JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2019; 3:113-120
https://doi.org/10.30621/jbachs.2019.625

Review

New Insight of Tumor Microenvironment in Non-Small Cell Lung Cancer

Tolga Sever[1], Asim Leblebici[1], Altug Koc[1,2], Cilem Binicier[1], Aybüke Olgun[1,3], Deniz Tuna Edizer[1,4], Taha Resid Özdemir[5,6], Gürdeniz Serin[5,7], Melek Erdem[5,8], Yasemin Basbinar[9]

[1]Dokuz Eylul University, Institute of Health Sciences, Department of Oncology, Translational Oncology, Izmir, Turkey
[2]Dokuz Eylul University, Faculty of Medicine, Medical Genetics, Izmir, Turkey
[3]Izmir Tepecik Training and Research Hospital, Internal Medicine, Izmir, Turkey
[4]Bozyaka Training and Research Hospital, Department of Ear Nose and Throat, Izmir, Turkey
[5]Dokuz Eylul University, Institute of Health Sciences, Department of Oncology, Basic Oncology, Izmir, Turkey
[6]Izmir Tepecik Training and Research Hospital, Genetic Diagnostic Center, Izmir, Turkey
[7]Ege University, Faculty of Medicine, Pathology, Izmir, Turkey
[8]Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, Pediatric Hematology and Oncology, Izmir, Turkey
[9]Dokuz Eylul University, Institute of Oncology, Department of Translational Oncology, Izmir, Turkey

Address for Correspondence: Tolga Sever, **E-mail:** severrtolga@gmail.com
Received: 20.04.2019; **Accepted:** 27.04.2019; **Available Online Date:** 28.05.2019

Cite this article as: Sever T, Leblebici A, Koc A, Binicier C, Olgun A, Edizer DT, Özdemir TR, Serin G, Erdem M, Basbinar Y. New Insight of Tumor Microenvironment in Non-Small Cell Lung Cancer. J Basic Clin Health Sci 2019; 3:113-120. https://doi.org/10.30621/jbachs.2019.625

Abstract

As in all living cells, cancer cells are evaluating is very important in the frames of tumor development and treatment. As in all evolutionary process, the environment of living cells has an important role. Tumor cells have an environment is called tumor microenvironment, affects the therapeutic response and clinical results. Tumor microenvironments involve various cell types, extracellular matrix substances that are in the niche of cancer cells. The microenvironment is not only important in tumorigenesis but it is effective on therapeutic efficacy. In this review, we carried out the interaction between non-small cell lung cancer and its microenvironment to point out the significance of the tumor environment.

Keywords: Tumor Microenvironment, Non-Small Cell Lung Cancer, Extracellular Matris

INTRODUCTION

Lung cancer, the leading cancer type, includes one of the five cancer cases of males and one of nine cancer cases of women. Recently, while the incidence of lung cancer is decreasing in men, is increasing in women. According to GLOBOCAN-2018 data shows, today the rate of lung cancer case is approximate 2.1 million and 1.8 million of the patients result with mortality. Lung cancer types commonly are aggressive. The main types of lung cancers are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Recently, it is discovered that tumor development not depends on the cancer cell individually but also on the tumor microenvironment and stromal components. Understanding the tumor microenvironment could direct to the discovery of new targets for immunological therapies, which is particularly important for patients with NSCLC who are diagnosed in progressed stages and have low survival rate with conventional chemotherapy. The tumor microenvironment (TME) is heterogeneous and interactions between genetically altered tumorigenic epithelial cells and intra-tumoral stromal cells regulate the main characteristics of cancer, including extracellular matrix (ECM), angiogenesis, inflammation, immune suppression, and metastasis (1). In the current review, the interaction between

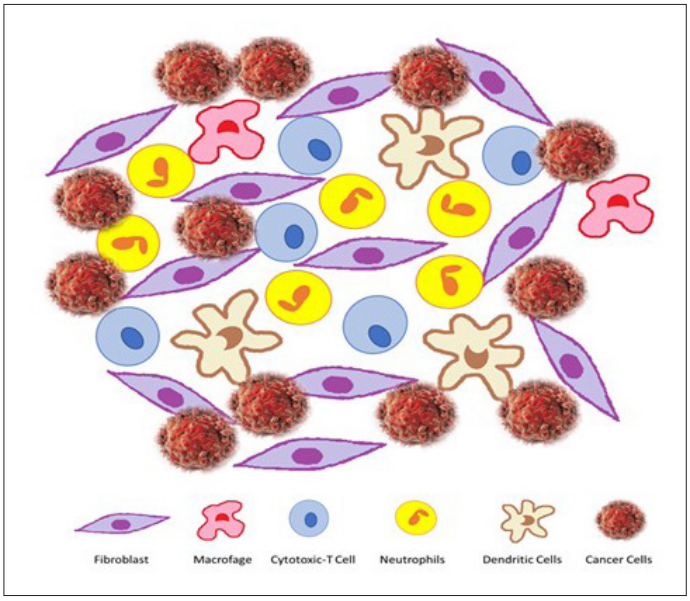

Figure 1. Tumor microenvironment components

NSCLC and TME which consist of ECM, the plasticity of cancer stem cells, immune suppression, invasion, inflammation, hypoxia, and metastasis, angiogenesis, has been carried out.

