<0.001
Papillary Predominant	0.213	0.804	-	0.003	<0.001
Solid Predominant	0.005	0.001	0.003	-	0.434
Micropapillary Predominant	0.001	<0.001	<0.001	0.434	-

Table 5a. Univariate analysis of the correlation between DFS, OS and patients' clinicopatholigical characteristics.

Variable	Univariate Analyses			
	DFS		OS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Sex	0.852 (0.536-1.355)	0.499	0.669 (0.370-1.209)	0.184
Age	1.001 (0.975-1.028)	0.922	1.010 (0.977-1.044)	0.549
Tumor Size	1.409 (0.916-2.167)	0.118	1.150 (0.675-1.957)	0.608
N Stage	2.638 (2.042-3.407)	<0.001	2.496 (1.826-3.411)	<0.001
CXCR5 expression	1.709 (1.023-2.856)	0.041	1.473 (0.791-2.745)	0.223
Histological Type	0.687 (0.419-1.127)	0.137	0.613 (0.336-1.118)	0.110
Tumor Differentiation	4.269 (2.794-6.522)	<0.001	4.155 (2.435-7.088)	<0.001
Lymphovascular Invasion	10.547 (5.781-19.241)	<0.001	7.089 (3.529-14.241)	<0.001
Tumor Location	0.879 (0.755-1.024)	0.099	0.913 (0.756-1.102)	0.342
Histological Subtype	2.069 (1.652-2.593)	<0.001	2.117 (1.583-2.830)	<0.001

DFS: Disease-free survival. *OS*: Overall survival.

Table 5b. Multivariate analysis of the correlation between DFS, OS and patients' clinicopatholigical characteristics.

Variable	Multivariate Analyses			
	DFS		OS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
N Stage	2.457 (1.717-3.516)	<0.001	1.627 (1.074-2.463)	0.022
CXCR5 expression	2.428 (1.157-5.095)	0.019	1.537 (0.628-3.761)	0.347
Tumor Differentiation	3.216 (1.671-6.190)	<0.001	2.097 (0.942-4.669)	0.070
Lymphovascular Invasion	8.791 (3.680-21.001)	<0.001	6.366 (2.129-19.028)	0.001
Histological Subtype	1.138 (0.869-1.490)	0.347	1.285 (0.907-1.820)	0.159

DFS: Disease-free survival. *OS*: Overall survival.

