The Microenvironment of Lung Cancer and Therapeutic Implications

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Abstract The tumor microenvironment (TME) represents a milieu that enables tumor cells to acquire the hallmarks of cancer. The TME is heterogeneous in composition and consists of cellular components, growth factors, proteases, and extracellular matrix. Concerted interactions between genetically altered tumor cells and genetically stable intratumoral stromal cells result in an "activated/reprogramed" stroma that promotes carcinogenesis by contributing to inflammation, immune suppression, therapeutic resistance, and generating premetastatic niches that support the initiation and establishment of distant metastasis. The lungs present a unique milieu in which tumors progress in collusion with the TME, as evidenced by regions of aberrant angiogenesis, acidosis and hypoxia. Inflammation plays an important role in the pathogenesis of lung cancer, and pulmonary disorders in lung cancer patients such as chronic obstructive pulmonary disease (COPD) and emphysema, constitute comorbid conditions and are independent risk factors for lung cancer. The TME also contributes to immune suppression, induces epithelial-to-mesenchymal

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transition (EMT) and diminishes efficacy of chemotherapies. Thus, the TME has begun to emerge as the "Achilles heel" of the disease, and constitutes an attractive target for anti-cancer therapy. Drugs targeting the components of the TME are making their way into clinical trials. Here, we will focus on recent advances and emerging concepts regarding the intriguing role of the TME in lung cancer progression, and discuss future directions in the context of novel diagnostic and therapeutic opportunities.

Keywords Microenvironment • Lung cancer • Inflammation • Immune cells • Angiogenesis • Endothelial cells • Bone marrow • Hypoxia • Therapy • Immunotherapy • Radiation • Resistance

1 The Tumor Microenvironment: An Overview

The TME has been recognized as a major contributor to tumor progression and metastasis [1–4]. The TME is heterogeneous in composition, and concerted heterotypic reciprocal interactions between genetically altered tumor epithelial cells and intratumoral stromal cells regulate major hallmarks of cancer including angiogenesis, inflammation, immune suppression, epithelial-to-mesenchymal transition (EMT), and metastasis [1, 3]. Importantly, strategies that target the TME are being considered in cancer prevention [5–7].

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The stromal cells recruited to the tumor beds are "educated" and "reprogramed" by the paracrine activity of tumor epithelial cells to acquire an "activated" protumorigenic phenotype [8–10]. Examples of tumor-activated stromal cells include macrophages (classically activated M1 to alternatively-activated M2 phenotype) [11, 12], neutrophils (N1 to N2 conversion) [11], fibroblasts (conversion to activated cancer-associated fibroblasts (CAFs)) [13], endothelial cells [14] and immune cells [15]. These activated stromal cells promote tumor growth and have begun to emerge as attractive targets for anti-cancer therapy [1, 5, 16, 17].

The "angiogenic switch" is a critical step in tumor growth and in the progression of micrometastasis to lethal macrometastasis [1, 18, 19]. The molecular players and mechanisms underlying the angiogenic switch have been intensely investigated, and a variety of pro-angiogenic factors and angiogenic inhibitors that play critical roles during the angiogenic switch have been identified and characterized. Insights from these investigations have led to the development of various pro- and anti-angiogenic therapies that are currently tested in clinical trials or are already in clinical use. Inhibition of angiogenesis by neutralizing antibodies against vascular endothelial growth factor (VEGF) is effective at reducing progression of certain tumors despite having little effect on most tumor cells [7]. In addition to endothelial cells, the inflammatory cells, particularly cells of the myeloid lineages (monocytes, macrophages, and neutrophils) and CAFs progressively accumulate in tumors, where they establish an inflammatory protumorigenic TME [12, 20]. Inflammation is now accepted as an underlying or enabling characteristic that contributes to key hallmarks of cancer, and non-steroidal anti-inflammatory drugs have shown a reduction in cancer risk [21, 22] and may prevent distant metastasis [23]. Myeloid cells also secrete VEGF, basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), placental growth factor (PIGF), and Bv8, that contribute to vascular remodeling during tumor progression [24, 25]. Myeloid cells also secrete proteases such as urokinase-type plasminogen activator (uPA) and matrix metalloproteinases (MMPs), which degrade extracellular matrix (ECM) components to release VEGF and other sequestered mitogenic factors that facilitate endothelial migration and tumor invasion [26].

Tumor-associated macrophages (TAMs) accumulate in regions of hypoxia [27] and support multiple aspects of tumor progression [28]. Studies from breast cancer and glioblastoma have shown that TAMs promote invasive cellular phenotypes [29], through a paracrine signaling loop that involves tumor-derived colony-stimulating factor 1 (CSF-1) and macrophage-derived epidermal growth factor (EGF) [30–32]. TAMs also secrete proteases, such as cysteine cathepsins, which support tumor progression and confer therapeutic resistance [33, 34]. The therapeutic potential of targeting TAMs has been demonstrated in breast cancer and in glioblastoma [6, 34, 35].

The stromal cells also generate inflammatory conditions that contribute to tumorigenesis [20, 36, 37]. The inflammation-responsive Ikappa B kinase (IKK)-beta and its target nuclear factor kappa B (NF-κB) have important tumor-promoting functions within malignant cells and inflammatory cells (macrophages, lymphocytes) [38]. From a clinical perspective, a strong tumor-associated inflammatory response can be initiated by cancer therapy. For example, radiation and chemotherapy cause massive necrotic death of cancer cells and surrounding tissues, which in turn trigger

an inflammatory reaction. Therapy-induced inflammation may have tumor-promoting functions [39, 40], or may enhance the cross-presentation of tumor antigens and subsequent induction of an anti-tumor immune response [41].

Cells and molecules of the immune system are a fundamental component of the TME. The tumor-infiltrating immune cells constitute two distinct compartments mediating the innate and adaptive immune responses. The innate immune system consists of phagocytes including neutrophils, mast cells/macrophages ($CD68^+$), dendritic cells (DC), natural killer NK cells ($CD56^+$ $CD3^-$), and NK T cells ($CD56^+$ $CD3^+$), and mainly serves as the first-line defense against both foreign pathogens and transformed cells. However, the tumor "reprogramed" innate immune system stimulates tumor growth by promoting tumor angiogenesis, invasion, and metastasis; whereas the adaptive immune system tends to repress tumor growth. The adaptive immune system is mediated by two major T lymphocyte subsets; cytotoxic T cells (CTL) ($CD8^+$) and helper T cells (Th) ($CD4^+$), and B cells ($CD20^+$). The adaptive immune system is the second-line defense, acting via antigen-specific molecules and requiring clonal expansion following the recognition of foreign antigens. However, in the TME, cancer cells often induce an immunosuppressive microenvironment, which favors the development of immunosuppressive populations of immune cells, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Treg). Understanding the complexity of immunomodulation by tumors is important for the development of immunotherapy, and among the most promising approaches to activating therapeutic antitumor immunity is the blockade of immune checkpoint pathways [42].