Extracellular Matrix in Non-Small Cell Lung Cancer Tumor Microenvironment

Cancer cells are not autonomously acting, but an ECM that contains non-cancerous cells such as immune cells, fibroblasts, endothelial and, immune cells, mesenchymal stem cells (MSCs) and collagen, elastin, fibronectin, and proteoglycans. These cells and stroma could be directed by malignant cells, to facilitate tumor growth and metastasis. Malignant cells could affect stromal cells' metabolism which allows the stroma to become proliferative (2).

Tumor stroma has similar features with stroma in wound healing. Fibroblasts are predominant cells in NSCLC stroma. Cancer-associated fibroblasts (CAFs) facilitate the development and invasion of lung cancer by simplifying tumor growth and influencing treatment responses. CAFs could be formed by the transformation of normal tissue fibroblasts. CAFs are migrated from the bone marrow to the tumor stroma, or epithelial-mesenchymal transition. The CAFs contribute to protease-associated ECM dissociation and to the development of invasion in NSCLC by secreting prometastatic factors such as hepatocyte growth factor, SDF-1/CXCL12, leading to a change in protein expression of cells at the border of invasion by factors that they secrete as (Tumor Growth Factor) TGF-β (3). CAFs could synthesize immunomodulatory cytokines such as vascular endothelial growth factor (VEGF) and TGF-β, leading to an increase in regulatory T cell (Treg) and immunosuppressive lymphocytes, thus leading to aggressive disease prognosis (4). Again, NSCLC has been reported to express proteins such as fibroblast activation protein, matrix metalloproteinases (MMPs), podoplanin, osteonectin/SPARC. The expression of these proteins is associated with treatment failure, early recurrence, and low survival (5).

The tumor-related macrophage is an important component of stroma and is examined in two functional classes. M1 type macrophages by secreting agents such as reactive oxygen radicals, TNF-α, shows the anti-tumor effect. M2-type macrophages contribute to tumor development and invasion by secreting degrading enzymes in matrix, angiogenic agents and immunosuppressive cytokines. The increase in M1 type macrophages in tumor stroma in NSCLC is demonstrated to be associated with a good prognosis (6).

The most common substance in ECM of lung tissue is collagen that provides tensile strength. With changes in expression in NSCLC, the collagen structure changes and contributes to forming a more suitable ECM structure for tumor growth. Alteration in collagen cross-linking is also seen in NSCLC. Lysizide-oxidase (LOX) is an enzyme that could cross-link collagen and increases its amount due to hypoxia. LOX-associated collagen binding in NSCLC may increase invasion. The laminin-5 expression has prognostic significance with increased phospho-EGFR/phospho-AKT expression (3).

Stem Cells in Cancer

The concept of cancer stem cell (CSC) was suggested about 40 years ago. It is proposed that there is an effective amount of CSCs in tumor growth as in the renewal of healthy cells. Although studies have pointed out that many cancer types, for instance brain, colorectal, breast cancer and, leukemia have a CSCs niche, the identification and destruction of these cells is a difficult process. Studies including lineage-tracing and cell-ablation strategies have provided insight into the plasticity, quiescence, and cell regeneration and treatment responses of CSCs.

The interesting aspect of the CSC theory is that after the first chemotherapy or radiotherapy treatments, cancer has an explanation for its dormancy and metastasis. More importantly in the treatment approach is targeting the group of cells that maintain the long-term presence of the tumor rather than targeting the tumor mass.

The four basic characteristics of the standard CSCs model are: First, the tumor tissue is heterogeneous and has a hierarchical structure, similar to the original tissue from which it originates. Second, a small portion of CSCs have an ability of self-renewing and are typically silent. Non-cancer stem cells, which make up a large part of the tumor mass, have only a temporary proliferation characteristic and therefore do not affect long-term tumor growth. Third, non-CSCs rarely cause tumor growth in xenograft studies. This indicates that the plasticity in the tumor hierarchy is limited. Finally, CSCs are mainly resistant to chemotherapy and radiotherapy targeting non-CSCs. This explains the relapses seen after treatment. Melanoma and pancreatic cancer have features which are not suitable to the standard model.

Mesenchymal stem cells (MSCs), are precursors of many stromal cells in the tumor microenvironment; they support to the construction of the tumor stroma and affects tumor growth. The effects of MSCs on NSCLC are complex. MSCs secretes pro-angiogenic factors such as FGF-2, PDGF, IL-6, FGF-6, VEGF, angiopoietin-1 which increase the angiogenesis of tumor. Moreover, causes immunosuppressive effects by inhibiting of dendritic cell maturation. Also, suppressing of T cell proliferation and NK/B cell stimulation. While many data support that MSCs protect NSCLC cells from apoptosis, there is also evidence that MSCs prevent NSCLC progression (3).

The Role of the Immune System in Non-Small Cell Lung Cancer Tumor Microenvironment

Immune system components of TME are essential. The immune system in TME consists of two separate parts, being an innate and acquired immune response. The innate immune system involves phagocytes containing neutrophils, dendritic cells (DC), natural killer cells (CD3-, CD56+), mast cells/macrophages (CD68 +), and natural killer T cells (CD56 + CD3 +), and is mainly against foreign pathogens. The innate immune system takes part in the first defense. However, reprogrammed innate immune system of cancerous cell stimulates tumor improvement by supporting tumor metastasis, invasion, and angiogenesis; In contrast, the adaptive immune system components try to suppress tumor

development. Two major T lymphocyte subgroups and B cells mediate to the acquired immune system; helper T cells (Th) (CD4 +), cytotoxic T cells (CTL) (CD8 +), and additionally B cells (CD20 +). The acquired immunity is a second-line protection acts through antigen-specific molecules and needs clonal proliferation which occurs after recognition of foreign antigens (1).

