The Role of Macrophages Within Microenvironment in a Lung Cancer Development and Progression

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Luka Brčić, Luka Opašić, and Helmut Popper

Contents

Abstract

Lung cancer, including carcinogenesis and therapy, is one of the main research focuses today. One of the main reasons for that is the very high mortality rate of patients with lung cancer. Cancer tissue is very heterogeneous, consisting of malignant tumor cells with many different cell types, proteins, and signaling molecules, all together forming the tumor microenvironment. The concept that tumor development is primarily based on mutations has been reapproached from the side of interaction between immune cells of the host, tumor cells, and tumor microenvironment. All components of the cancer microenvironment interact with each other and with tumor cells in a complex manner, both promoting tumor cell growth and development, as well as suppressing it. This interplay is very complicated and today still not completely understood. The most prevalent cells

L. Brčić (✉) • H. Popper
Institute of Pathology, Medical University of Graz, Auenbruggerplatz 25, Graz 8036, Austria
e-mail: luka.brcic@medunigraz.at

L. Opašić
Max Planck Institute for Evolutionary Biology,
August-Thienemann-Str. 2, Plön 24306, Germany

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among leukocytes in the cancer microenvironment are macrophages. These are called tumor-associated macrophages and are still very difficult to differentiate and identify by single markers. However, it is clear that they have a very important role in tumor development and progression in lung cancer, as in many other cancers. In patients with lung carcinoma, there is a correlation between tumor-associated macrophages and prognosis, although not uniform.

17.1 Introduction

Today, lung cancer is still the number one cause of cancer-related death throughout the world. The discovery of targetable/drugable mutations, primarily in lung adenocarcinomas, had a huge impact on the quality of life, however, only to a small subset of patients, and has not much changed the overall survival of patients with lung carcinomas. In addition, many of these patients treated with these new drugs will develop resistance to this therapy. And while enthusiasm about these targeted therapies is slowly melting away, more and more concentration, research, and therapeutical attempts are being redirected toward tumor microenvironment. Microenvironment, with its plethora of different cells, extracellular matrix, and very complicated interactions and impact on tumors, will be shortly presented, with emphasis on tumor-associated macrophages and their role in lung cancer.

17.2 Microenvironment of Lung Cancer

Cancer tissue is very heterogeneous, and within it, we can always differentiate many components. Apart from tumor cells, there are many other different cell types, proteins, and signal molecules, which all together form the tumor microenvironment (TME). Usually, the most abundant component in tumor mass consists of cancer cells, including cancer stem cells. Other cell types found are tumor-activated fibroblasts with altered phenotype, called cancer-associated fibroblasts (CAF; in reality myofibroblasts); endothelial cells, forming the vasculature within tumor; and infiltrative immune cells (Fig. 17.1). They all together are embedded in the extracellular matrix of the tumor stroma. Extracellular matrix is composed of structural molecules like collagen, fibronectin, laminin, tenascin, and other glycoproteins and proteoglycans which are produced by cancer-associated fibroblasts [1]. In the last 10 years, research has focused on other cells within the tumor. All those components of cancer microenvironment closely interact with each other in a complex manner, both inducing or promoting tumor cell growth and development and suppressing it. Lung tissue is physiologically highly oxygenized and vascularized, and is in close contact with the outer environment. It can easily recruit immune inflammatory cells to manage injuries. On the other hand, additional factors, such as tobacco smoke, increase injury incidence and promote chronic inflammation, which increases the probability of malignant alteration of epithelial cells [1, 2]. When such alteration occurs, malignant cells start to recruit and alter the phenotype of the surrounding stroma cells. They do that by secreting many different factors, such as interleukins,