MDSCs are a heterogeneous population of immature myeloid progenitors, and precursors of macrophages, granulocytes and dendritic cells [43, 44]. In general, MDSCs from cancer patients express the common myeloid markers CD33 and CD11b, display heterogeneous expression of CD14 (monocytic) and CD15 (granulocytic) markers, but lack mature myeloid or lymphoid markers such as HLA-DR [45, 46]. Clinical correlation studies in breast, colorectal, pancreatic, esophageal, and gastric cancer patients demonstrated that MDSC levels confer an independent prognostic factor for survival [47, 48]. Since MDSCs the major regulators of the immune response due to their ability to suppress both the cytotoxic activities of natural killer (NK) and NKT cells, and the adaptive immune response mediated by $CD4^+$ and $CD8^+$ T cells [44, 49, 50], this cell type has generated much attention. While the mechanism of NK cell inhibition is currently not well understood, multiple pathways are responsible for MDSC-mediated T cell suppression including: (1) production of Arginase 1 (ARG1), which depletes L-Arginine from the microenvironment, and (2) production of nitric oxide synthase 2 (NOS2). Both pathways block translation of the T cell CD3 zeta chain, inhibit T cell proliferation, and promote T cell apoptosis [51]. Not much is known regarding upstream regulators of these suppressive mediators. However recent studies have demonstrated the importance of key signaling pathways such as PI3K, Ras, JAK-STAT, and TGFβ—STAT3 signaling [52, 53]. In mice, MDSCs have been defined as $CD11b^+$ $Gr1^+$ cells and can be subdivided into granulocytic ($CD11b^+$ $Ly6G^+$ $Ly6C^{low}$) or monocytic ($CD11b^+$ $Ly6G^-$ $Ly6C^{hi}$) [54]. The mechanisms by which MDSCs are generated and contribute to immune suppression is being exploited for developing anti-MDSC agents [55]. Approaches to inhibit MDSCs include use of phosphodiesterase (PDE) inhibitors,

nitroaspirins, synthetic triterpenoids, COX2 inhibitors, ARG1 inhibitors, anti-glycan antibodies, CSF-1R antagonists, IL-17 inhibitors, and histamine-based approaches. In another approach, MDSCs differentiate by using all-trans retinoic acid (ATRA), vitamins A or D3, or IL-12 [56]. Some compounds, such as ATRA, PDE5 inhibitors, nitroaspirins (e.g. NCX-4016), or tyrosine kinase inhibitors, are being tested in clinical trials to mediate suppression of MDSCs, and improve the efficacy of immune modulating therapies (immune checkpoint inhibitors or cancer vaccines). Notably, pre-clinical evidence suggests that cancer vaccines are more effective in tumor-bearing mice that have been depleted of MDSCs.

2 TME in Lung Cancer Prognosis

Lung cancer is the leading cause of cancer-related deaths worldwide [57]. Lung cancer is generally classified into two histopathological subtypes, small-cell lung carcinoma (SCLC) and non–small cell lung carcinoma (NSCLC). NSCLC accounts for 80% of all lung malignancies, and the overall 5-year survival of patients with this disease remains approximately 15% [58]. A major research focus in lung cancer has been directed to cancer cell intrinsic properties [59–61], which has led to the discovery of important driver mutations and the development of targeted therapies, such as the receptor tyrosine kinase (RTK) inhibitors gefitinib/erlotinib (EGFR inhibitors) and crizotinib (EML4-ALK inhibitor) [62–64]. However, these treatments benefit only a small proportion (15–20%) of patients harboring these driver mutations, and acquired resistance to these therapies presents a major impediment to the effective treatment of NSCLC patients with these mutations [65–67]. More recent studies have begun to elucidate the prognostic and pathophysiological role of the TME in lung cancer.

Many studies have examined the contribution of tumor epithelial molecular markers for prognosis and guidance of cancer therapy, yet only a few have focused on the analysis of the tumor-associated stroma for the identification of prognostic and predictive markers in cancer therapy. More recent studies have begun to demonstrate the prognostic role of TME in cancer with the promise to advance discovery of prognostic and predictive molecular markers for patient management and cancer therapy. For example, stromal gene signatures have been shown to predict clinical outcome and resistance to therapy in breast cancer [68, 69], and fibroblast-derived transcriptional signatures were associated with cancer progression and poor outcome in human breast and lung cancer [70, 71]. In patients with stage I NSCLC, the presence of CAFs is a poor prognostic indicator typically associated with nodal metastases and a higher risk of recurrence [72]. Interestingly, a specific 11-gene expression signature in CAFs stratified NSCLC patients into low and high-risk groups, and was associated with survival [71]. Similarly, prognostic gene signatures from bulk NSCLC tissue analysis included prominent stromal genes such as glypican 3, ICAM-1, laminin B1, L-selectin, P-selectin, and SPARC [73, 74]. High numbers of circulating endothelial cells (CECs) and high levels of soluble CD146 (sCD146) in the plasma have been shown to correlate with poor prognosis and may be useful for the prediction of clinical outcome in patients undergoing surgery for NSCLC [75].

Recently, several groups have demonstrated that the immune fraction of the TME has prognostic value in lung cancer. Elevated numbers of MDSCs have been associated with poor clinical outcomes [76, 77]. Similarly, leukocyte infiltrates, particularly increased numbers of neutrophils, were significantly associated with a worse outcome in patients with bronchioalveolar carcinoma [78–80]. Tumor-infiltrating mature dendritic cells have been suggested to identify patients with early-stage NSCLC who have a high risk of relapse [81, 82]. High density of stromal $CD56^+$ NK cells was shown to be an independent factor associated with improved prognosis in resected NSCLC [81]. TAMs are abundant components of NSCLC, and clinical data correlating the apoptotic index and/or macrophage densities and polarization status (M1/M2) with outcome in NSCLC patients has been recently reviewed [83]. The number of macrophages in NSCLC stroma is an independent predictor of survival time in NSCLC patients [84]. Similarly, mast cells [85], cytotoxic T cells [86], and helper T cells [87] have been reported as potential prognostic factors following resection in patients with NSCLC. Recently, tumor-infiltrating $FOXP3^+$ Treg cells were positively correlated with intratumoral COX-2 expression and were associated with a worse recurrence-free survival (RFS), especially among patients with node-negative NSCLC [88]. Stromal CD99 expression has been described as a novel prognostic marker in human NSCLC [89], and humoral immune response immunoglobulin kappa C (IGKC) expression in tumor-infiltrating plasma cells was shown to have prognostic value in NSCLC [90].

3 TME in Lung Cancer Progression and Metastasis

The stroma in NSCLC is heterogeneous, comprised of many different populations of cells, including bone marrow-derived immune and inflammatory cells, fibroblasts, and endothelial cells (Fig. 1). The contribution of these cell types to tumor growth is illustrated below.

3.1 Cancer-Associated Fibroblasts (CAFs)

As is the case for many solid tumors, the TME of human NSCLC often demonstrates significant desmoplasia, which is characterized by stromal changes depicted by the presence of activated stromal fibroblasts [91–93]. In addition, several mouse explant studies have suggested a pro-tumorigenic role for tumor-derived lung fibroblasts in NSCLCs [94–96]. CAFs, which differ morphologically and functionally from normal fibroblasts (NFs), exhibit similar activities with wound-activated fibroblasts, suggesting that the supportive and reparative roles of activated fibroblasts in wound healing contribute to the pro-tumorigenic activities of CAFs. The origin of CAFs is not clear, yet it is likely that they arise from a reprogramming of tissue resident fibroblasts [97] as well as differentiate from BM cells recruited to the tumor [98].