The organization, which ensures that the immune cells are systematically dispersed within the cancerous tissue, is due to the occurrence of tertiary lymphoid structures (TLSs), the ectopic lymph node-like structures. Some adhesion molecules, chemokines and integrins, have a significant influence in the recruitment of T cells from blood to TLS. The role of TLSs is demonstrated in respiratory immunity in viral infections and the presence of TLSs is illustrated to affect the local immune microenvironment in the lung. Primary, high TLS intensities are related with activated CD8 + T cells within the tumor. The importance of TLSs in the antitumor immune reaction has been also demonstrated by the presence of IgA and/or IgG against tumor antigens from B cells in lung cancer tissue which involves TLS. The intensity of TLS and the increase in survival supports that TLS is important in defensive response (7–9). While the immune system varies in lung cancer metastases of other tumors or in different organ metastases to the lung, there is a significant correlation between the immune cell density between the primary and metastatic regions (10).

Myeloid-derived suppressor cell (MDSC) is a significant ingredient of the immunosuppressive system. MDSCs are able to suppress host antitumor immunity. MDSCs constitute tumor growth factor-β (TGF-β), vascular endothelial growth factor (VGFR), hypoxia-induced factor 1 (HIF 1), fibroblast growth factor, reactive oxygen species and also MMP-9 to form a suitable environment for neo-angiogenesis for tumor development (10). These myeloid cells expressing L-arginase (ARG-1), which consume L-Arginine, and inducible nitric oxide synthase (NOS), show an effect on the suppression and apoptosis of CD8 + T cell proliferation and in the reduction of CD3 + expression. MDSC numbers are related with negative response to short survival and chemotherapeutic treatments.

TME usually harbors Tumor-infiltrating lymphocytes (TILs). They are associated in an immune response to the tumor. TILs include CD8 + cytotoxic T lymphocytes (CTLs) that could directly kill tumor cells, and CD4 + T helper lymphocytes (Th), which are secreting heterogeneous cytokine. Th1 subtypes induce CTLs, while Th2 lymphocytes activate humoral immunity. In addition to Th1 and Th2 lymphocytes, a subset of the CD4 + regulatory T lymphocyte (Treg) is involved in the secretion of IL-10, IL-35, and TGF-β. Moreover, in the suppression of effector T lymphocytes subset of Treg has an important role (11).

As in various neoplastic cases, lung tumor cells could improve different escape mechanisms. Tumor cells possibly cause loss of Human Leukocyte Antigen (HLA) class I molecules in tumor progression by modulating their sensitivity to breakdown by NK cells and cytotoxic T cells. It is pointed out that lung cancer cells could perform the production of immunosuppressive factors in the TME. Also, tumor cells are known to secrete a catabolic enzyme indoleamine 2.3-dioxygenase that is related with inhibiting of immune responses, or immunosuppressive cytokines, such as TGF-β or IL-10, and which is effective in increasing the proliferation of cancer cells. Lung cancer cells express their chemokine receptors besides other components of the TME. Additionally, it is signified that, C-X-C motif chemokine receptor 4 (CXCR 4), which provides survival, proliferation, invasion, and metastasis in tumor cells, has a high expression level in tumor cells opposed to normal cells (12). The effect of CXCR4-C-X-C motif chemokine ligand 12 (CXCL12) is significant in the metastasis in patients with NSCLC. C-C motif chemokine ligand 20 (CCL20)′C-C motif chemokine receptor 6 (CCR6) is known to be effective in the progression of NSCLC with the pro-inflammatory and proliferative effects (13). Finally, NSCLC cells can express CXCL8 which takes on an important role as a growth factor with autocrine/paracrine action on tumor cells. CXCL8 is a ligand for CXCR1 and CXCR2 which are secreted by the cancer cells.

Some oncogenes in lung cancers (alteration in TP53, K EGFR, RAS, CDKN2, FHIT, MYC, LKB1 and RB genes) are shown to affect tumor microenvironment to promote immune escape (14). Cancer germline antigens genes indicate poor prognosis in lung cancer or antigens encoded by mutated genes have been identified.

Tumor cells form a specific immune control point to protect itself from the immune system. In particular, they escape from the control of T cells which are specific for tumorigenic antigens. These points involve programmed death protein-1 (PD-1), and cytotoxic T-lymphocyte-associated protein-4 (CTLA4) and T-cell immunoglobulin and mucin associated with cytotoxic T-lymphocyte domain-3 (TIM-3) and inhibitory substances that are secreted by T cells. Additionally, these points involve ligands such as programmed death ligands PD-L1–2, and also B7-H2 which are expressed by APCs. Cancer cells are able to express the death ligands. (15). In NSCLC, 20–60% of tumors are known to be positive for the death ligands in low levels. (16). The expression of the ligands in tumor cells is signified on the cell membrane and/or the cytoplasm. High PD-1 level is related with the impaired T-cell function. Suppression of the PD-1/PD-L1 pathway resulted in increment of T-cell proliferation. Also while the pathway is blocked, cytokine production may increase. High PD-L1 tumor expression indicates poor prognosis (16). In approximately 50% of NSCLC patients, CTLA-4 is present in the tumor cell membrane and cytoplasm. It is known that CTLA-4 overexpression has a positive correlation with survival (17). Since various immune control points are starting by ligand-receptor interactions, they could be inhibited and in oppose could be promoted by unique antibodies (17).

