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Cytokines and chemokines expression in non-small cell lung carcinoma: diagnosis and therapeutic responses

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Abstract

Non-small cell lung carcinoma (NSCLC) being multifaceted, it acquires genetic and epigenetic variations. These changes tend to control differentiation, proliferation, invasion, and metastasis of tumors. Surgery considered the most effective treatment for NSCLC in the initial stage, about 70 to 80% of patients are not convinced about it essentially because of locoregional tumor extension, extrathoracic spread, or poor physical and functional condition when diagnosed. Cytokines have proved to be successful in cancer treatment, but the impact of certain promising targets on different immune cell populations is still unknown. In the present study, a total of 250 papers including 230 research papers and 20 review papers, extracted from PubMed and Scopus and published from December 31, 1995, to December 31 2021, are reviewed. The most important involved-chemokines in lung cancer including α-chemokine (CC), β-chemokine (CXC), γ-chemokine (C), and δ-chemokine (CX3C). While essential cytokines in lung cancer including TNF-α, IFN- γ, TGF-β and interleukins such as IL-6, IL-1β, IL-8, and IL-18 are introduced. The pathological role of such chemokines and cytokines in cancer signaling pathways are investigated.

Keywords: Cytokines; Lung cancer; Chemokines; Interleukins

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Introduction

Lung cancer is a major worldwide public health problem and the most frequently diagnosed cancer. Non-small cell lung carcinoma (NSCLC) histology represents roughly 85% of all lung cancer. Despite some improvements in the detection and therapy of NSCLC patients, the 5-year overall survival rate for NSCLC is ≤15%, demonstrating the need for new biomarkers and new targets for NSCLC therapy. Specifically, a focus on molecules regulating the tumor-favorable microenvironment could open new strategies against NSCLC. The presence of biologically active effector mechanisms and extensive crosstalk exists among immune cells within the tumor microenvironment that support disease development and progression. Cytokines, in particular, modulate multiple components of the tumor microenvironment, such as the immune system, stroma cells, and tumor cells. A subfamily of cytokines called chemokines was first identified by their property to influence the biological processes which control leukocyte trafficking. These molecules bear an important role in the carcinogenesis

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processes by controlling several cellular biological aspects, such as cell proliferation, angiogenesis, inflammation, and cell trafficking. Cytokines and chemokines are involved in NSCLC histogenesis and they are promising biomarkers influencing diagnosis, prognosis, and predictive response to therapy in NSCLC.

Epidemiology and Risk Factors

Lung cancer is a major health problem worldwide in terms of incidence, morbidity, and mortality. Nowadays, lung cancer is the leading cause of death from cancer for both men and women in Europe and the United States. In Europe, more than 400,000 new lung cancer cases are diagnosed every year, representing around 20% of all cancer diagnoses. In the US, the American Cancer Society estimated that 18% of all cancer cases come from lung cancer and 23% of all cancer deaths are due to the disease. The major cause of principal lung cancer mortality is a late diagnosis. The overall 5-year survival rate is 15%, with the majority of the patients dying within one year following diagnosis. The treatment of non-small lung cancer cell carcinoma (NSCLC) is based on surgical resection, but in more than 30% of patients, diagnosis is made when local symptoms, such as cough or dyspnea related to poor lung function, arise and surgery is only possible in early stages.

In addition to surgical resection, NSCLC therapy is based on different strategies such as chemotherapy, radiotherapy, targeted therapy using either epidermal growth factor receptor (EGFR) inhibitors or the more recent programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) immune checkpoint inhibitors. In literature, many studies report that immune checkpoint programs such as PD-1, its ligand PD-L1, and CTLA-4 can inhibit the ability of immune cells to recognize and destroy cancer cells, and this cancer mechanism facilitates cancer cell proliferation. Consequently, the analysis of PD-1 and PD-L1 expression in NSCLC is essential to guide clinicians in cancer prognosis and therapy. Nonetheless, the control that induces PD-1/PD-L1 signaling activity represents the last step of a complex network, and in the future, cancer therapy may benefit from a strategy addressing these interactions.