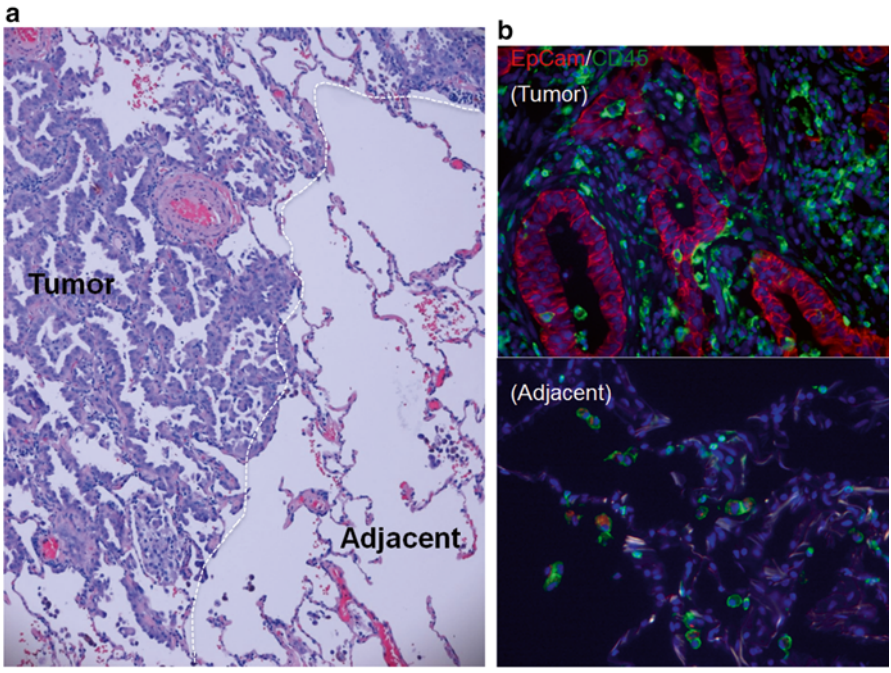

Fig. 1 Infiltration of BM hematopoietic cells in the adenocarcinoma and matched adjacent lung. (**a**) H&E staining of lung tissue from an adenocarcinoma patient (×20 magnification). (**b**) Representative immunofluorescence image of tumor and matched adjacent non-neoplastic lung of adenocarcinoma patient stained for epithelial cells ($EpCAM^+$, *red*) and BM-derived hematopoietic cells ($CD45^+$, *green*). DAPI (*blue*) was used to label cell nuclei

CAFs have been reported to support tumor progression, metastasis, and chemotherapy resistance by a wide variety of mechanisms, including direct paracrine support of cancer cells via the secretion of growth factors, cytokines, and chemokines, through pro-angiogenic effects, as well as by remodeling the extracellular matrix [99–102].

A number of different mechanisms have been specifically reported for the protumorigenic activity of CAFs in NSCLC. A paracrine crosstalk between fibroblasts and NSCLC cells involves IL-6 and TGFβ-enhanced EMT and tumor progression [97, 103]. Cross-species functional characterization of mouse and human lung CAFs identified a secreted gene signature, and functional studies identified important roles for cardiotrophin-like cytokine factor 1 (CLCF1)-Ciliary Neurotrophic Factor Receptor (CNTFR) and interleukin (IL)-6–IL-6R signaling in promoting growth of NSCLCs [104]. A paracrine network was described, involving Insulin-like growth factor-II (IGFII)/IGF1 receptor (IGF1R)-Nanog signaling pathway by which CAFs contributed to cancer stem cell enrichment in NSCLC [105]. Importantly, this paracrine signaling predicted overall and relapse-free survival in stage I NSCLC patients. Similarly, pulmonary fibroblasts induced EMT and stem cell potential in NSCLC [106]. Fibroblast-derived hepatocyte growth factor (HGF) was shown to induce EGFR-tyrosine kinase inhibitor (TKI) resistance in NSCLC with EGFR-activating mutations [107, 108].

The lack of a single pro-tumorigenic activity likely reflects the heterogeneity of CAFs within a tumor. Although there are several markers of CAFs (e.g. α-smooth muscle actin (αSMA), fibroblast-activating protein (FAP), and fibroblast-specific protein (FSP)), no distinct single marker of CAFs exist, and none of the commonly used markers for CAFs are unique to CAFs [94]. Compounding this heterogeneity of CAFs within a tumor is the heterogeneity of CAFs among different tumors. It is likely that specific cancer cells require distinct support from CAFs. For example, in a recent study, metabolic reprogramming in NSCLC-CAFs was shown to correlate with increased glycolytic metabolism of the tumor, indicating tumor-specific specialization of CAFs [109].

3.2 Endothelial Cells

Endothelial cells that form the vasculature have key functions in providing nutrients and oxygen to the tumor. However, emerging studies have begun to describe "angiocrine" regulation as a major endothelial function in cancer [110]. Vascular endothelial cells actively participate in and regulate the inflammatory response in both normal and diseased tissues [111], and emerging data suggests that endothelial cells directly influence tumor behavior [18, 112]. In NSCLC, the degree of tumor-associated angiogenesis correlates with disease progression and predicts unfavorable survival outcome [113]. In particular, high vascularity at the tumor periphery has been correlated with tumor progression [114]. However, high steady state vessel density in the lung has imposed challenges in accurate identification and quantification of neoangiogenic microvessels in the tumor tissue. Notably, some NSCLCs do not display an angiogenic phenotype and these tumors are invasive, exploiting the pre-existing alveolar vessels for growth [115, 116].

In a recent study, endothelial-derived angiocrine signals were shown to induce regenerative lung alveolarization. Particularly, activation of VEGFR2 and FGFR1 in pulmonary capillary endothelial cells induced MMP14 expression that unmasked EGF receptor ligands to enhance alveologenesis [117]. Lung endothelial cells also control lung stem cell differentiation, as bone morphogenetic protein 4 (BMP4)-BMPR1A signaling triggers calcineurin/NFATc1-dependent expression of thrombospondin-1 (Tsp-1) in lung endothelial cells to promote alveolar lineage-specific bronchioalveolar stem cell differentiation [118]. Using a mouse model of lung adenocarcinoma, it was shown that perlecan, a component of the ECM, secreted by endothelial cells in a paracrine fashion blocked proliferation and invasiveness of lung cancer by impacting pro-inflammatory pathways [112].

3.3 Hypoxia in Lung Cancer

Hypoxia is typically present in solid tumors, like lung cancer, and is known to enhance tumor progression and therapy resistance [119]. The effects of hypoxia are largely mediated by the hypoxia-inducible factors (HIFs) HIF-1α and HIF-2α, as

they activate the transcription of genes implicated in tumor angiogenesis, cell survival, and resistance to chemotherapeutic drugs [120]. The overexpression of HIF-1α confers cellular resistance to the EGFR-blocking mAb cetuximab in epidermoid carcinoma cells. In addition, knocking down HIF-1α substantially restores cellular sensitivity to cetuximab-mediated antitumor activities [121]. These findings suggest that HIF-1α expression is associated with the therapeutic responses of cancer cells to EGFR-targeted therapies. More recently, the involvement of hypoxia in the resistance to EGFR-TKIs, such as gefitinib and erlotinib, in NSCLC with an EGFR-sensitive mutation was shown to be mediated by TGFβ [122]. The hypoxic microenvironment is an important stem cell niche that promotes the persistence of cancer stem cells (CSCs) in tumors. Importantly, hypoxia was shown to increase the population of lung CSCs resistant to gefitinib in EGFR mutation-positive NSCLC by activating IGF1R [123].