In NSCLC, many investigations have been performed to evaluate the relationship between the existence and intensity of immune cells and the survival of patients. In most of the test, high CD3+, CD8+ or CD4+ T cell infiltrations have been related with good prognosis and Tregs were related with poor prognosis. The proportion of stromal FoxP3 to CD3 + cells have been signified as related to higher relapse risk. When immunohistochemically

specific Treg markers are not detected, FoxP3 or CD25 could also be used as carrier markers of Tregs since their effects on prognosis are important (18).

Unlike T cells, the clinical effect of B cells is not fully proved. In NSCLC, every step of B-cell alteration was illustrated. High B cell density is shown to correlate with increased survival. It could be assumed that B cells are important in their antitumor immunity, by producing tumor antigen-specific antibodies by presenting tumor antigens directly to T cells. Additionally, it could be assumed that professional Antigen Presenting Cells (APCs) play a role by producing tumor antigen-specific antibodies which aim to tumor antigens such immune complexes (19).

Natural killer (NK) cells have been found in the TME of NSCLC. In studies which utilize NKp46 marker proposed that the intensity of NK cells have no correlation with clinical result in the initial phase of NSCLC (20).

Tumor-associated macrophages (TAMs) signify other important substance of the TME in NSCLC (32). Basically, TAMs are categorized as pro-inflammatory M1 macrophages which show anti-tumor effect and M2 macrophages demonstrate proangiogenic activity. In NSCLC, M1 phenotype is related with good prognosis and M2 macrophages identified as signs of poor prognosis (21).

High level of neutrophil is found to be related with a higher risk of recurrence (22) but, not related to survival (23). Dendritic cells (DC) are the key controllers of the immune response and may create T-cell responses, which differ from other APCs. Matured DCs are only found in TLS which are signs for good prognosis (9). Since the intensity of mature DCs and TLSs are correlated which has been suggested that the presence of TLS in patients with NSCLC is a related with a good prognosis (7).

As a result, the immune environment has a big significance in the outcomes of patients with NSCLC Additionally, the environment should be considered in the clinical management of cancer patients.

Inflammation and Non-Small Cell Lung Cancer Interaction

Inflammatory cells and mediators are an important constituent of the TME. Whatever the origin, the inflammatory process in the TME has many tumor-supporting effects. The Inflammatory process helps progression and proliferation of tumor cells, promotes angiogenesis and metastasis, disrupts the adaptive immune response, and modifies responses to therapeutic agents (24).

Inflammation is a physiological progression encouraged by inflammatory cells to fight against to infections and heal wounds. Additionally, prolonged inflammation due to chronic inflammation is able to cause cellular proliferation due to continuous tissue damage. This may result in metaplasia and dysplasia (25). Thus, there is an important relationship between infection, and chronic inflammation in early stages of neoplastic growth. Although it is clear that inflammation does not cause malignant proliferation alone, it may increase the risk of developing rich malignant microenvironment with inflammatory cells and growth factors. This tumor microenvironment consists not only of local tissue cells such as endothelium and fibroblasts but also of tumor-infiltrating leukocytes (26). In clinical and epidemiological studies, 20% of tumors were illustrated to be related with chronic infections (27).

Tumor-associated fibroblasts are thought to inhibit the function of inflammatory cells in the TME. They contribute to tumor proliferation, angiogenesis, invasion, and metastasis by secreting various cytokines and growth factors. It is also demonstrated to increase the PDL-1 expression from tumor cells in lung cancer.

It has been known that tumor-infiltrating neutrophils can exhibit both pro-tumoral effect and anti-tumoral effect. It has been reported that high neutrophil density represents poor prognosis in NSCLC patients (28).

All of the cells that make up the cancer microenvironment may produce various cytokines, chemokines, reactive oxygen radicals, metalloproteinases, interleukins or interferons which mediate the development of lung cancer. In addition, tumor cells produce various cytokines and chemokines which cause leukocytes to enter the environment. Some cytokines are carried out to mediate various stages of carcinogenesis. Interleukins are a family of cytokines that can cause growth, differentiation of tumor cells and activation of inflammatory cells, so that play an important role in tumor development. They have effects as autocrine and paracrine growth factors that help to growth by blocking apoptosis in the inflammation site (29). Novel researches have performed that IL6, and IL17 gene polymorphisms is effective on the survival rate. Additionally IL12A, IL13, IL16 genes play an important role on the survival of NSCLC patients (30, 31). IL1B is a cytokine which responds to inflammation and stimulates the expression of genes related to inflammation. IL1B could be produced by lung epithelial cells. Changes in ILB1 gene are revealed to have an impact on the development of NSCLC. IL6 is an important inflammatory cytokine that plays a role in tumor development stages such as proliferation, angiogenesis, and apoptosis. It is secreted from both lymphoid cells and non-lymphoid cells (13). The polymorphism in the IL6 gene is related with a poor prognosis in NSCLC (32). IL12A is a cytokine produced in macrophages, neutrophils, and B lymphocytes. The cytokine regulates the immune response and shows anti-angiogenic activity. In germline mutations are thought to contribute to tumor progression due to decreased anti-angiogenic activity. Again, polymorphisms in the IL13 gene have been reported to support tumor progression (30). IL16 T is a pro-angiogenic cytokine which is lymphocyte stimulating, it has been reported that the polymorphisms in this gene may have an effect on the prognosis of NSCLC patients (30). IL17 is specifically secreted from activated CD4 (+) T-helper cells are known as Th17, macrophages, and CD8 (+) T lymphocytes. IL17 has other functions such as induction of other cytokines (IL6, IL8, IL18, TNF-alpha) and stimulation of new vessel formation/endothelial migration. Therefore, it plays an important role in tumor progression. The high expression level of IL17 in lung cancer is shown to be an indicator for poor prognosis (31).