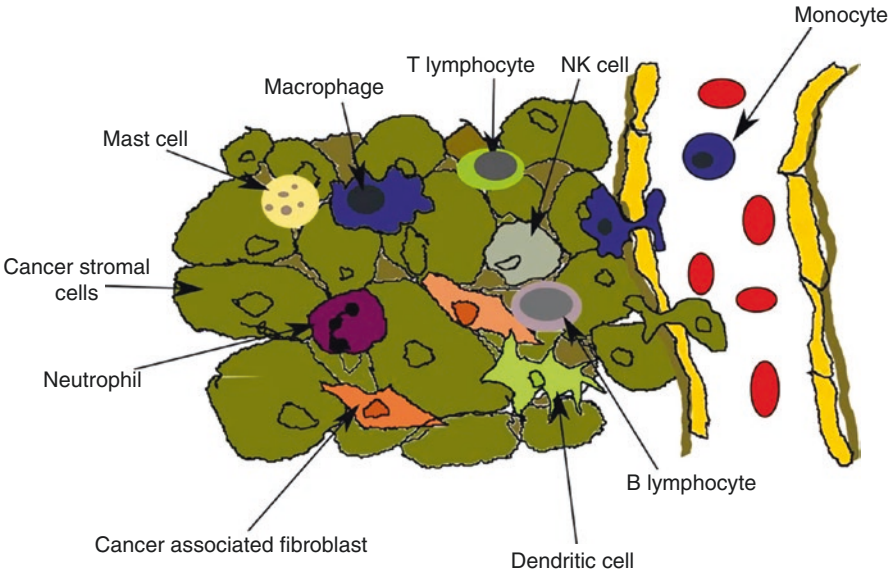

Fig. 17.1 Cellular components of tumor microenvironment

transforming growth factor β (TGFβ), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), inducing in this way stromal reactions [3].

17.2.1 Cancer-Associated Fibroblasts (CAF) in Lung Carcinogenesis at the Border of Supply

Fibroblasts are cells responsible for the production of structural components of extracellular matrix and, in healthy tissues, are usually dormant until homeostasis is compromised. Once activated, they secrete mediators of inflammation, growth factors, and pro-migratory extracellular matrix components, all of which can contribute to carcinogenesis [4]. Studies on other tumors and on mouse models have shown that most CAF are myofibroblasts and develop from locally present precursor cells. They can also differentiate from bone marrow-derived precursor cells, however, in much smaller numbers. Interestingly, epithelial cells can also be the source of CAF after epithelial-mesenchymal transition [5]. In some pulmonary carcinomas, the desmoplastic stroma is in part formed by the tumor cells themselves using EMT. It is known that desmoplasia, defined as increased fibrocytic component within the tumor, is strongly associated with NSCLC. Vincent [6] has shown that both normal fibroblast and CAF promote growth of NSCLC and that CAF are derived from locally present normal myofibroblast. Normal fibroblasts under long exposure to cancer cells start to express the same cytokines that are expressed by CAF. Cardiotrophin-like cytokine factor 1 (*CLCF1*) and interleukin 6 (*IL6*) are genes that are upregulated in CAF in comparison with normal fibroblast. CLCF1

and IL6 are members of the interleukin 6 family that activate JAK-STAT and MAPK cascade via gp130 (glycoprotein 130), LIF receptor, and OSM receptor [7–9]. They exhibit paracrine effects on promotion of lung cancer growth and thus are considered an important component of molecular microenvironment. Alongside signaling molecule secretion, CAF also produce serine proteases and matrix metalloproteases which remodel the matrix and therefore facilitate migration of both immune cells into cancer and malignant cells out of the primary tumor mass [1].