3.4 Inflammation

Chronic lung inflammation has been associated with an increased risk of lung cancer. Carcinogens including asbestos, cigarette smoke, and other pollutants are known to cause a chronic inflammatory state, which in turn promotes tumorigenesis [20]. Moreover, pulmonary disorders such as COPD/emphysema and pulmonary fibrosis, which are associated with greater risk for developing lung cancer, are characterized by copious inflammation [124–126]. It remains unclear whether inflammation affects the incidence of driver oncogenic mutations. However, inflammation has been shown to enhance tumor progression. Lipopolysaccharide (LPS), a potent endotoxin eliciting chronic lung inflammation, significantly increased the risk of carcinogen-mediated lung tumorigenesis in mice through K-ras gene activation by point mutations [127]. Recently, it was demonstrated that mucin 1 (MUC1) contributes to smoking-induced lung cancers that are driven by inflammatory signals from macrophages, and a signaling pathway involving PPAR-γ, ERK, and MUC1 resulted in TNFα secretion in macrophages [128].

Inflammation has also been described in the generation of lung metastasis from extrapulmonary neoplasms. Clinical studies suggested a correlation between smoking and an increased risk of lung metastasis in patients with breast cancer [129, 130] and esophageal cancer [131]. In addition, inflammation caused by smoke inhalation in mice was also correlated with increased incidence of lung metastasis [132]. Data on autoimmune arthritis showed that lung inflammation in arthritic mice, characterized by neutrophil and mast cell infiltration, as well as increase in circulating levels of pro-inflammatory cytokines, was associated with enhanced lung metastasis [133, 134]. Recently, several mechanisms explaining the metastasis-promoting effects of inflammation have been elucidated. LPS-induced acute lung inflammation dramatically increased breast cancer cell metastasis to lung via a ubiquitin/CXCR4-dependent mechanism [135]. Systemic LPS-induced inflammation led to elevated levels of E-selectin expression in lung tissue and enhanced lung metastasis of breast

cancer cells [136]. Induction of lung inflammation by specific NF-κB activation in airway epithelial cells increased lung metastasis via a macrophage-dependent mechanism [137]. Bladder cancer cells expressing the proteoglycan versican metastasize to the lungs via a mechanism involving increased lung CCL2 chemokine expression and macrophage infiltration [138]. The recruitment of CCR2 (the receptor for chemokine CCL2)-expressing monocytes/macrophages to the metastatic site in response to CCL2 enhances breast tumor metastasis to lungs [139]. Lewis lung carcinoma (LLC) cells express versican and subsequently activate TLR2: TLR6 complexes on myeloid cells, inducing TNFα secretion and thus enhancing LLC metastatic growth [140]. Another study showed that $CD11^+$ $Gr1^+$ $Ly6C^{high}$ myeloid progenitor cells express versican in the premetastatic lung, leading to stimulation of mesenchymal-to-epithelial transition of metastatic tumor cells, increasing cell proliferation and accelerating metastasis [8]. Furthermore, these pre-metastatic niches are characterized by the induction of chemoattractants such as, S100A8, growth factors, ECM proteins including fibronectin, and ECM-modifying proteins like lysyl oxidase [141–144], creating a permissive microenvironment for metastasis [145]. Importantly, S100A8/A9 expression in the pre-metastatic niche in turn induces expression of serum amyloid A (SAA) 3, which through the Toll-like receptor 4 (TLR4) leads to the activation of NF-κB signaling and further amplification of inflammatory responses, accelerating lung metastasis [146].

3.5 Immune Cells

Tumors utilize various mechanisms to evade destruction by the immune system. One of the key immunomodulatory mechanisms is via immune checkpoint pathways, which play a key role in regulating T-cell responses. Under normal circumstances, the immune checkpoints are important to maintain self-tolerance by preventing autoimmunity and protecting the tissue from damage when the immune system is activated. The expression of immune checkpoint proteins are usually exploited by the tumor cells to develop resistance mechanisms.

3.5.1 T-Cells

Tumor-infiltrating lymphocytes (TILs) are often found in the TME, suggesting an immune response against the tumor. Among the TILs, $CD8^+$ cytotoxic T lymphocytes (CTLs) are directly capable of killing tumor cells, whereas $CD4^+$ T helper lymphocytes (Th) are a heterogeneous cytokine-secreting class of T lymphocytes. Th1 subtypes activate CTLs, whereas Th2 lymphocytes stimulate humoral immunity. Besides the Th1 and Th2 subsets, the $CD4^+$ regulatory T lymphocyte (Treg) subset suppresses effector T lymphocytes. In cancer, Tregs preferentially traffic to tumors, as a result of chemokines produced by tumor cells and microenvironmental macrophages. While active immunotherapy such as adoptive T cell-transfer

represents one promising therapeutic approach in lung cancer, more recently, immune checkpoint blockade has received tremendous attention as a potential therapy in solid tumors including lung cancer. The two major immune checkpoint inhibitory pathways involve the programmed cell death-1, PD-1/ PD-L1 pathway and the cytotoxic T-lymphocyte antigen-4, CTLA-4 pathway [147]. PD-1 is a surface receptor member of the B7-CD28 superfamily. It is expressed on many cell types, including activated T cells, B cells, NK cells, and host tissues. PD-1 binds with its ligand PD-L1 (B7-H1, CD274) on antigen presenting cells (APCs), and this interaction inhibits downstream NF-κB transcription and downregulates interferon (IFN)-γ secretion, resulting in T-cell tolerance. Similarly, PD1 can also interact with PD-L2 on dendritic cells, and PD-L2 also has effective inhibitory activity upon T cells. CTLA-4 is expressed on the surface of activated cytotoxic T cells, and it competes with the costimulatory molecule CD28 for mutually shared ligands, B7-1 (CD80) or B7-2 (CD86), and these interactions inhibit the antitumor activity of T-cells.

Recent understanding of the functioning of the immune system and its relation to tumor evasion have led to the development of novel agents that have promising results in the treatment of NSCLC. These agents include immune checkpoint inhibitors such as anti-PD-1 antibodies (nivolumab and MK-3475), anti-PD-L1 antibody (MPDL3280A, MEDI4736), and CTLA-4 inhibitors (tremelimumab and ipilimumab), as well as vaccines.

3.5.2 γδ T Cells

γδ T cells contribute to lymphoid antitumor surveillance and bridge the gap between innate and adaptive immunity [148]. γδ T cells constitute 1%–5% of peripheral blood T lymphocytes and recognize phosphoantigens via polymorphic γδ T-cell antigen receptors (TCR), and develop strong cytolytic and Th1-like effector functions [149]. Therefore, γδ T cells are attractive candidate effector cells for cancer immunotherapy, as they can secrete cytokines abundantly and exert potent cytotoxicity against a wide range of cancer cells. Clinical trials have been conducted to evaluate the safety and efficacy of γδ T-cell-based immunotherapies for non-Hodgkin's lymphoma, multiple myeloma, and solid tumors. In lung cancer, the therapeutic impact of adoptive immunotherapy with expanded γδ T-cells is being assessed [150, 151], and in one study, remission of lung metastasis following adoptive immunotherapy using activated autologous γδ T-cells in a patient with renal cell carcinoma was observed [152].

3.5.3 Myeloid-Derived Suppressor Cells

Increase in the number of MDSCs induces a strong immunosuppressive activity in cancer patients [153–155]. In a mouse model of lung cancer, MDSC depletion increased APC activity and augmented the frequency and activity of NK and T cell effectors that led to impaired tumor growth, enhanced therapeutic vaccination

responses, and conferred immunological memory [156, 157]. Immune suppressive MDSCs, defined as $Lin^{-}HLA\text{-}DR^{-}CD33^{+}$ and $CD14^{-}CD11b^{+}$ $CD33^{+}$ [158] were increased in patients with lung cancer. Analysis of 89 patients with NSCLC showed an increase in both frequency and absolute number of MDSCs in the peripheral blood and indicated an association with metastasis, response to chemotherapy, and progression-free survival [159].