PD-L1 (programmed cell death ligand-1) is the main ligand of PD-1 (programmed cell death-1) and is widely expressed in dendritic cells, macrophages, mast cells, T lymphocytes, B lymphocytes, endothelial and epithelial cells. It is known that PDL-1 binding to PD-1 induces T cell tolerance and thus the tissues are protected from autoimmune attack. Tumor cells are also protected from the immune system by demonstrating PDL-1 overexpression with similar mechanisms. In NSCLC, the association between increased expression of PDL-1/PD-1 and positive response to anti-PDL1/PD1 treatments is illustrated shown (33).

As a result, NSCLC is an important component of the tumor microenvironment consisting of inflammation, inflammatory cells, and mediators. While some components of inflammation show anti-tumoral effects, many of them show features that support tumor progression, angiogenesis, invasion, and metastasis. In recent years, studies on targeting therapies for tumor microenvironment and inflammation have gained speed and positive results have been obtained from these treatments.

Hypoxia Effects in Non-Small Cell Lung Cancer

Hypoxia, low oxygen status, is a condition in solid tumors and typical features of the TME. Hypoxia occurs when the oxygen requirement of the tissue exceeds the oxygen supply. The reasons for this are aberrant blood vessel formation, fluctuations of blood and high oxygen demand due to the rapid growth of the tumor. It is known that hypoxia activates genes those involve in angiogenesis and metastasis and predisposes to metastasis. Chronic hypoxia affects the motility and indirectly invasive characteristics of tumor cells, resulting in poor prognosis. Tumor cells exposed to hypoxia activate various transcription factors, triggering signals that regulate important events such as apoptosis, cell proliferation, and angiogenesis (34).

One of the most important mediator in the presence of hypoxia is hypoxia-induced factor-1 (HIF-1). The factor is responsible for the regulation of genes which has an important role in metastasis, metabolism, angiogenesis, and invasion (23).

HIF-1 is a transcription factor containing alpha, beta subunits. Although beta subunit is constitutively synthesized, alpha subunit is demolished by prostheses after ubiquitination under normoxic conditions. HIF-1 alpha shows HIF activity as it is expressed fast in hypoxia. HIF-1 alpha is revealed to perform a crucial effect in angiogenesis. Once the HIF-1 alpha protein reaches the nucleus, it is coupled with HIF-1 beta to produce a heterodimer. The HIF-1 heterodimer causes activation of the gene encoding vascular endothelial growth factor-A (VEGF-A) (35). Increased expression of HIF-1 in the presence of hypoxia increases oxygenation in the affected area.

In the presence of normal oxygen concentration, proteolytic degradation of the HIF-1 alpha subunit is very fast. In cases where the concentration of oxygen decreases, the degradation of HIF-1 alpha decreases. In the presence of cancer, HIF-1 alpha also provides for the expression of a large number of genes. VEGF is an important stimulant in angiogenesis (36). Cytokines, growth factors and oncogenes that activate p42/p44 mitogen-associated protein kinase (MAPK) and/or phosphoinositidyl-3 kinase (PI-3K) pathways could rise HIF-1 alpha activity. This activity increase may be in the form of phosphorylation or an increase in HIF-1 alpha expression independent of oxygen (37).

HIF-1 binds to a region (5'-CGTG-3 ') located in the promoter region of the target gene and known as the hypoxic response element. These target genes are important in various processes such as angiogenesis, vasodilation and anaerobic metabolism, which are responsible with increasing cell survival. HIF-1 alpha activity is indicated to increase tumor growth and cause chemotherapy resistance in many studies. Dephosphorylated HIF-1 alpha stabilizes p53 and induces apoptosis.

HIF-1 is highly expressed in solid tumors and is associated with poor prognosis. The relationship between HIF-1 and poor prognosis is accepted independently of the histopathological stage (37). Carbonic anhydrase IX and glucose transporter-1 are among the transcriptional targets of HIF-1 and are used as hypoxia markers in different tumors. High HIF-1 expression reflects chronic hypoxia, and it is thought to affect prognosis in the long term. On the other hand, acute hypoxia is a factor affecting radiotherapy response, but it is known that it is less important than long term response to surgery and chemotherapy compared to chronic hypoxia.

Some biological agents have been approved to eliminate the effects of VEGF (38). Similarly, the use of HIF-1 alpha as a new target in the treatment of lung cancer is also considered.

Although it is more difficult in lung cancer than tumors that are more superficially located, there are usually three methods for assessing hypoxia: measurement of partial oxygen pressure, detection of proteins in tumor tissue or hypoxia in the blood, and visualization of hypoxia and tumor vessels.