Figures

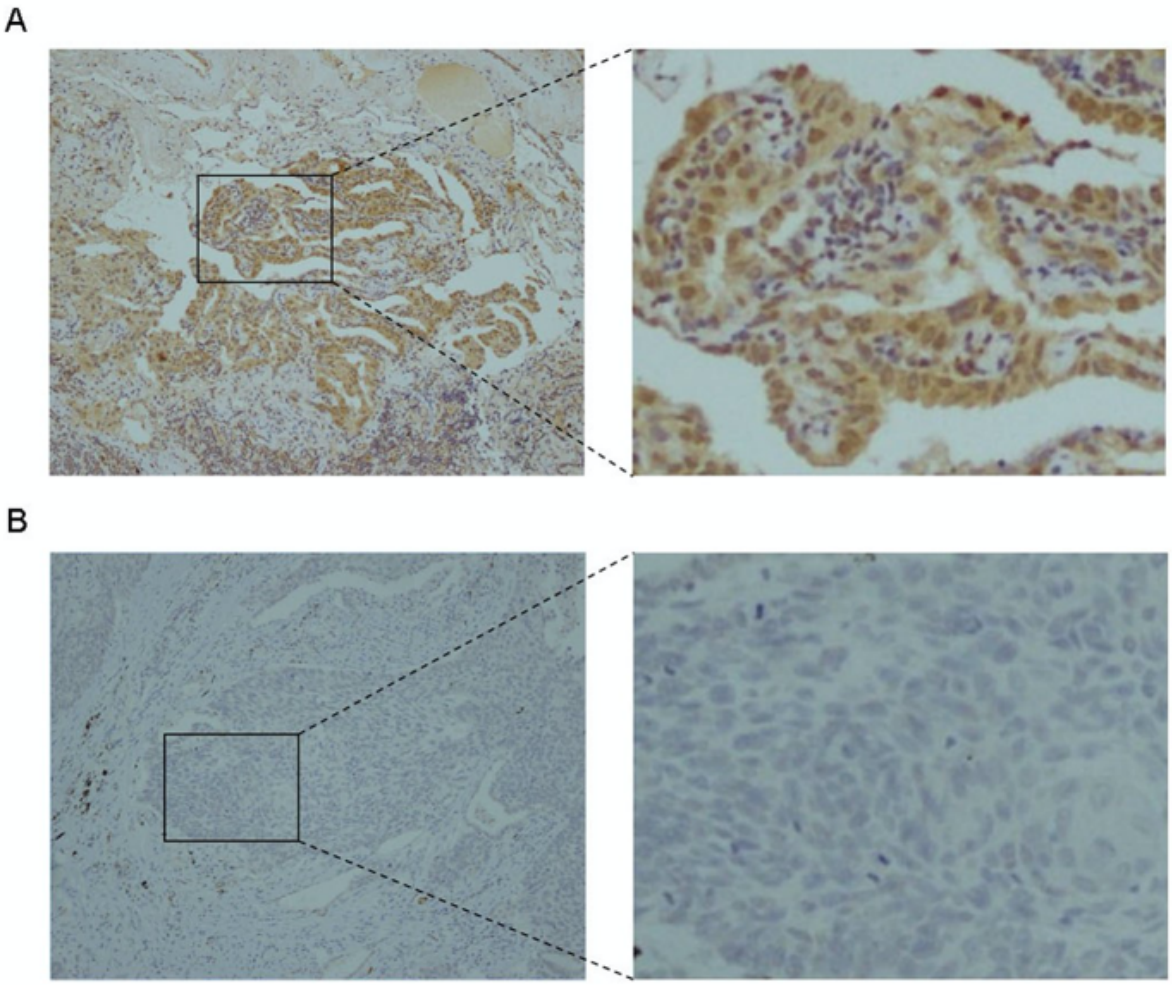

Figure 1

CXCR5 immunohistochemical staining in tumor tissues. (A) Positive CXCR5 expression in AC. (B) Negative CXCR5 expression in SCC. AC: Adenocarcinoma. SCC: Squamous cell carcinoma.