4 TME of Premetastatic Niche in the Lung

The lung is one of the most frequent sites of metastasis from extrapulmonary neoplasms including breast and colon cancer. As early as 1889, Steven Paget proposed his "seed" and "soil" hypothesis establishing the concept that primary tumors metastasize to specific organs which harbor a receptive microenvironment [160]. More recently, experimental support for this hypothesis has been provided by studies showing that primary tumors release specific cytokines such as VEGF, SDF-1, TGFβ, and TNFα, which systemically initiate premetastatic niches. These premetastatic niches are characterized by the accumulation of BM-derived cells, and selective induction of organ-specific chemoattractants, growth factors, and ECM-related proteins, which provide permissive local microenvironments for recruiting the incoming tumor cells, leading to the initiation and establishment of micrometastases [145]. Pioneering studies by Lyden and colleagues have shown that the premetastatic niche is comprised of BM-derived $VEGFR1^{+}$ hematopoietic progenitor cells, which express VLA-4 (also known as integrin α4β1), and that tumor-specific growth factors upregulate fibronectin, a VLA-4 ligand in resident fibroblasts, suggesting a possible mechanism by which the permissive niche recruits incoming tumor cells [143, 161]. Similarly, Hiratuska et al. have demonstrated that tumor-secreted factors including VEGF-A, TGFβ, and TNFα induce expression of chemoattractants, such as S100A8 and S100A9 by lung endothelial cells and $Mac1^{+}$ myeloid cells [143, 161], that facilitate the homing of tumor cells to the premetastatic sites, via induction of serum amyloid A3 (SAA3). Notably, SAA3 stimulated NF-κB signaling in the macrophages via TLR4 and facilitated metastasis [146], suggesting the therapeutic potential of blocking SAA3-TLR4 for the prevention of pulmonary metastasis. Giaccia and colleagues have shown that lysyl oxidase (LOX) secreted by hypoxic tumors accumulates in the lungs and supports premetastatic niche formation. LOX remodels ECM by crosslinking collagen IV, which recruits $CD11b^{+}$ myeloid cells that cleave collagen by secreting MMP2, enhancing the invasion and recruitment of BM cells and metastasizing tumor cells. LOX inhibition prevents $CD11b^{+}$ cell recruitment and metastatic growth. $CD11b^{+}$ cells and LOX were also shown to colocalize in biopsies of human metastases [142, 162, 163].

In another mechanism, within the premetastatic niche, fibroblasts expressed periostin which contributed to cancer stem cell maintenance and expansion through Wnt signaling leading to metastasis [164]. In a similar study, metastatic tumor cells, by secreting tenascin C, enhanced stem cell signaling via Notch in the metastatic

niche [165]. In the premetastatic lung, BM-derived myeloid progenitor cells were shown to secrete the proteoglycan versican, which induced mesenchymal -to-epithelial transition (MET) of disseminated metastatic tumor cells, accelerating tumor outgrowth in the lungs [8, 166]. Notably, this tumor outgrowth was facilitated by BM-derived endothelial progenitor cells (EPCs), which by initiating the angiogenic switch resulted in the progression of micro- to macrometastases [167]. The premetastatic niche has become an exciting area of research in the quest for novel therapeutic and prophylactic strategies against metastasis [168]. In contrast, a novel mechanism was recently described, whereby metastasis-incompetent tumors generate metastasis-suppressive microenvironments in the lungs by inducing the expression of a potent antiangiogenic factor, thrombospondin 1 (Tsp-1), in the recruited BM-derived myeloid cells [169]. Tsp-1 induction is mediated by the activity of prosaposin (PSAP), a protein secreted by poorly metastatic cells, which acts systemically to reprogram myeloid cells into metastasis-inhibitory cells [169].

5 The Contribution of TME to Therapeutic Resistance

A major research focus to determine the mechanisms of therapeutic resistance has largely been the analysis of tumor cells, and resistance mechanisms involving secondary pathway mutations or bypass mechanisms within the tumor cells, such as EGFR (T790M) mutations or MET receptor amplification have been identified. Importantly, more recent studies have begun to unravel that heterologous cell types within tumors can actively influence therapeutic response and elicit resistance [170, 171].

5.1 Contribution of TME to Resistance to Radiation Therapy

Given that lung cancer is one of the leading causes of death from cancer worldwide, new and effective treatments are urgently needed [172, 173]. Approximately 70% of NSCLC patients receive radiotherapy (RT), either alone or in combination with other treatment modalities such as surgery or chemotherapy [174]. In patients who are unable to tolerate surgical resection because of medical co-morbidities, conventional RT is an alternative, but with poor long-term survival of 15–30% and local failure of up to 50% [175–177]. Retrospective and nonrandomized prospective data suggest that further dose escalation in NSCLC may be associated with better outcomes [178–181]. Additional improvement of the therapeutic ratio for NSCLC will likely come from different radiation dosing schedules. However, for patients with locally advanced disease, the benefit of dose escalation beyond 60 Gy has not been supported by level I evidence. A recent randomized study by the Radiation Therapy Oncology Group (RTOG) in patients with locally advanced NSCLC showed worse survival rates for patients receiving 74 Gy versus 60Gy with concurrent chemotherapy [182].

Accurate delivery of the ionizing radiation (IR) that allows more precise deposition of dose in the tumor while progressively reducing any unwanted dose to surrounding normal tissues has motivated hypofractionated radiation schedules [174]. Stereotactic body RT (SBRT) takes advantage of this favorable dose distribution and gained credence recently as a result of phase II studies with promising outcomes for early-stage medically inoperable NSCLC [183]. However, lack of pathological confirmation of primary tumor control, different definitions of NSCLC control after SBRT, and serious toxicity, particularly for centrally placed tumor, raises concerns about the utility of dose escalation [184, 185]. Clinical factors can explain some of the failures, such as a large tumor and/or advanced tumor stage, but many failures still go unexplained, for tumors with apparently similar sizes, stages, grades, and delivered doses.

It is clear from such clinical considerations and from a wealth of experimental research, that biological factors also have a crucial role in determining treatment success. The main biological factors affecting outcome after RT [186] include intrinsic radioresistance of the tumor cells [187], the ability of the surviving cells, including cancer stem cells, to repopulate [188], and the extent of hypoxia. Sensitizing strategies commonly focus on either targeting intrinsic properties of tumor cells or the vasculature. Recently, targeting the TME has become an even more compelling option to impede tumor progression and augment RT responses [189, 190]. For example, the recognition that tumor infiltration by inflammatory cells and other BM-derived cells contributes to RT responses, particularly tumor regrowth, provides a new route to augment RT efficacy [191, 192].

There is considerable evidence that the microenvironment regulates many tumor responses to radiation, thus providing novel routes for manipulating the response to radiotherapy [193–195]. Of particular interest is the activity of TGFβ, which is a critical signal in cancer and plays a detrimental role to tumor responses to RT. In NSCLC, increased TGFβ activity correlates with tumor progression, increased tumor growth and angiogenesis [196]. TGFβ signaling activation in TME has been identified as a key factor for chemotherapy resistance in NSCLC [197]. Although little is known about how TGFβ modulates the irradiated TME, given its pleiotropic roles in NSCLC, TGFβ inhibition may increase tumor cell radiosensitivity and shift the microenvironment to augment NSCLC response to radiotherapy. TGFβ ligands are enriched in the TME, where their production by stromal or tumor cells varies according to tumor phenotype [198]. The use of clinically viable TGFβ inhibitors in oncology is motivated by rationales to reduce metastasis, augment existing cancer therapies, and to improve tumor vaccines [199]. TGFβ signaling blockade enhances glioblastoma (GBM) response to chemoradiation in preclinical models [200, 201], and specifically inhibits GBM cancer stem cell renewal in vitro and in vivo [202].