17.2.2 Immune Contexture

Interestingly, Virchow was the first to describe leukocyte infiltration within cancers back in [10]. It is known today, primarily from the research on colorectal cancer (and some other tumors), that differences in type, number, and level of mutual interactions between immune cells are strongly associated with behavior of cancer, response to therapy, and patient survival. That link is so strong that, according to some authors, type and density of T cells within certain tumors are regarded as a better prognostic factor than standard pathological criteria [11]. Yet, in lung tumors, immune cells are not evenly distributed. Instead, those cells are organized into tertiary lymphoid structures composed out of dendritic cells (DC), T-cells clusters, and B-cell follicles. Those structures are not present in healthy adult lung, but are found in diseased fetal and infant lung [12]. Interestingly, the number of those tertiary lymphoid structures and density of mature dendritic cells within are in positive correlation with patient survival [13]. A subset of dendritic cells known as killer dendritic cells also promote elimination of cancer cells via apoptosis and necrosis in some cancers [14]. That is the reason why immunotherapy for lung cancer containing dendritic cells is currently undergoing clinical trials [15]. Dendritic cells, together with mast cells, macrophages, neutrophils, eosinophils, and basophils, comprise the innate immune microenvironment. They are the first line of defense against altered/malignant cells. NK cells have direct cytotoxic effects on cancer cells and positive role in immunosurveillance. They kill cells that fail to present MHC class I molecules, "missing self-cells" [16]. NK cells play an ambiguous role in cancer. Type 1 NK cells promote immunosurveillance, whereas type 2 downregulates immunosurveillance and suppresses immune response against cancer. Other cell types that promote cancer growth and development are myeloid-derived suppressor cells [17]. They are a heterogeneous population of immature myeloid cells that inhibit the function of T and NK cells. One suppressing mechanism is overexpression of arginase 1, which depletes L-arginine required for T-cell development [18]. Furthermore, they promote angiogenesis by secreting proangiogenic mediators, produce transforming growth factor β (TGFβ) and reactive oxygen species, reduce antigen presentation capability of dendritic cells, disrupt polarization of M1 macrophages, and are involved in other processes promoting cancer growth [17, 11]. Lymphocytes usually comprise up to two thirds of all nonmalignant cells inside tumor mass [19], with T cells being the most abundant (around 80% of all lymphocytes).

Al-Shibli and his colleagues [20] have shown in a series of resected NSCLC that the number of $CD4^+$ and $CD8^+$ lymphocytes in stroma is positively correlated with

prolonged survival of patients, yet Wakabayashi and colleagues, few years before [21], demonstrated just the opposite, correlating the number of intratumoral $CD8^+$ lymphocytes with shorter overall survival (OS) in NSCLC. Some research suggests that $CD8^+$ lymphocytes which infiltrate cancer do not secrete IFNγ and are thus not able to deal with malignant cells [22]. Regulatory T cells are known to suppress immune reactions, which potentially benefit tumor development. This suppression is mediated by cyclooxygenase-2 (COX-2), and by its inhibition, tumor burden can be decreased [23, 24].

17.3 Role of Macrophages in Cancer

The concept that tumor development is primarily based on mutations has been reapproached from the side of interplay between immune cells of the host, tumor cells, and tumor microenvironment [25, 26]. Very important in these interactions are macrophages, which are involved in innate immunity and are important for immunological reactions, as well as for tissue healing processes. Macrophages play an important role in maintaining tissue homeostasis. Macrophages within tumor, tumor-associated macrophages (TAMs), have also important roles in cancer development. Meta-analysis by Zhang et al. [27, 28] showed statistically significant correlation between the $CD68^+$ macrophage densities in solid tumors and decreased overall survival.

Tumors use factors produced by macrophages for their progression and metastasis, in other words to avoid potentially harmful reaction of the host's immune system. TAM is a very interesting cell population, because TAMs can present with various phenotypes and comprise heterogeneous population [29, 30]. They are close to tumor cells, and they produce and release many different cytokines, growth factors, and chemokines, such as VEGF and IL-10. Many of these are important for tumor progression (local growth and metastatic potential) [31]. They are activated by different molecules, such as IFN-gamma, TNF, IL-4, and IL-10, and are profiled as M1 or M2 macrophages [31–35]. In which direction they differentiate depends mainly on the signals they receive from microenvironment. Macrophages are attracted to tumor microenvironment by hypoxia [36], but also by chemokine secretion, like CCL2 [37, 38].