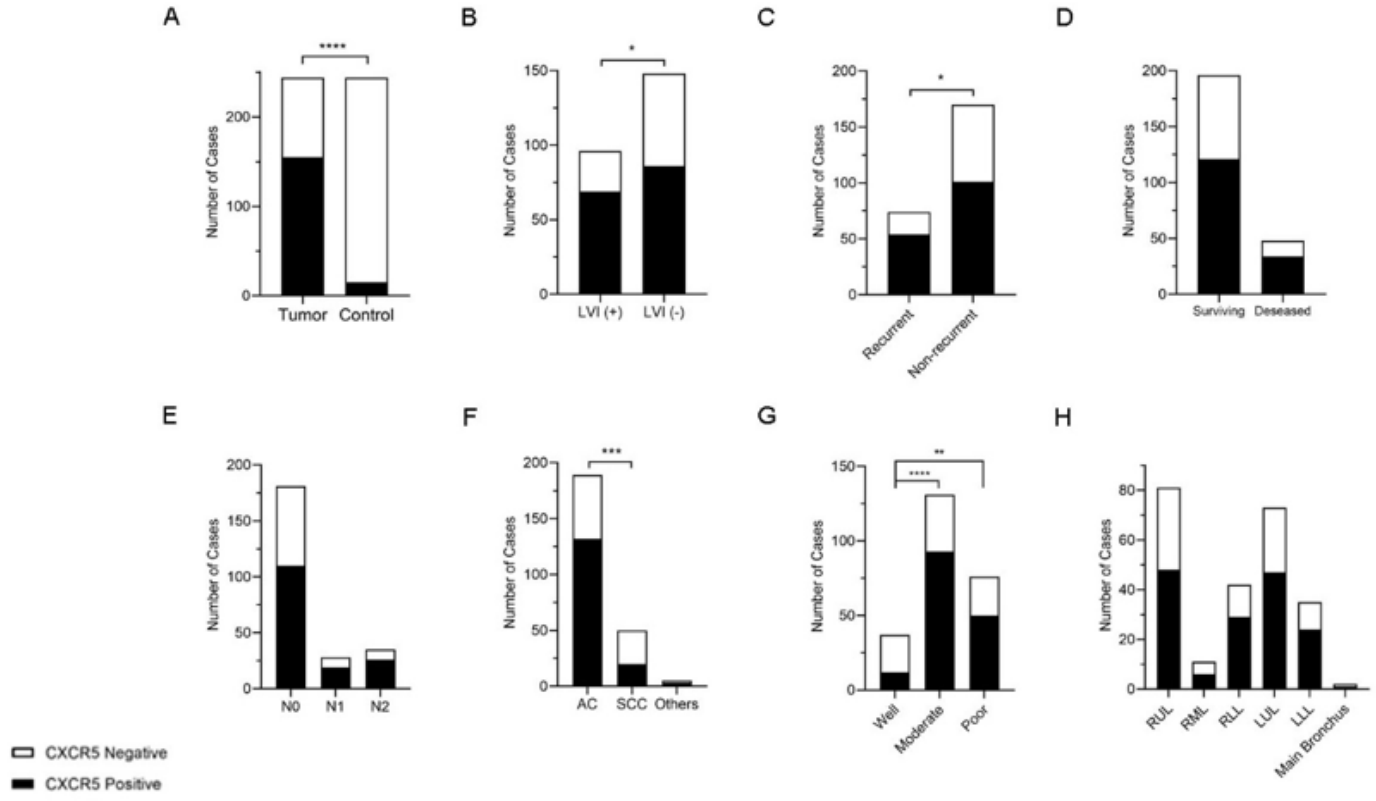

Figure 2

Correlation between CXCR5 expression and patients' clinicopathological characteristics. (A) 155/244 (63.5%) patients in tumor group and 15/244 (6.1%) patients in control group were with positive CXCR5 expression. (B) 69/96 (71.9%) patients in LVI (+) group and 86/148 (58.1%) patients in LVI (-) group were with positive CXCR5 expression. (C) 54/74 (73.0%) patients in recurrent group and 101/170 (59.4%) patients in non-recurrent group were with positive CXCR5 expression. (D) 121/196 (61.7%) patients in surviving group and 34/48 (70.8%) patients in deceased group were with positive CXCR5 expression. (E) 110/181 (60.8%) patients in stage N0 group, 19/28 (67.9%) patients in stage N1 group and 26/35 (74.3%) patients in stage N2 group were with positive CXCR5 expression. (F) 132/189 (69.8%) patients in AC group, 20/50 (40.0%) patients in SCC group, and 2/5 (40.0%) patients in Others group were with positive CXCR5 expression. (G) 12/37 (32.4%) patients in Well differentiated group, 93/131 (71.0%) patients in Moderate differentiated group and 50/76 (65.8%) patients in Poor differentiated group were with positive CXCR5 expression. (H) 48/81 (59.3%) patients in RUL group, 6/11 (54.5%) patients in RML group, 29/42 (69.0%) patients in RLL group, 47/73 (64.4%) patients in LUL group, 24/35 (68.6%) patients in LLL group and 1/2 (50.0%) patients in Main Bronchus group were with positive CXCR5 expression. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. LVI: Lymphovascular invasion. AC: Adenocarcinoma. SCC: Squamous cell carcinoma. RUL: Right upper lobe. RML: Right middle lobe. RLL: Right lower lobe. LUL: Left upper lobe. LLL: Left lower lobe.