In addition to a well recognized phenomenon of the impact of TGFβ on tumor-promoting effects and metastasis [203], TGFβ mediates an effective DNA damage response in epithelial cells via control of ATM kinase activity [204]. TGFβ activity is controlled by production as a latent complex that requires extracellular modification to initiate ligand binding to ubiquitous receptors; this activation is efficiently

induced by ionizing radiation, in part due to the presence of a redox sensitive motif in the latency associated peptide (reviewed in [189]). As a consequence, we have shown that inhibiting TGFβ promotes clonogenic cell death of mouse and human breast cancer and GBM cells in vitro and that systemically neutralizing TGFβ enhances RT action in GBM and breast cancer preclinical models [205, 206]. Given that radiation-induced TGFβ is also a significant factor in lung fibrosis, a late tissue toxicity that limits effective tumor control [207], the application of TGFβ antagonists in radiation treatment of NSCLC is clinically viable.

Recent preclinical studies support the potential for improving radiotherapy by use of TGFβ inhibitors (Du and Barcellos-Hoff, unpublished data). As observed for brain and breast tumors [205, 208], most murine and human lung cancer cells were sensitized by TGFβ inhibition prior to radiation, as measured by in vitro clonogenic assays. Using the Lewis lung cancer syngeneic subcutaneous tumors, tumor growth control was significantly improved by use of TGFβ neutralizing antibodies concurrent with single or fractionated radiation treatment. Notably, even though irradiated tumors treated with TGFβ inhibition were significantly smaller at experiment termination, hypoxia was higher and vessel density was also significantly more decreased than that of non-irradiated, bigger tumors. Martin Brown has shown that hypoxia promoted mobilization of $CD11b^+$ monocytes, which secrete the pro-angiogenic factor MMP9 into the TME in preclinical GBM, and blockade of this crucial event prevents tumor recurrence [207]. The combined treatment of radiation and TGFβ inhibition decreased $CD11b^+$/MMP9 monocytes, suggesting that TGFβ is necessary for the recruitment of the $CD11b^+$/MMP9 cells and tumor regrowth.

Given that radiation-induced immunity is critical for long term benefit [209], we also studied the effect of combined treatment of fractionated radiation and TGFβ inhibition on the peripheral anti-tumor immune response. Analysis of monocyte maturation and activation markers CD11b and F4/80 in tumors suggests that distinct BM cells are recruited as a function of treatment: the $F4/80^+$ macrophage population is more differentiated, while $CD11b^+$ cells are more immature. TGFβ inhibition concurrent with radiation treatment also affects systemic maturation as evidenced by analysis of cells from spleens of treated mice. These preliminary data suggest that TGFβ inhibition concurrent with fractionated radiation treatment may cooperate in directing both the microenvironment and the immune system towards an anti-tumor response, which could lead not only to better control of primary tumor growth but also to abrogation of relapse.

5.2 Contribution of TME to Resistance to Antiangiogenic Therapies and EGFR-TKIs

BM-derived cells have also been shown to provide resistance to cancer therapeutics. For example, BM-derived $Gr1^+$ myeloid cells [210] have been shown to make tumors refractory to anti-VEGF treatment [211], by obviating the necessity for VEGF signaling and reinitiating angiogenesis. In another study, administration of

vascular disruptive agents (VDA) or chemotherapeutics caused acute hypoxia and necrosis in tumors and triggered an accumulation of endothelial progenitor cells at the tumor leading edge to reinitiate angiogenesis [212]. This appears to be an adaptive response of the tumor to develop evasive resistance to potent anti-angiogenesis therapy. In lung cancer, the tumor-stroma cross talk was implicated in mediating resistance to EGFR-TKIs. For example, fibroblast-derived hepatocyte growth factor (HGF) was shown to induce EGFR-TKI (gefitinib) resistance in NSCLC with EGFR-activating mutations [107, 108].

6 The TME as a Therapeutic Target in Lung Cancer

Lung cancer is a global public health problem with an estimated 1.3 million new cases each year [213]. In the United States, approximately 226,160 new cases of lung cancer are diagnosed per year with over 160,000 deaths. Despite advances in treatment options, including minimally invasive surgical resection, stereotactic radiation, and novel chemotherapeutic regimens, the 5-year survival rate in NSCLC remains at approximately 15%. Available targeted therapies such as EGFR TKIs (erlotinib and gefitinib) and EML4-ALK inhibitor (crizotinib) benefit only 15–20% of NSCLC patients who carry specific drug-sensitive mutations. Even in these patients, acquired resistance is a major impediment to a durable therapeutic response [65–67]. Moreover, a majority of the patients with lung cancer patients do not exhibit an actionable molecular aberration. Therefore, traditional standard cytotoxic chemotherapies remain the only treatment option for the majority of advanced NSCLC patients, and these treatments also usually fail, resulting in an aggressive metastatic relapse. As such, there is an unmet medical need for the development of additional targeted therapies for lung cancer patients. In this context, more recent studies have begun to focus on the TME as an unexplored target for drug discovery, with an increased interest in evaluating anti-angiogenic, immunomodulatory, and anti-inflammatory agents in the treatment of various malignancies, including NSCLC [214] (Table 1).

6.1 Antiangiogenic Therapies in Lung Cancer

Drugs that either block tumor vascularization or interfere with the activity of growth factor receptors and molecular pathways that are triggered by activation of these receptors have already been used in clinical practice [215]. Bevacizumab, a humanized monoclonal antibody against VEGF, has been approved in many countries for use in combination with first-line platinum-based chemotherapy (carboplatin and paclitaxel) for the treatment of NSCLC patients with advanced stage disease [216, 217]. Approvals were based upon an improvement in response rate (RR) and progression-free survival (PFS) observed with the addition of bevacizumab to

Table 1 Stromal therapy in lung cancer

Drug	Type	Mode of action	Clinical trials	Results
CTLA-4 antibodies (Ipilimumab)	Immune checkpoint inhibitor	Blocks PD-L1 interaction with PD-1 and allows T cells to perform antitumor activities	Phase III, NSCLC (NCT01285609)	PFS 5.7 months for ipilimumab + chemo vs 4.6 months for placebo + chemo
			Phase III, SCLC (NCT01450761)	
Tremelimumab			Phase II, Mesothelioma	
PD-1 antibodies	Immune checkpoint inhibitor	Blocks PD-L1 interaction with PD-1 and allows T cells to perform antitumor activities	Phase III, NSCLC (NCT01673867)	
Nivolumab			Squamous cell (NCT01642004)	
MK-3475			Phase III, in PD-L1-positive NSCLC (NCT01905657)	
PD-L1 antibodies	Immune checkpoint inhibitor	Targets the ligand PD-L1 and allows T cells to perform antitumor activities	Phase II in PD-L1-positive NSCLC (NCT01846416)	
MPDL3280A				
MEDI4736			Phase I NSCLC (NCT01693562)	
VEGF antibody Bevacizumab	Anti-angiogenic therapy	Targets VEGF ligand	Phase III	PFS and OS positive with Carbo/PXL
VEGF trap	Anti-angiogenic therapy (Soluble decoy receptor)	Targets VEGFA, VEGFB and PIGF	Phase III	PFS positive with DXI
Aflibercept				OS negative
Endostatin	Anti-angiogenic therapy-natural inhibitor of angiogenesis	Targets bFGF, VEGF	Phase III, in combination with chemotherapy (NCT00657423)	
			Phase II, in combination with chemoradiation in NSCLC (NCT01218594)	