Cytokines and Chemokines: Functions and Roles in NSCLC

Cytokines and chemokines are small proteins that work as ligands for G-protein-coupled transmembrane receptors. Upon binding to the receptor, cytokines act in an endocrine and/or paracrine manner, as ligands for signaling responses, modulating cellular behavior in different physiological conditions, including angiogenesis, lymphangiogenesis, erythropoiesis, and bone marrow stem cell self-renewal. Chemokines, in turn, play important roles in the activation and migration of leukocytes to inflammatory sites or during inflammatory resolution. Since they were discovered, several studies have highlighted the value of cytokines and chemokines as regulatory proteins, involved in the progression, behavior, metastasis, and prognosis of a variety of human malignancies, including lung cancer.

Lung cancer is the most common cancer worldwide and the leading cause of cancer-related deaths. Approximately 85% of patients screened and diagnosed with lung cancer are characterized as non-small cell lung carcinoma (NSCLC). In the tumor microenvironment, an increase in pro-tumorigenic cytokine and chemokine expression, which is correlated with the progression of NSCLC, has been observed. In tumor-associated macrophages, high levels of pro-inflammatory mediators such as CXCL12 and CCL2 can induce proliferation, angiogenesis, and metastasis through the activation of the CXCR4 receptor and recruitment of endothelial progenitor cells, a subset of myeloid angiogenic cells.

Overview of Cytokines and Chemokines

Cytokines represent a large group of soluble proteins produced by different cells, including macrophages, lymphocytes, mast cells, endothelial cells, fibroblasts, and various stromal cells. Functionally, the cytokines serve as classical modulators of host responses, archetypically involved in innate immunity, inflammatory responses, and regulation of homeostasis (e.g., cellular differentiation, growth, and tissue control). Their multiple functions are partly explained by the complexity of their receptors, allowing a cytokine to act on many cell types and signaling pathways.

In contrast to the cytokines, the chemokines are a defined subfamily of structurally and functionally related molecules. Their core function is in the control of leukocyte traffic in homeostatic processes of leukocyte influx to tissues and in the enhancement of inflammatory reactions following tissue injury, infection, or autoimmune responses. Chemokine receptors are typical G protein-coupled receptors, further divided functionally into CC, CXC, XC, and CX3C receptors, and each can be designated on the basis of sequence homology. Overall, these molecular groups are involved in the orchestration of different immunological processes and have been shown to play critical immunological roles in a number of pathological states, including tumorigenesis.

In microenvironmental responses to human non-small cell lung carcinoma (NSCLC), the activation of specific immune cells results in the tissue expression of related cytokines and chemokines. The sum effect of local cytokine/chemokine expression creates an orchestrated response mechanism, which serves to influence the function of the various immune cell types within and around the tumor sites. These molecules control leukocyte traffic and cytokine activity in the tumor sites. Such inflammatory responses to cancer cells, aggregated within individual tissues, are integral to the generation of tissue-specific inflammatory cancers.

Inflammatory Microenvironment in NSCLC

Patients with non-small cell lung cancer (NSCLC) face poor clinical outcomes due to the lack of sensitive diagnostic biomarkers, a high proportion of resistance to classical therapies, and the absence of cancer-specific symptoms. Similar to other diseases, chronic inflammation promotes the development of lung tumors. Various cell types, including macrophages, T and B lymphocytes, natural killer cells, mast cells, and neutrophils, communicate with tumor cells through different pathways, such as cell-to-cell contact or paracrine signaling. Numerous

studies have demonstrated both pro- and antitumor effects of immune cells in cancer. Therefore, due to the complexity and heterogeneity of cell-to-cell interactions, the design and application of immune therapy yield different responses.

Cytokines and chemokines play crucial roles in inflammation and innate immunity and significantly influence each stage of NSCLC development. These molecules can serve as essential elements for new strategies aimed at combating NSCLC. There are two fundamental approaches: in vivo quantitative and qualitative estimation of chemokines and cytokines produced using ELISA, Luminex, or PCR laboratory tests for diagnostic/prognostic purposes and for predicting response before and during targeted therapy; ex vivo studies on tumor cell cultures to evaluate the effects of active substances in the tumor microenvironment on NSCLC. In this context, a series of data, if further confirmed, could be a crucial factor in designing a bioactive multimodal platform that combines antitumor agents for cancer treatment, with the goal of personalized medicine.

Immunohistochemistry

Immunohistochemistry allows describing the cellular localization and distribution of a specific molecule, which is necessary to identify the immediate cellular source and ultimately allows determining the rationale behind treatment. The commonly used protocol is based on the incubation of the slice covered by the commercial antibodies towards the target antigen. These are typically polyclonal and monoclonal fluorophils, which will bind the primary antibodies. The secondary antibodies are species-specific to the primary antibody and are covalently attached to the proteins that produce fluorescence.