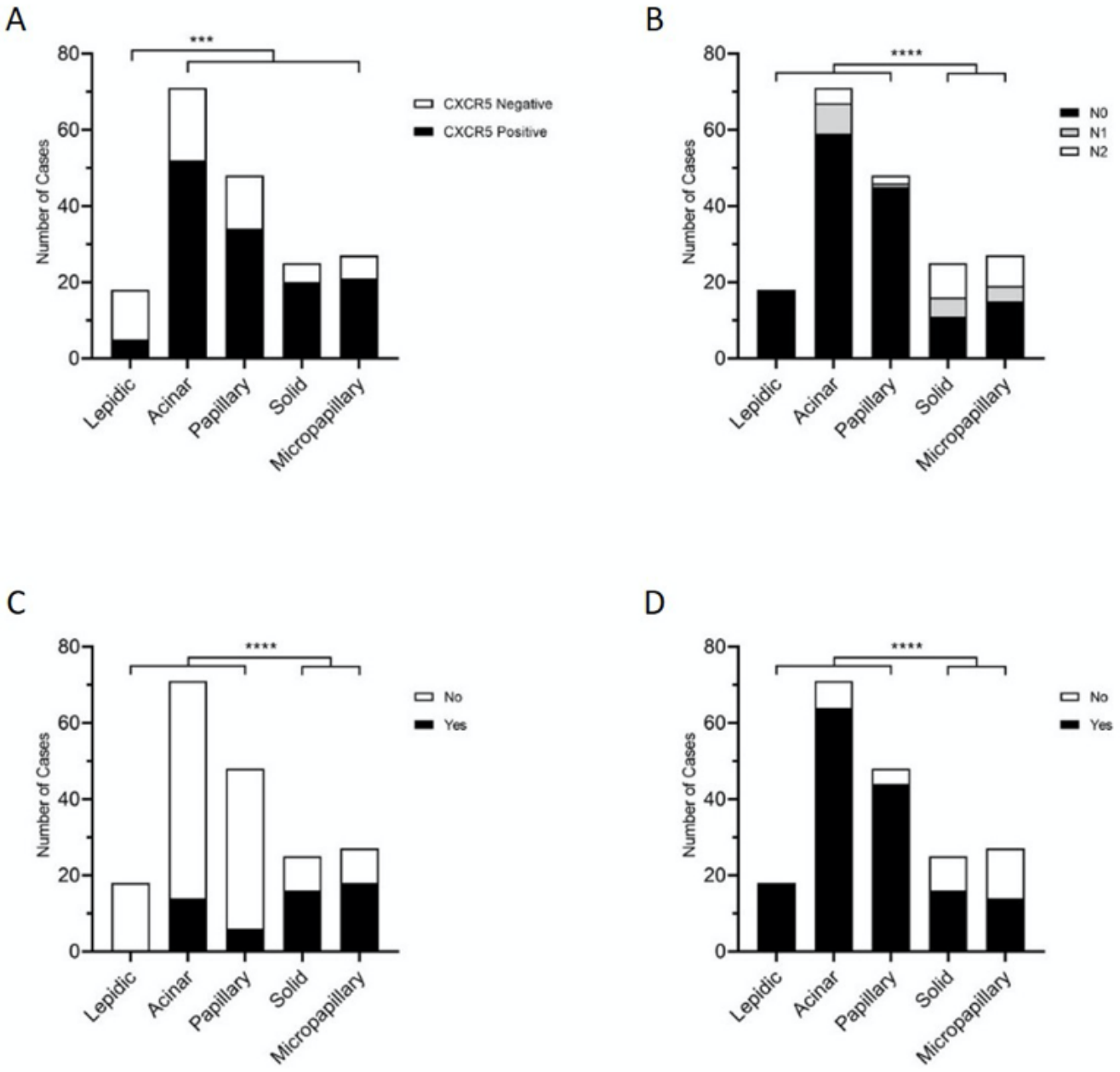

Figure 3

Correlation between CXCR5 expression and patients' pathological subtypes. (A) 5/18 (27.8%) patients in LP group, 52/71 (73.2%) patients in AP group, 34/48 (70.8%) patients in PP group, 20/25 (80.0%) patients in SP group and 21/27 (77.8%) patients in MPP group were with positive CXCR5 expression. (B) 18/18 N0 patients were in LP group; 59/71 (83.1%) N0 patients, 8/71 (11.2%) N1 patients and 4/71 (5.6%) N2 patients were in AP group; 45/48 (93.8%) N0 patients, 1/48 (2.1%) N1 patients and 2/48 (4.2%) N2 patients were in PP group; 11/25 (44.0%) N0 patients, 5/25 (20.0%) N1 patients and 9/25 (36.0%) N2 patients were in SP group; 15/27 (55.6%) N0 patients, 4/27 (14.8%) N1 patients and 8/27 (29.6%) N2 patients were in MPP group. (C) 0/18 (0.0%) recurrent patients were in LP group; 14/71 (19.7%) recurrent patients were in AP

group; 6/48 (12.5%) recurrent patients were in PP group; 16/25 (64.0%) recurrent patients were in SP group, and 18/27 (66.7%) recurrent patients were in MPP group. (D) 18/18 (100.0%) surviving patients were in LP group; 64/71 (90.1%) surviving patients were in AP group; 44/48 (91.7%) surviving patients were in PP group; 16/25 (64.0%) surviving patients were in SP group, and 14/27 (51.9%) surviving patients were in MPP group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. LP: Lepidic predominant. AP: Acinar predominant. PP: Papillary predominant. SP: Solid predominant. MPP: Micropapillary predominant.