(continued)

Table 1 (continued)

Drug	Type	Mode of action	Clinical trials	Results
Pazopanib	TKI, Antiangiogenic	Targets c-KIT, FGFR, PDGFR and VEGFR	Phase II/III in NSCLC patients who have received first line therapy (NCT01208064)	
			Phase II in Refractory small cell lung cancer (NCT01253369)	
Motesanib	TKI, Antiangiogenic	Targets VEGFR-1, 2, 3, PDGFR, RET, kit	Phase III	PFS positive with Carbo or PXL
				OS negative
Sorafenib	TKI, Antiangiogenic	Targets, VEGFR-2, 3 and PDGFR-b	Phase III, Advanced NSCLC in combination with chemo	PFS and OS negative with chemo
				Monotherapy pending
Cediranib	TKI, Antiangiogenic	Targets VEGFR-1,2, 3, c-kit, Flt-3	Phase III, Advanced NSCLC in combination with chemo	PFS pending with DXI
				OS pending with DXI
Vandetanib	TKI, Antiangiogenic	Targets VEGFR-2, VEGFR-3, RET, EGFR	Phase III, in advanced NSCLC in combination with chemo (NCT00312377)	PFS positive with DXI
				OS negative with DXI
Nintedanib	Antiangiogenic	Targets VEGFR, FGFR, PDGFR	Phase III (LUME-Lung-1)	PFS positive with DXI
				OS not significant

References: (1) Hilbe W, Manegold C, Pircher A. Targeting angiogenesis in lung cancer—Pitfalls in drug development. Transl Lung Cancer Res 2012;1(2):122-128. (2) http://www.cancer.gov/clinicaltrials/results/type/lung

DXl docetaxel, *PXL* paclitaxel, *Carbo* carboplatin, *TKI* tyrosine kinase inhibitor

chemotherapy in two large phase III studies, the North American Eastern Cooperative Oncology Group (ECOG) 4599 [218] and the European AVAiL [219]. The encouraging results with bevacizumab has led to approval of Aflibercept (VEGF Trap), which is a recombinant VEGF receptor-antibody protein fusion with affinity for VEGF-A, VEGF-B and placental growth factor (PlGF), which acts as a decoy receptor preventing angiogenesis [220]. Aflibercept, has been approved for metastatic colorectal cancer, and it has been evaluated in second-line therapy of NSCLC. A randomized phase III trial of second-line docetaxel with or without aflibercept in platinum-pretreated patients with advanced non-squamous NSCLC

failed its primary endpoint of overall survival, despite higher response rates and progression free survival in the experimental arm [221].

Other promising anti-angiogenic agents include small molecule TKIs targeting the VEGF receptor (VEGFR). Motesanib, a selective oral inhibitor of VEGF receptors-1, 2, and 3, platelet-derived growth factor receptor (PDGFR), and c-Kit was tested in a randomized phase II trial in combination with carboplatin/paclitaxel as frontline therapy for patients with advanced NSCLC, and results showed that RR, PFS, and OS were comparable in those patients receiving either motesanib or bevacizumab [222]. However, an international randomized phase III trial with carboplatin/paclitaxel either alone or in combination with motesanib in patients with advanced NSCLC showed no improvement in overall survival compared with placebo; despite an improvement in PFS and overall response [223, 224]. Another phase III trial evaluated the addition of the multi-kinase inhibitor (including VEGFR2) sorafenib to chemotherapy in patients with advanced non-squamous NSCLC. Again, despite a slight but statistically significant improvement in PFS, there was no improvement in OS, the trial's primary end-point [225]. A recently reported phase III trial assigned patients with advanced NSCLC who failed first-line therapy to docetaxel with and without nintedanib, a multi-angiogenic kinase inhibitor (VEGFR1-3/FGFR1-3/PDGFR/FLT3). Nintedanib in combination with docetaxel was associated with significant improvement in PFS and OS especially in patients with adenocarcinomas [226]. This is the first and only trial to demonstrate an improvement in OS using a targeted agent in the second-line setting. Finally, a phase III placebo-controlled trial of carboplatin and paclitaxel with and without the vascular disrupting agent vadimezan (ASA404) as first-line therapy for patients with advanced lung cancer did not meet the specified primary and secondary endpoints of OS and PFS [227–229]. Results from recently completed and ongoing phase III trials will determine if these newer antiangiogenic agents will be incorporated into clinical practice [230].

6.2 Anti-inflammatory Therapies in Lung Cancer

Compared to advances with antiangiogenic therapies, success with anti-inflammatory treatments have been less impactful. Previous clinical trials have indicated that long-term use of aspirin or other NSAIDs decreases the incidence of colorectal, esophageal, breast, lung, and bladder cancers [231]. While initial studies had focused on various broad-spectrum NSAIDs (which non-specifically inhibit both COX-1 and COX-2), more recent studies have examined COX-2 specific agents, such as celecoxib [125]. Significant pre-clinical and clinical data support the importance of COX-2 in the development and progression of NSCLC. Despite this, a protective effect of NSAIDs was not observed on lung cancer development in either the general or high-risk COPD populations [232]. Moreover, clinical trials of COX-2 inhibition in NSCLC have been disappointing [233]. The lack of clinical benefit in the Cancer and Leukemia Group B (CALGB) 30203 trial may be that COX-2 inhibition would be of value in COX-2-overexpressing tumors, emphasizing

the need for a prospective, randomized trial that selects patients for therapy on the basis of COX-2 expression [234]. CALGB 30801 is a randomized phase III double-blind trial evaluating selective COX-2 inhibition in COX-2-expressing advanced NSCLC. However, given the gastrointestinal (GI) toxicity and non-specific activity of NSAIDs, and the cardiotoxicity of specific COX-2 inhibitors, the use of such agents continues to remain controversial [235].

Two recent studies have shed light on the future therapeutic potential of the NF-κB-mediated inflammatory pathway in lung cancer. Logsdon and colleagues found that in the presence of oncogenic Ras, inflammatory stimuli initiate a positive feedback loop involving NF-κB that further amplifies Ras activity to pathological levels [236]. Because a large proportion of lung cancer patients possess Ras mutations, disruption of this positive feedback loop may be an important strategy for cancer prevention. In another study, using mouse models of lung cancer, Verma and colleagues found that therapies targeting the enzyme IKK2 (involved in inflammation) and Timp1, which help activate the body's inflammatory response, may effectively treat certain lung cancers [237].