M1 macrophages are involved in inflammation and infection, mainly as antigen-presenting cells and activators of inflammation [39], but are also directed against tumor cells [35, 40, 41]. M1 macrophages are activated by interferon γ (IFNγ) and lipopolysaccharide to act bactericidally and promote inflammation and T helper 1 (TH1) responses. TAM with phenotype similar to M1 can be found in early stages of cancer development and chronic inflammation that precedes it. Macrophages are generally not tumoricidal. Only when activated, they can destroy cancer cells directly or indirectly by stimulation of other cells. Direct cytotoxicity can be macrophage-mediated tumor cytotoxicity (MTC) in which toxic factors, such as TNFα, are secreted onto cancer cells, causing lysis or antibody-dependent cellular cytotoxicity (ADCC) [42]. In contrast, M2 macrophages, activated through IL-10 and TGFβ, influence angiogenesis through VEGF [32], and indirectly increase the expression of angiogenin via IL-1b and TNFα [42, 43] in vitro,

as well as basic fibroblast growth factor (bFGF, also known as FGF2), chemokine (C-X-C motif) ligand 8 (CXCL8; also known as IL-8), cyclooxygenase 2 (COX2, also known as PTGS2), plasminogen activator, urokinase (uPA, also known as PLAU), and platelet-derived growth factor β (PDGFβ) [44]. Matrix metalloproteinases (MMP) excreted by TAM increase cancer growth also by promotion of angiogenesis (MMP7, MMP9, and MMP12) and, on the other hand, along with serine proteinases such as urokinase, metalloproteases as collagenase (MMP-1), gelatinase A and B (MMP-2, MMP-9), stromelysin (MMP-3), and macrophage elastase (MMP-12) degrade basal membrane and connective tissue, facilitating in this way tumor growth and migration of tumor cells [42, 45]. They also influence tissue remodeling [35, 40]. M2 macrophages improve tumor cell growth in in vitro conditions [46], and in vivo they act anti-inflammatory, preventing T-cell proliferation and antigen presentation and secreting IL-10 as an anti-inflammatory cytokine [35, 47, 48]. M2 macrophages are immunosuppressive and promote T helper 2 (TH2) responses. As tumor develops, TAM starts to express genes typical of M2 phenotype to become M2-skewed TAM, a predominant type of TAM (Fig. 17.2) [44].

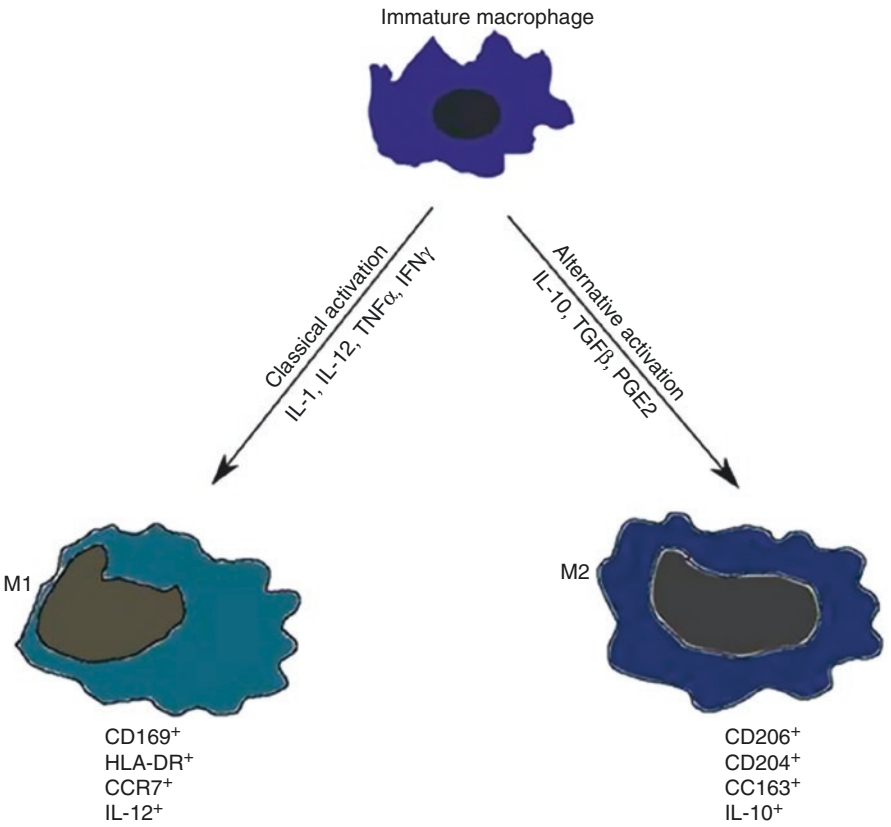

Fig. 17.2 Two different ways of macrophage activation and transformation into M1 and M2 subtypes

Although there is an overlap, generally two subpopulations of macrophages can be distinguished by immunohistochemistry. CD68 is a general macrophage marker, and CD163 and iNOS can be used as markers for M2 and M1, respectively. M1 macrophages express IL-1, IL-12, and iNOS [31, 32]. M2 are characterized by CD204 and CD206 (Fig. 17.3) [35, 40].