The partial oxygen pressure (pO_2) in non-small cell lung cancer (NSCLC) was evaluated in vivo. In all except one of the twenty patients who underwent intraoperative measurement with polarographic electrode during NSCLC resection, the pO_2 value in the tumor tissue was shown to be lower than the pO_2 value of lung tissue (35). In plasma, osteopontin (OPN) and carbonic anhydrase IX (CA-IX) levels in tumor tissue were investigated and these variables were found to be strongly correlated with the ratio of pO_2 in tumor tissue to pO_2 in the lung.

Proteins associated with hypoxia in solid tumors include VEGF, BNIP3, lysyl oxidase, plasminogen activator inhibitor-1, lactate dehydrogenase isoenzyme-5, and galectin-1. However, the clinical significance of these proteins in lung cancer has not been fully established.

HIF-1 alpha expression in NSCLC is proved to be associated with T staging and poor prognosis. The expression is related with vascular invasion, pathological stage and VEGF expression (39).

Tumor Microenvironment in Angiogenesis

Endothelial cells, which are the basic building blocks of tumor angiogenesis, have very important functions in enhancement the nutrients and related gases necessary for tumor growth, continuity, and metastasis. Vascular endothelial cells actively modulate inflammatory processes in both normal and diseased tissues and directly affect tumor behavior in carcinogenesis. In particular, high vascularity around the tumor is associated with progression of tumor (40).

Angiogenesis is controlled by stimulating factors and inhibitory factors that balance them. Among the stimulating factors were VEGF, PDGF (platelet-derived growth factor), bFGF (basic fibroblast growth factor), TP (thymidine phosphorylase)/PDEC-GF (thrombocyte endothelial cell growth factor). Tumors could regulate these factors up or down to form an environment in which angiogenesis will occur. In lung cancer, activation of the VEGF/VEGFR signaling pathway is increased and VEGF overexpression is associated with poor prognosis. The increase in bFGF and PDEC-GF/TP expression was also related with poor prognosis in NSCLC patients (40). HIF-1α and HIF-2α, which have a significant role of the regulation of many genes involved in the regulation of angiogenesis, are expressed in tissue samples of NSCLC patients, and the presence of HIF-2α expression is a sign of poor prognosis (40). Expression of MMP-12 from the MMP family involved in the rearrangement of the ECM during the angiogenesis process is related with a poor prognosis in NSCLC. Increased expression of IL-8, another angiogenic factor, is associated with increased microvessel concentration and is an important prognostic marker.

Tumor development and metastasis are firmly associated with angiogenesis, potential targets include VEGF, receptor tyrosine kinases (TK) and MMPs. The first of these is the VEGF-VEGFR pathway. One of these pathways inhibiting agents is Bevacizumab, the anti-VEGF recombinant human monoclonal antibody. TKIs inhibit receptor tyrosine kinases, particularly VEGFR and EGFR.

Microenvironment Effects in Invasion And Metastasis In Non-Small Cell Lung Cancer

In the lung, anatomical and cellular properties demonstrate defensive barrier against foreign pathogens and particles. Especially in inflammatory conditions such as Chronic Obstructive Pulmonary Disease (COPD), lung microenvironment supports carcinogenesis. Lung adenocarcinomas include subtypes with different cellular and mutation heterogeneity (41). Heterogeneity is not limited to tumor epithelial cells, but also includes TME, which includes vascular, CAFs, ECM, and infiltrative immune cells. TME involves to lung cancer growth and may provide prognostic benefit. Biomarkers with the potential to identify the stage and/or type clinical and therapeutic responses of TME are described (42). CAFs are associated with cancer cell angiogenesis, proliferation, metastasis, invasion, and drug resistance. They are characterized by an increased risk of metastases and recurrence (43). At the same time, CAFs were also isolated in NSCLC expressing PD1 receptor-associated PDL1. In various studies, it was emphasized that the ECM network containing hyaluronan, MMP14 and lysyl oxidase may have therapeutic effect (44). Angiogenesis is the key sign of cancers. Tumor-derived angiogenetic factors support to endothelial cell proliferation and migration. This contributes to the progression, spread of the tumor and the formation of new capillaries which have an important role in metastasis. Anti-angiogenetic therapies, such as bevacizumab, have been included in the treatment of NSCLC (45). Combinations of bevacizumab may be preferred in non-squamous NSCLC with negative EGFR and ALK.

20–40% of patients with NSCLC occur brain metastases that is a sign of poor survival throughout their lives (46). Astrocytes produce specific inflammatory cytokines such as IL-1beta, IL-8, IL-6, and TNF into the brain microenvironment, contributing to the brain metastasis from the lung. At the same time, astrocytes secrete MMP-2 and MMP-9 also contribute to the formation of cell invasion and metastasis (47). Sprinkle expression from lung cancer cells also facilitates vascular co-option and death in the reactive brain microenvironment of these cells signal (48). Early stage of lung cancer metastasize to the brain. In the animal models of lung cancer, activation of the WNT-T cell factor (TCF) pathway leads to the activation of WNT and TCF target genes, homeobox B9 (HOXB9) and lymphoid enhancer-binding factor 1 (LEF1) also modulate invasion and growth of bone metastases. CXC-chemokine ligand12 (CXCL12; also known as SDF1alfa) of osteoclast origin, activates the pathway of ERK-nuclear factor-KB (NF-SDB) and secretion of MMP-9 by signaling through the CXC4 receptor in lung cancer cells. Thus, invasion and metastasis of the cancer cell is possible. CD8: CD68 T cell and CD8: CD4 T cell in primary and metastatic lesions in patients with NSCLC. found low (49). In another study, lung cancer cells are signified to secrete IL-6 to transport galectin-3-expressing inflammatory myeloid cells into the blood; it follows the interaction of myeloid cells in the metastatic niche, through cell surface expression of an oncofetal galectin-3 carbohydrate ligand (T antigen) of metastatic cancer cells (50).