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a sensitive and specific method to detect and measure proteins. In this technique, signal amplification is achieved with the addition of an enzyme-labeled secondary antibody which can produce a visible or fluorescent colorimetric signal based on an enzymatic reaction. It is essentially a plate-based immunoassay, where a monoclonal or polyclonal antibody is adsorbed to a polystyrene microplate well. Cytokine that is being measured can be captured by this antibody.

In a direct ELISA, antibody pairs are used. In a sandwich ELISA, after the coated antibody captures the cytokine of interest, another antibody that will bind to a different site on the same molecule is used. The second antibody has an enzyme attached to it, usually alkaline phosphatase or peroxidase. The amount of cytokine in the sample can be quantified by detecting and measuring the colored product, which is directly proportional to the amount of cytokine present in the sample. A standard curve is then constructed to determine the cytokine concentration of the unknown samples.

Mainly, ELISA is used in cytokine profiling studies, including in lung cancer research, to assess the expression of a panel of cytokines and chemokines in cell culture supernatants, serum, or plasma from patients or study participants. These profiles, overall, can provide significant

information about the relationship of these clinically relevant cytokines with lung cancer. They are useful in understanding the biology of NSCLC and how it might inform the use of targeted therapy in future combinatorial trials.

ELISA technology, which is a gold standard for the measurement of molecules in body liquids or culture, has mainly driven profiling studies in which a panel of cytokines have been quantified using commercial kits for larger numbers of samples, and previously measured expression levels have been correlated with survival. The main purpose of using detection technology in NSCLC in relevant therapeutic research is due to the diagnosis and personalized medicine of the disease. The current goal and aim are the detection of the effectiveness of the ongoing trials. The cytokines that have been defined in serum can also provide information about the diagnosis and be a tumor marker of NSCLC.

Diagnostic Significance of Cytokines and Chemokines in NSCLC

Work on promising biological factors that provide a more accurate differential diagnosis between neoplastic and non-neoplastic lung diseases, as well as prognosis, has become a particularly urgent problem. It has been shown that the protein markers of cancer and tumor-associated immune inflammation among cytokines in patients with non-small cell lung carcinoma (NSCLC) are identified. However, cytokines and chemokines may be used as biomarkers for the prediction of carcinomas' behavior. They can modulate the development and function of the immune system and participate in the formation of antitumor resistance. An increase or decrease in the expression of cytokines and chemokines reflects the body's immune response to the tumor, contributing to the increase in the survival rate of patients. Numerous studies have demonstrated that cytokines and chemokines play an important role in the initiation, promotion, and progression of lung carcinomas. Therefore, the study of the immune system and tumor microenvironment is an urgent medical and biological problem. Based on the above facts, the study of immunity in NSCLC patients has acquired a new development vector in solving diagnostic and prognostic problems related to the study of cytokines and chemokines.

Biomarkers for Early Detection

One of the major issues with NSCLC is that it is initially asymptomatic. Chronic cough, chest pain, recurrent bronchitis, coughing up blood, swallowing difficulty with choking, weight loss, and loss of appetite are among the nonspecific symptoms that appear at the advanced stage of NSCLC, which include neurological symptoms such as headache, mental status change, arm/leg weakness, seizures or problems with wobbling gait (areas of the brain). Indeed, 65% of patients are diagnosed when the metastases are already advanced and when surgical operations are no longer applicable. There is an unmet need to detect these primary cancers early to result in a positive prognosis. Nodules and masses, however, are observed from chest X-ray and CTPA.

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Therapeutic Targeting of Cytokines and Chemokines in NSCLC

NSCLC is the leading cause of cancer death around the world and globally, it accounts for around 85% of all LC cases. Its yearly incidence has increased in both men and women, even with substantial progression in prevention, early detection, and treatment methods, including surgery, radiotherapy, chemotherapy, targeted agents, and immunotherapy. The treatment decisional process includes the clinical stage, anatomical location, adverse factors, and patient's interest. Given the significance of the tumor microenvironment in modulating long-term disease remission, this has created a new generation of 'drugable' targets which lay within the tumor and non-tumor fraction of the responsive cells and a variety of agents that block these-related cytokines and chemokines are currently undergoing clinical trial testing in NSCLC. These encompass the CCL2/CCR2, CXCR2/IL-8, IKK/NF kappa-B, LIF/LIFR, IL4, and IL-6/IL6R pathways.