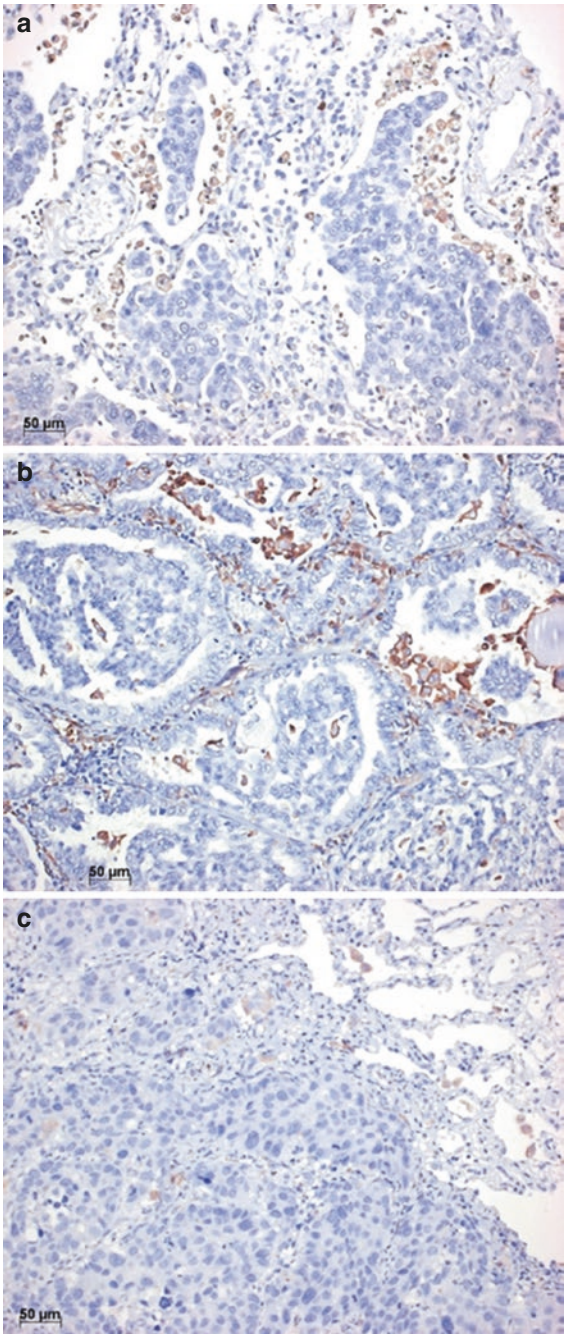

Fig. 17.3 Immunohistochemical presentation of squamous cell carcinoma (**a**) and adenocarcinoma (**b**) with many CD206-positive macrophages and solid area of another lung adenocarcinoma (**c**) with only few CD206-positive macrophages

In a study by van Overmeire et al. [49], the authors clearly demonstrated the importance of hypoxia for macrophage differentiation toward M1 or M2 line. Masumoto et al. [50] showed that increased metabolic activity in tumor, as well as not adequate vascularization, resulted in hypoxia (chronic or transient). And hypoxia is important for invasion of tumor cells, as well as for the resistance to therapy. Macrophages, especially M2 subpopulations, are present in hypoxic areas of the tumors. However, some authors [51] showed that improving hypoxic conditions, by regulation of blood vessel formation in tumor, does not decrease the number of M2 in the hypoxic areas, but only regulates the production of factors like VEGF A, GLUT 1, GLUT3, and iNOS, stimulating angiogenesis induced by TAMs [51].