CONCLUSION

Despite the progress in surgery, radiotherapy, chemotherapy and targeted therapies, the prognosis in the local advanced and metastatic stage at the time of diagnosis is poor in most patient. In the frame work analysis of TME in lung cancer is a new area of investigation. This is the most important obstacle given the high heterogeneity of genetic and epigenetic mutations present in the related tumor, differences in host genetic background, as well as tissue-specific responses. Understanding the cellular and molecular mechanisms underlying these processes will provide novel avenues leading to the discovery of biomarkers for development and treatment in lung cancer.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - YB; Design - AL, TS; Supervision - YB, CB, AK, AO; Literature Search - DTE, TRO, GS, ME; Writing Manuscript - TS, AL; Critical Review - YB, AL, TS

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Ahmad A, Gadgeel SM. Lung Cancer and Personalized Medicine: Novel Therapies and Clinical Management. Preface. Adv Exp Med Biol. 2016;890:v-vi. [CrossRef]
2. Donnem T, Andersen S, Al-Saad S, Al-Shibli K, Busund LT, Bremnes RM. Prognostic impact of angiogenic markers in non-small-cell lung cancer is related to tumor size. Clin Lung Cancer 2011;12:106-15. 10.1016/j.cllc.2011.03.00
3. Wood SL, Pernemalm M, Crosbie PA, Whetton AD. The role of the tumor-microenvironment in lung cancer-metastasis and its relationship to potential therapeutic targets. Cancer Treat Rev 2014;40:558–566. [CrossRef]
4. Kinoshita T, Ishii G, Hiraoka N, et al. Forkhead box P3 regulatory T cells coexisting with cancer associated fibroblasts are correlated with a poor outcome in lung adenocarcinoma. Cancer Sci 2013;104:409–415. [CrossRef]
5. Paulsson J, Micke P. Prognostic relevance of cancer-associated fibroblasts in human cancer. Sem Cancer Biol 2014;25:61–68. [CrossRef]
6. Ma J, Liu L, Che G, Yu N, Dai F, You Z. The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. BMC Cancer 2010;10. [CrossRef]
7. Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 2008;26:4410–4417. [CrossRef]
8. Chaisemartin L, Goc J, Damotte D, et al. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res 2011;71:6391–6399. [CrossRef]
9. Goc J, Germain C, Vo-Bourgais TKD, et al. Dendritic cells in tumor-associated tertiary lymphoid structures signal a th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ t cells. Cancer Res 2014;74:705–715. [CrossRef]
10. Remark R, Alifano M, Cremer I, et al. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: Influence of tumor origin. Clin Cancer Res 2013;19:4079–4091. [CrossRef]
11. Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol 2006;6:295–307. [CrossRef]
12. Wald O, Shapira OM, Izhar U. CXCR4/CXCL12 axis in non small cell lung cancer (NSCLC) pathologic roles and therapeutic potential. Theranostics 2013;3:26–33. [CrossRef]
13. Kirshberg S, Izhar U, Amir G, et al. Involvement of CCR6/CCL20/IL-17 axis in NSCLC disease progression. PLoS One 2011;6:e24856. [CrossRef]
14. Ramalingam SS, Owonikoko TK, Khuri FR. Lung cancer: New biological insights and recent therapeutic advances. CA Cancer J Clin 2011;61:91–112. [CrossRef]
15. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–264. [CrossRef]
16. Mu CY, Huang JA, Chen Y, Chen C, Zhang XG. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol 2011;28:682–688. [CrossRef]
17. Salvi S, Fontana V, Boccardo S, et al. Evaluation of CTLA-4 expression and relevance as a novel prognostic factor in patients with non-small cell lung cancer. Cancer Immunol Immunother 2012;61:1463–1472. [CrossRef]
18. Ménétrier-Caux C, Gobert M, Caux C. Differences in tumor regulatory T-cell localization and activation status impact patient outcome. Cancer Res 2009;69:7895–7898. [CrossRef]
19. Germain C, Gnjatic S, Tamzalit F, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am J Respir Crit Care Med 2014;189:832–44. [CrossRef]
20. Al-Shibli K, Al-Saad S, Donnem T, Persson M, Bremnes RM, Busund LT. The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma. Histopathology 2009;55:301–312. [CrossRef]
21. Hanahan D, Coussens LM. Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment. Cancer Cell 2012;21:309–322. [CrossRef]
22. Ilie M, Hofman V, Ortholan C, et al. Predictive clinical outcome of the intratumoral CD66b-positive neutrophil-to-CD8-positive T-cell ratio in patients with resectable nonsmall cell lung cancer. Cancer 2012;118:1726– 1737. [CrossRef]
23. Graves E, Maity A, Le Q. The tumor microenvironment in Non-Smal-Cell Lung Cancer. Semin Radiat Oncol 2010;20:156–163. [CrossRef]
24. Sica A, Allavena P, Mantovani A. Cancer related inflammation: The macrophage connection. Cancer Lett 2008;267:204–215. [CrossRef]
25. Kumar S, Chan CJ, Coussens LM. Inflammation and Cancer. In: Immunity to Pathogens and Tumors . Elsevier Inc.; 2016. pp.406–415. [CrossRef]
26. Ben-Baruch A. Inflammation-associated immune suppression in cancer: The roles played by cytokines, chemokines and additional mediators. Semin Cancer Biol 2006;16:38–52. [CrossRef]
27. Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: Short-term friend, long-term foe. Clin Cancer Res 2009;15:425–430. [CrossRef]
28. Lin GN, Peng JW, Liu PP, Liu DY, Xiao JJ, Chen XQ. Elevated neutrophil-to-lymphocyte ratio predicts poor outcome in patients with advanced non-small-cell lung cancer receiving first-line gefitinib or erlotinib treatment. Asia Pac J Clin Oncol 2017;13:e189–e194. [CrossRef]
29. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 2004;4:71–78. [CrossRef]
30. Woods NT, Monteiro AN, Thompson ZJ, et al. Interleukin polymorphisms associated with overall survival, disease-free survival, and recurrence in non-small cell lung cancer patients. Mol Carcinog 2015;54:E172–E184. [CrossRef]
31. Wang XF, Zhu YT, Wang JJ, et al. The prognostic value of interleukin-17 in lung cancer: A systematic review with meta-analysis based on Chinese patients. PLoS One 2017;12:e0185168. [CrossRef]
32. Gomes M, Coelho A, Araújo A, et al. IL-6 polymorphism in non-small cell lung cancer: a prognostic value? Tumor Biol 2015;36:3679–3684. [CrossRef]
33. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the Treatment of Non-Small-Cell Lung Cancer. N Engl J Med 2015;372:2018–2028. [CrossRef]
34. Semenza GL. Hypoxia-inducible factors: Mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci 2012;33:207–214. [CrossRef]
35. Karetsi E, Ioannou M, Kerenidi T, et al. Differential expression of hypoxia-inducible factor 1α in non-small cell lung cancer and small cell lung cancer. Clinics (Sao Paulo) 2012;67:1373–1378. [CrossRef]
36. Adini A, Adini I, Chi Z, Derda R, et al. A novel strategy to enhance angiogenesis in vivo using the small VEGF-binding peptide PR1P. Angiogenesis 2017;20:399–408. [CrossRef]
37. Swinson DEB, Jones JL, Cox G, Richardson D, Harris AL, O'Byrne KJ. Hypoxia-inducible factor-1α in non small cell lung cancer: Relation to growth factor, protease and apoptosis pathways. Int J Cancer 2004;111:43–50. [CrossRef]
38. Frezzetti D, Gallo M, Maiello MR, et al. VEGF as a potential target in lung cancer. Expert Opin Ther Targets 2017;21:959–966. [CrossRef]