6.3 Immune Checkpoint Inhibitors in Lung Cancer

Utilizing the immune system to eliminate cancer holds great potential, and therefore understanding the complexity of immunomodulation by tumors is important for the development of immunotherapy. A large numbers of different factors have been implicated in the inhibition of tumor-specific immune responses. These include regulatory T cells (Treg), MDSCs, various soluble factors and cytokines, and inhibitory molecules expressed by immune and tumor cells. As such, various strategies are being developed to enhance anti-tumor immune responses, including DC-based vaccines and antagonists of inhibitory signaling pathways to overcome 'immune checkpoints'. The immune checkpoint pathway is a series of cell-cell interactions that inhibit effector T cells from being overactive under normal conditions [147, 238]. A major arm of the immune checkpoint pathway consists of the T cell surface receptor CTLA-4. CTLA-4 is an inhibitory receptor expressed upon activation of a cytotoxic T cell, competing with the co-stimulatory receptor CD28 for their shared ligands CD80 and CD86 on antigen-presenting cells (APCs) [239]. Lung cancer can co-opt this mechanism to evade immune surveillance by stimulating abnormal expression of CTLA-4 on T-cells, leading to T cell anergy. The monoclonal antibodies, tremelimumab and ipilimumab, which inhibit CTLA-4, are being tested for the treatment of lung cancer. Although tremelimumab treatment did not enhance PFS in a phase II trial, objective radiological responses in 5% of participants was observed using tremelimumab. Ipilimumab treatment, on the other hand, showed slight improvement in immune-related progression-free survival (irPFS) in NSCLC patients when administered in a phased manner with platinum-based chemotherapy [240]. Interestingly, ipilimumab treatment showed high activity in squamous carcinomas

[241]. These results prompted the phase III trial, testing ipilimumab in squamous NSCLC using the phased ipilimumab schedule [147].

PD-1 pathway is a major immune checkpoint by which tumors suppress lymphocyte function within the TME. PD-1 is a surface receptor on activated T cells, B cells, and NK cells. It binds to its ligands PD-L1 and PD-L2 on the surface of APCs or dendritic cells, leading to T cell anergy. Cancers can co-opt this pathway and aberrantly express PD-L1 on their cell surface, leading to T cell inactivation. It has been reported that sarcomatoid and adenocarcinoma subtypes of lung cancer express PD-L1, and its expression correlated with poor prognosis [242, 243].

Antibody blockade of PD-1 with its ligands (B7-H1/PD-L1 and B7-DC/PD-L2) showed promising activity in several malignancies [42]. In particular, blocking antibodies against PD-1 and PD-L1 have shown clinical activity in NSCLC [244, 245]. Nivolumab, a monoclonal antibody targeting PD-1, as been shown to restore cytokine secretion and proliferation of $CD8^+$ T cells within lung tumors [246]. A phase I trial of Nivolumab showed a response rate of 17% in previously treated patients with advanced NSCLC, with responses persisting for a median duration of 17 months [244, 247]. As with any type of therapy, a main consideration for the implementation of an immunotherapy regimen is toxicity. For instance, Ipilimumab in combination with chemotherapy exhibited 14% to 17% higher incidence of all-cause grade 3/4 adverse events (AE) compared to chemotherapy alone [248]. Furthermore, a fatal side effect that occurs in a small proportion of patients following anti-CTLA-4 antibody treatment is hypophysitis, inflammation of the pituitary gland [249]. Nivolumab treatment exhibited 9% rate of treatment-related grade 3/4 AE [250], with three drug-related deaths due to pneumonitis [147]. Nivolumab treatment in combination with platinum-based chemotherapy yielded an objective response rate of 33% and a grade 3/4 AE rate of 49% [147]. A current phase I trial is testing the combination of nivolumab with ipilimumab for SCLC [147]. Another antibody targeting PD-1 is MK-3475. A phase I trial in 38 NSCLC patients showed an objective response rate of 24%, with a median PFS of 9.7 weeks and median OS of 51 weeks. 53% of patients had drug-related AEs, most of which were mild. Another approach to targeting the PD-1/PD-L1 pathway is using antibodies that target PD-L1 on cancer cells. One such antibody, MPDL3280A, yielded a 23% overall response rate, with only 11% drug-related grade 3–4 AEs in a phase I trial that included 85 patients with NSCLC [147].

Another avenue being explored to block tumor-driven immunosuppression is based on NK cell activity. NK cells express killer cell immunoglobulin-like receptors (KIRs) that downregulate NK cytotoxic activity, in response to HLA class I molecules on target cells. A higher incidence of the suppressive KIR2DL3 and its ligand HLA-C2 is observed in NSCLC [251] leading to reduced NK activity and protection of cancer cells from NK-mediated killing. A monoclonal antibody to KIR, Lirilumab (IPH2102), has demonstrated efficacy in combination with nivolumab in preclinical models. A trial combining nivolumab with lirilumab in human solid tumors, including 32 NSCLC patients is being conducted, as well as a trial combining lirilumab with ipilimumab [147].

6.4 MDSC as a Therapeutic Target in Lung Cancer

MDSCs have prognostic importance in multiple solid tumors. Emerging data has begun to support the utility of circulating MDSCs as a predictive marker for cancer immunotherapy and for predicting clinical response to systemic chemotherapy in patients with advanced solid tumors [252]. An increase in the number of MDSCs evokes strong immune suppressive activity in cancer patients [153–155], and greatly limits the efficacy of immune therapy. In a randomized phase II clinical trial of advanced stage SCLC, depletion of MDSCs with ATRA substantially improved the immune response to vaccination, suggesting that this approach can be used to enhance the effect of immune interventions in cancer [253]. These studies are consistent with the demonstration that targeting MDSCs augments antitumor activity against lung cancer in mice [157].

7 Future Directions

Analysis of TME in lung cancer is a relatively new area of investigation. Therefore, major efforts are required to identify individual stromal components and unravel heterotypic reciprocal crosstalk signaling pathways between the stroma and tumor cells in NSCLC. This is a major challenge given the high heterogeneity of genetic and epigenetic alterations present in the 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 disease stratification, molecular diagnosis and prognosis, and devising therapeutic strategies against lung cancer. Over 10 years ago, it was suggested that treatments options for NSCLC other than chemotherapy needed to be investigated [254]. So far, only one phase III clinical trial showed survival benefit of combining an anti-angiogenic agent to standard platinum-based chemotherapy in patients with advanced stage NSCLC. Selected groups of patients responded to anti-angiogenic therapies that result in tumor shrinkage and disease stabilization; however, in aggregate, antiangiogenic therapy has not yet had a major clinical impact in most of the trials conducted so far [215]. Many clinical benefits are short-lived; while numerous trials have shown an increase in survival of patients treated with antiangiogenic therapy, the increase for many has been a matter of months [255]. Several possibilities have been suggested to explain why anti-angiogenic trials have not yielded significant benefit in NSCLC. For example, lack of predictive biomarkers continues to be a major hurdle in the selection of adequate patient cohorts that are most likely to benefit. In fact, some studies have alluded to a possible link between antiangiogenic therapy and increased metastasis in multiple tumor types [256, 257].

Immunotherapy has been heralded as a new era of lung cancer therapy. Blocking PD1-PDL1 or CTLA-4 immune checkpoints has resulted in striking and durable responses, with global overall response rates of 20% to 25% as monotherapy in metastatic NSCLC. In order to increase response rates, it has been suggested that

identifying patients who might respond to immunotherapy would be particularly useful, as correlations between PD-L1 expression and EGFR mutation, and PD-1 expression and KRAS mutations has been observed (D'Incecco et al. Journal of Thoracic Oncology 2014). Notably, activation of the PD-1 pathway was shown to contribute to immune escape in mutant EGFR-driven lung tumors in mice, and blockade of this escape pathway improved survival [258]. These findings support further investigation of anti-PD-L1 or anti-PD-1 agents in combination with various targeted therapies, including epigenetic therapy. While immune checkpoint inhibitors such as ipilimumab (anti-CTLA-4 antibody) have been approved for the treatment of melanoma, they have yet not been approved for lung cancer. However, several classes of new drugs appear to be active in various ongoing clinical trials, and their impending approval for use in lung cancer is presumed. At present, several new therapeutic agents are being tested in more than 600 clinical trials in patients with advanced NSCLC, and based on early phase data exhibiting potential, some of these new agents have the capacity to translate to phase III trials, and eventually benefit patients.

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