17.4 Macrophages in Lung Cancer

Data on the importance of TAM in lung cancer has emerged, especially in the last 15–20 years, since it has been found that they have an interesting, but not easily understandable role in lung cancer. For example, some studies have clearly shown that patients with higher numbers of macrophages in primary lung carcinomas have shorter survival, while, on the other hand, others showed total lack of significance in correlation between TAM and survival. Studies concentrating on the localization of the macrophages, with regard to tumor cells and tumor stroma, showed favorable outcomes for the patients who had higher density of macrophages between tumor cells and an unfavorable one if the density was higher in the stroma [52, 53]. However, macrophage density was higher in adenocarcinomas than in other lung carcinomas analyzed (squamous and large cell) [52]. In one study comparing survival after platinum-based first-line therapy and macrophage infiltration in between tumor cells, the author found no association with the number of macrophages and survival [54], but also showed significantly better survival in patients with lower number of stromal macrophages. Only one of the previously mentioned studies [55] did not find any significance of stromal macrophages density in relation to the patient survival. Although studies used different approaches and methods, and in spite of studies demonstrating no relation of macrophages and survival, we can conclude (based on the all available data) that a higher number of macrophages between tumor cells is prognostically better, while the opposite is true for the macrophage numbers in tumor stroma. It is evident that looking only into the number of macrophages infiltrating lung carcinomas is not enough, and that macrophage differentiation toward M1 and M2 has to be evaluated. A single marker which can reliably and specifically detect M1 macrophages does not exist [30, 56], and in further studies, a panel of antibodies has to be used for distinction and characterization of macrophages in lung carcinomas. In a very nice and a clear-cut study by Ohri and his colleagues [32] using patients with long survival (mean 92.7 ± 7.2 months) and short survival (7.7 ± 0.7 months), the authors showed that in the prognostically better group, there are more M1 than M2 macrophages, and that patients whose M1 density was under the median had a 5-year survival rate of <5%, in comparison with the ones with M1 density over

the median whose 5-year survival rate was >75% [32]. Some studies showed that higher number of TAM ($CD68^+$) is correlated with survival in NSCLC patients [53, 57], while others were not able to find this connection [58, 59]. M2 macrophages ($CD204^+$), in a study by Ohtaki et al. [60], where only adenocarcinomas were included, demonstrated significant correlation with survival, but also with vascular and pleural invasion and stage.

On the other hand, Zhang and his colleagues [61] demonstrated that the number of M2 macrophages is to be an independent prognostic factor for overall survival. The confusion does not end here: while Ohri et al. [32] clearly showed that M1 macrophages (CD68 iNOS+) are significantly increased between tumor cells of the patients with longer survival, making them good prognostic markers, Almatroodi et al. [62] compared the same M1 population in tumor and non-tumor tissue, demonstrating decreased iNOS expression in squamous cell lung carcinomas and adenocarcinomas, but not in large cell carcinomas (in comparison with the matched non-tumorous tissue). Decreased iNOS expression is associated with deregulation of NF-KB signaling pathway having as a consequence non-adequate immunological response [63]. M2 macrophages (CD163+) stimulate proliferation of tumor, mainly through angiogenesis activation [31]. Higher number of M2 inside tumor cell aggregates correlates with metastatic tumor potential [61] and is very high in progressive disease [64]. Almatroodi et al. [62] showed that in all NSCLC types (adenocarcinomas, squamous cell lung carcinomas, large cell lung carcinomas), CD163 macrophages are present in a greater number than in non-tumor tissue. M2 have been in positive correlation with poor prognosis, TNM staging, and metastases to lymph nodes [60, 61]. This great variability of presented results is probably the consequence of a nonexistent specific marker for TAM (M2). They can be characterized by cell surface proteins, transcription factors, enzyme, and cytokine production. However, these markers change their expression over time, depending on cell activity, depending on the tumor type in which they are analyzed, and even depending on the smoking status of patient.

It is very important to stress once more that TAMs, like macrophages in general, are a very heterogeneous population and that there is a wide continuum of possible phenotypes between M1 and M2 macrophages. Many potential factors have influence on this differentiation process, among which tumor type and stage, as well as microenvironment, play an important role [30, 31].