39. Enatsu S, Iwasaki A, Shirakusa T, et al. Expression of hypoxia-inducible factor-1 alpha and its prognostic significance in small-sized adenocarcinomas of the lung. Eur J Cardio-thoracic Surg 2006;29:891–895. [CrossRef]

40. Herbst RS, Onn A, Sandler A. Angiogenesis and lung cancer: Prognostic and therapeutic implications. J Clin Oncol 2005;23:3243–3256. [CrossRef]

41. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong KK. Non-small-cell lung cancers: A heterogeneous set of diseases. Nat Rev Cancer 2014;14:535–546. [CrossRef]

42. Kargl J, Busch SE, Yang GHY, et al. Neutrophils dominate the immune cell composition in non-small cell lung cancer. Nat Commun 2017;8:14381. [CrossRef]

43. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer 2016;16:582–598. [CrossRef]

44. Banat GA, Tretyn A, Pullamsetti SS, et al. Immune and Inflammatory Cell Composition of Human Lung Cancer Stroma. PLoS ONE 2015;10:e0139073. [CrossRef]

45. Salmon H, Franciszkiewicz K, Damotte D, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. J Clin Invest 2012;122:899–910. [CrossRef]

46. Villano JL, Durbin EB, Normandeau C, Thakkar JP, Moirangthem V, Davis FG. Incidence of brain metastasis at initial presentation of lung cancer. Neuro Oncol 2015;17:122–28. [CrossRef]

47. Wang L, Cossette SM, Rarick KR, et al. Astrocytes directly influence tumor cell invasion and metastasis in vivo. PLoS One 2013;8:e80933. [CrossRef]

48. Valiente M, Obenauf AC, Jin X, et al. Serpins promote cancer cell survival and vascular Co-option in brain metastasis. Cell 2014;156:1002–1016. [CrossRef]

49. Müller P, Rothschild SI, Arnold W, et al. Metastatic spread in patients with non-small cell lung cancer is associated with a reduced density of tumor–infiltrating T cells. Cancer Immunol Immunother 2016;65:1–11. [CrossRef]

50. Reticker-Flynn NE, Bhatia SN. Aberrant glycosylation promotes lung cancer metastasis through adhesion to galectins in the metastatic niche. Cancer Discov 2015;5:168–181. [CrossRef]