Conclusion

The last decade has witnessed a significant rise in the number of scientific publications that delve into the investigation of cytokines and chemokines expression in non-small cell lung carcinoma (NSCLC) with the objective to furnish information indispensable for patient diagnosis and eventual therapeutic approaches employing cytokines and chemokines.

Competing interests

The authors declare no conflict of interest.

Ethics Statement

This study has been approved by the Ethical Review Committee. The publication of any potentially identifiable images or data contained in the article requires personal written informed consent.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

References

1. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008; 83:584-594.
2. Thommen DS, Koelzer VH, Herzig P, et al. A transcriptionally and functionally distinct PD-1+ CD8+ T cell pool with predictive potential in non-small- cell lung cancer treated with PD-1 blockade. Nature Medicine 2018; 24(7):994-1004.
3. P. Sarode MB. Schaefer, F. Grimminger, W. Seeger, and R. Savai, "Macrophage and tumor cell cross-talk is fundamental for lung tumor progression: we need to talk," Frontiers in Oncology 2020;10:1–11.

4. Joshi BH, Hogaboam C, Dover P, Husain SR, Puri RK. Role of interleukin-13 in cancer, pulmonary fibrosis, and other TH2-type diseases. In: Interleukins. Elsevier 2006; 479-504.
5. Matanić D, Beg-Zec Z, Stojanović D, Matakorić N, Flego V, Milevoj-Ribić F. Cytokines in patients with lung cancer. Scand J Immunol 2003 ;57(2):173–178.
6. Carpagnano GE, Spanevello A, Curci C et al. IL-2, TNF-alpha, and leptin: local versus systemic concentrations in NSCLC patients. Oncol Res 2007; 16(8):375–381.
7. Wu FY, Fan J, Tang L, Zhao YM, Zhou CC. Atypical chemokine receptor D6 inhibits human non-small cell lung cancer growth by sequestration of chemokines. Oncol Lett 2013; 6:91-95.
8. Schmall A, Al-Tamari HM, Herold S, Kampschulte M, Weigert A, Wietelmann A. et al. Macrophage and cancer cell cross-talk via CCR2 and CX3CR1 is a fundamental mechanism driving lung cancer. Am J Respir Crit Care Med 2015; 191:437-447.
9. Kee JY, Arita Y, Shinohara K, Ohashi Y, Sakurai H, Saiki I. et al. Antitumor immune activity by chemokine CX3CL1 in an orthotopic implantation of lung cancer model. Mol Clin Oncol 2013; 1:35-40.
10. Travis WD. Pathology of lung cancer. Clin Chest Med 2011; 32:669-692.
11. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-867.
12. Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ. et al. International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol Rev 2014; 66:1-79.
13. Singh S, Sadanandam A, Singh RK. Chemokines in tumor angiogenesis and metastasis. Cancer Metastasis Rev 2007; 26:453-467.
14. Koontongkaew S. The tumor microenvironment contribution to development, growth, invasion and metastasis of head and neck squamous cell carcinomas. J Cancer 2013; 4:66-83.
15. Lee HJ, Kim YT, Park PJ, Shin YS, Kang KN, Kim Y. et al. A novel detection method of non-small cell lung cancer using multiplexed bead-based serum biomarker profiling. J Thorac Cardiovasc Surg 2012; 143:421-427.
16. Xu Y, Liu L, Qiu X, Liu Z, Li H, Li Z. et al. CCL21/CCR7 prevents apoptosis via the ERK pathway in human non-small cell lung cancer cells. PLoS One 2012; 7:e33262.
17. Gupta P, Sharma PK, Mir H, Singh R, Singh N, Kloecker GH. et al. CCR9/CCL25 expression in non-small cell lung cancer correlates with aggressive disease and mediates key steps of metastasis. Oncotarget 2014; v5:10170-10179.
18. Zhu YM, Webster SJ, Flower D, Woll PJ. Interleukin-8/CXCL8 is a growth factor for human lung cancer cells. Br J Cancer 2004; 91:1970-1976.
19. Zhang Y, Wang L, Zhang M, Jin M, Bai C, Wang X. Potential mechanism of interleukin-8 production from lung cancer cells: an involvement of EGF-EGFR-PI3K-Akt-Erk pathway. J Cell Physiol 2012;227: 35-43.
20. Yousif NG, Sadiq AM, Yousif MG, Al-Mudhafar RH, Al-Baghdadi JJ, Hadi N. Notch1 ligand signaling pathway activated in cervical cancer: poor prognosis with high-level JAG1/Notch1. Arch Gynecol Obstet 2015; 292(4):899-904.
21. Long GV, Schachter J, Ribas A, Arance AM, Grob J-J, Mortier L, et al. 4-year survival and outcomes after cessation of pembrolizumab (pembro) after 2-years in patients (pts) with ipilimumab (ipi)-naive advanced melanoma in KEYNOTE-006. J Clin Oncol 2018; 36(15 Suppl.):9503.
22. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015; 348:124–8.
23. Mathew M, Safyan RA, Shu CA. PD-L1 as a biomarker in NSCLC: challenges and future directions. Ann Transl Med 2017; 5:375.
24. Balkwill F. Cancer and the chemokine network. Nat Rev Cancer 2004; 4:540.
25. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12:252.
26. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol 2017; 18:e143–52.
27. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007; 19:813–24.
28. Blank CU, Haanen JB, Ribas A, Schumacher TN. The "cancer immunogram". Science 2016; 352:658–60.
29. Byrne EH, Fisher DE. Immune and molecular correlates in melanoma treated with immune checkpoint blockade. Cancer 2017; 123:2143–53.