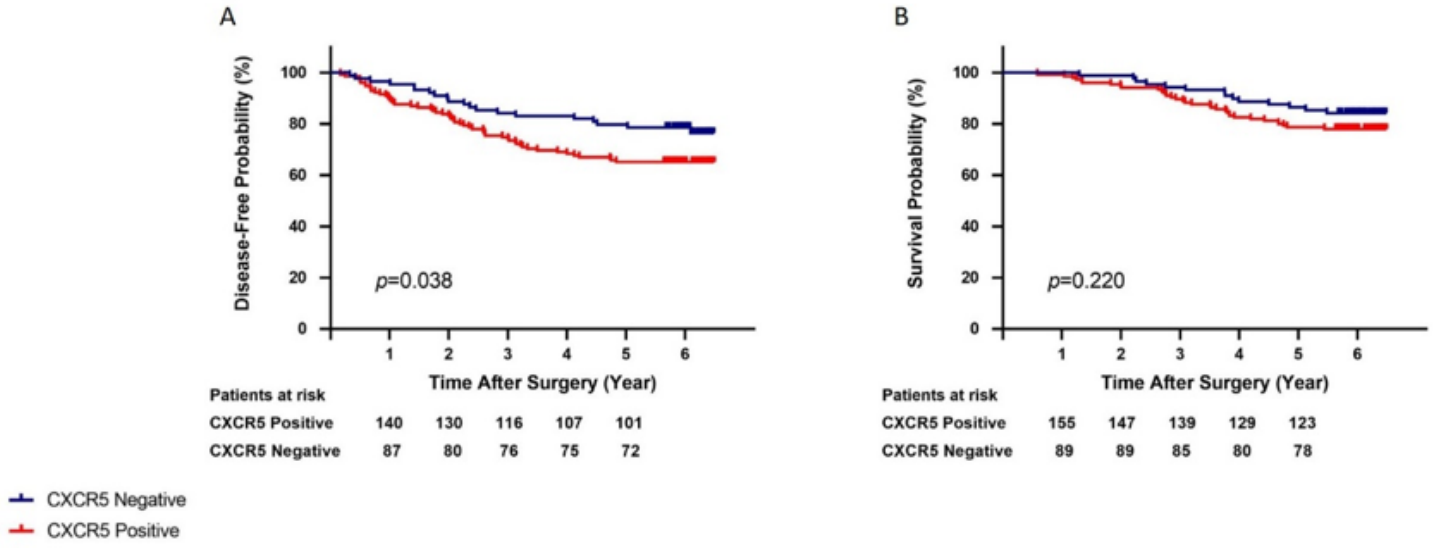

Figure 4

Survival outcomes based on CXCR5 expression. (A) 5-year DFS of patients with positive/negative CXCR5 expression. (B) 5-year OS of patients with positive/negative CXCR5 expression. DFS: Disease-free survival. OS: Overall survival.

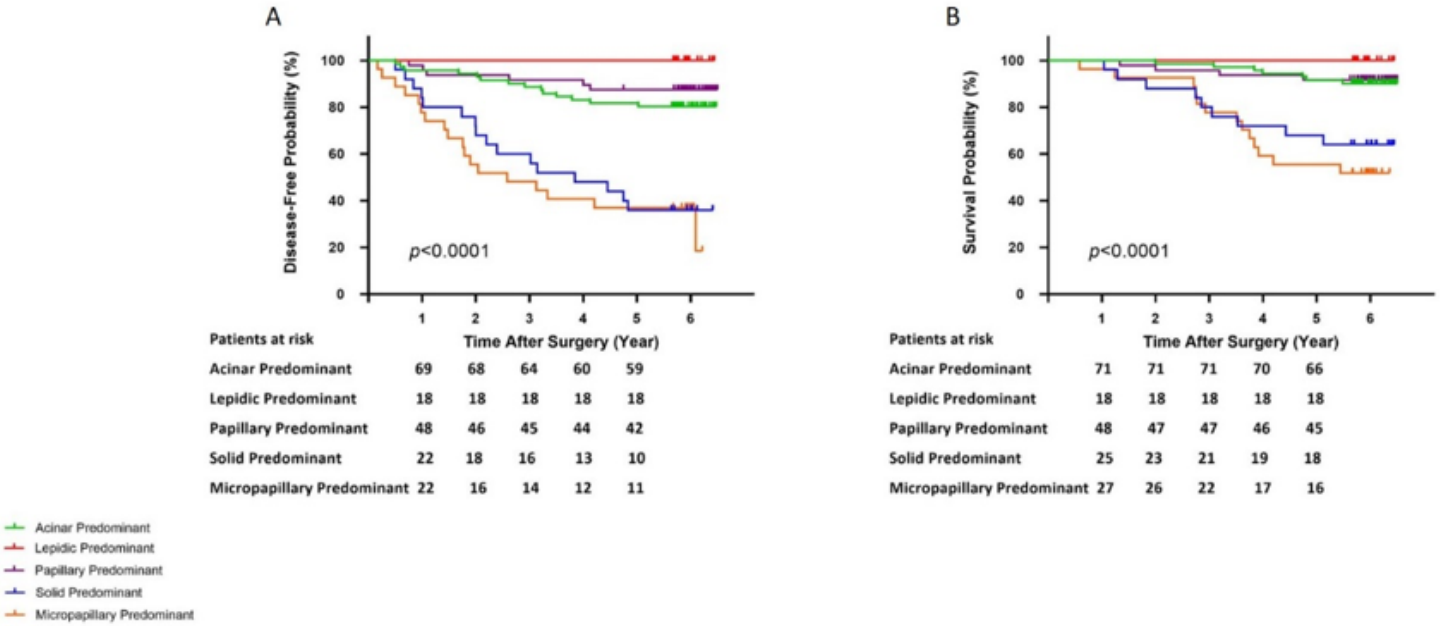

Figure 5

Survival outcomes based on pathological subtypes of lung adenocarcinoma. (A) 5-year DFS of patients with different pathological subtypes of lung adenocarcinoma. (B) 5-year OS of patients with different pathological subtypes of lung adenocarcinoma. DFS: Disease-free survival. OS: Overall survival.