M2 accelerates proliferation and migration of Lewis lung carcinoma (LLC1) cells [65] and activates lymphangiogenesis [61]. Zhang showed accelerated proliferation and invasiveness of LLC cells after cultivation with mouse macrophages (cell line Raw264.7), equivalent to M2 macrophages [27, 28]. A possible mechanism of M2 macrophage (CD206+ macrophages) activation according to the Unver experimental model [66] is also through chemokine ligand 7 (CXCL7) (which is a member of ELR+ CXCL chemokines promoting tumor progression mainly through angiogenesis [67]), indicating once again the importance of chemokine interplay in microenvironment that is crucial for early tumor development and progression. Furthermore, CCR2 and CX3CR1 are two receptors present on the macrophages and, when bonded to their ligand CCL2 and CX3CL1, influence signaling pathways

such as JAK-STAT, PI3-K, and MAPK [68–71]. In a very nice and comprehensive study by Schmall et al. [72], the authors showed importance of CCR and CX3CR1 signaling for lung cancer progression. In mice without host CCR2 and CX3CR1, LLC1 tumor and progression decreased, and M2 macrophages were repolarized in M1 direction, influencing also angiogenesis and resulting in better survival. In the same study, they showed significant correlation between CCR2 expression with tumor stage and metastasis. They reconfirmed M2 as major player for tumor progression. They showed for the first time that through IL-10 secreted from macrophages, upregulation of CCR2 and CX3CR1 occurs in lung cancer cells. In this way, lung cancer cells behave like the macrophages, and with CCL2 and CX3CL1, secretion attracts more macrophages creating amplification loop, resulting in cancer cell proliferation and migration, as well as metastasis and creation of new blood supply network for microenvironment.

17.5 Tumor-Associated Macrophages and Antitumor Therapy

Another emerging and interesting interaction exists between TAMs and applied antitumor therapy, since it is now evident that TAMs influence response to chemotherapy, both positively and negatively, depending on the cytotoxic agent. Doxorubicin promotes M1 population, having a positive antitumoral effect, while on the other hand, different therapeutical protocols might induce PD-L1 expression on macrophages, followed by CD8+ T lymphocyte inhibition and unsuccessful therapy outcomes [73, 74]. On the other hand, there are some recent promising studies [75] in mouse models of breast carcinoma where TAMs were used as gene delivery vehicles for interferon alpha, activating immunity and inhibiting progression of the breast carcinoma. Another possible approach is reversing polarization of M2 toward M1, as in the study by Chen et al. [76], where M1 macrophages were induced by neuropeptide methionine enkephalin, resulting in antitumor activity or macrophage depletion as in study by Fritz et al. [77]. Latter macrophage depletion in mouse models of lung carcinoma was induced by clodronate-encapsulated liposomes, resulting in lower tumor burden and lower tumor cell proliferation. Especially interesting are immunotherapies using monoclonal antibodies against immune checkpoints [78, 79], where repolarization toward M1 might also improve efficacy [56].

Conclusion

After the above, only shortly, presented overview of macrophages and their role in cancer, it is clear that macrophages have a great plasticity and impressive variety of secreted molecules, such as growth factors, enzymes, cytokines, and chemokines. Today, it is known that not only the majority of cancer types contain macrophages but that they also form a symbiotic relationship in which cancer cells recruit macrophages and support their growth in exchange for factors that promote angiogenesis and tumor growth produced by macrophages. Most of this complicated interplay is yet to be revealed and then transferred to lung carci-

noma diagnosis and treatment. It is clear that macrophage polarization into M2 is crucial for angiogenesis in tumor, tumor growth, and metastasis. The majority of the studies have demonstrated negative correlation with M2 macrophages and prognosis of patients with lung carcinoma. What makes the integration of presented as well as future studies' results and cross-integration very hard is still nonexistent single marker for M2 population, resulting in many different combinations of selected markers for presumably the same cells. However, even with the knowledge we have accumulated so far, new promising therapeutical options and treatment approaches are emerging.

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