30. Ribas A. Adaptive immune resistance: how cancer protects from immune attack. Cancer Discov. 2015; 5:915–9.
31. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016; 387:1540–1550.
32. Tripathi SC, Peters HL, Taguchi A, et al. Immunoproteasome deficiency is a feature of non-small cell lung cancer with a mesenchymal phenotype and is associated with a poor outcome. Proc Natl Acad Sci USA 2016;113: E1555–E1564.
33. Sadique AM, Al-Huseini LM, Noaman M, et al. High-Level of Notch 1/Jagged 1 Level up Regulated Chemo-Resistance of Cisplatin in NSCLC. Sys Rev Pharm 2020; 11(5):917-922.
34. Critchley-Thorne RJ, Simons DL, Yan N, et al. Impaired interferon signaling is a common immune defect in human cancer. Proc Natl Acad Sci USA 2009; 106:9010–9015.
35. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. J Thorac Oncol 2017; 12:208–222.
36. Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. JAMA Oncol 2017; 3:1051–1058.
37. Prat A, Navarro A, Pare L, et al. Immune-related gene expression profiling after PD-1 blockade in non-small cell lung carcinoma, head and neck squamous cell carcinoma, and melanoma. Cancer Res 2017; 77:3540–3550.
38. Reguart N, Teixido C, Gimenez-Capitan A, et al. Identification of ALK, ROS1 and RET fusions by a multiplexed mRNA-based assay in formalin-fixed, paraffin-embedded samples from advanced non-small-cell lung cancer patients. Clin Chem 2017; 63:751–760.
39. Peters S, Cappuzzo F, Horn L, et al. OA03.05 Analysis of Early Survival in Patients with Advanced Non-Squamous NSCLC Treated with Nivolumab vs Docetaxel in CheckMate 057. J Thorac Oncol 2017;12:S253.
40. Rebelatto MC, Midha A, Mistry A, et al. Development of a programmed cell death ligand-1 immunohistochemical assay validated for analysis of non-small cell lung cancer and head and neck squamous cell carcinoma. Diagn Pathol 2016; 11:95.
41. Yousif NG, Mohammed KG, Mohammed SM, Hadi NR. Association between Natural Killer Cell Cytotoxicity and the Progression of Non-Small Cell Lung Cancer. Sys Rev Pharm 2020; 11(4):543-551.
42. Rimm DL, Han G, Taube JM, et al. A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. JAMA Oncol 2017; 3:1051-58.
43. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. Science 2013; 339:1546-58.
44. Chen N, Fang W, Zhan J, et al. Upregulation of PD-L1 by EGFR Activation Mediates the Immune Escape in EGFR-Driven NSCLC: Implication for Optional Immune Targeted Therapy for NSCLC Patients with EGFR Mutation. J Thorac Oncol 2015; 10:910-23.

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