



The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses

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The success of cancer immunotherapy relies on the knowledge of the tumor microenvironment and the immune evasion mechanisms in which the tumor, stroma, and infiltrating immune cells function in a complex network. The potential barriers that profoundly challenge the overall clinical outcome of promising therapies need to be fully identified and counteracted. Although cancer immunotherapy has increasingly been applied, we are far from understanding how to utilize different strategies in the best way and how to combine therapeutic options to optimize clinical benefit. This review intends to give a contemporary and detailed overview of the different roles of immune cells, exosomes, and molecules acting in the tumor microenvironment and how they relate to immune activation and escape. Further, current and novel immunotherapeutic options will be discussed.

Keywords: tumor, tumor microenvironment, immune cells, immune response, immunosuppression, antitumor

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INTRODUCTION

The tumor microenvironment (TME) has a decisive role in tumor differentiation, epigenetics, dissemination, and immune evasion. In fact, the TME is a highly heterogeneous milieu consisting of different cell types and many abundant molecules produced and released by tumor cells, stromal cells, and immune cells. In this review, the TME will be discussed from the immunologist's point of view.

Concrete evidences support that both innate and adaptive immunity are involved in immune surveillance. This is referred to as the elimination phase and is reviewed elsewhere (1). If the transformed cells evade immune control during the elimination phase, tumors are formed. As tumor cells and its stroma progress, the immunosuppressive mechanisms increase in magnitude. Although tumor cells can be recognized and eliminated by the immune system, the tumor continues to grow (equilibrium phase) and escapes surveillance (escape phase) later. Lymphocytes including natural killer (NK) cells, CD8⁺ T cells and CD4⁺ helper T (Th) cells together with proinflammatory macrophages (M1) and dendritic cells from the anti-tumor immune responses while the heterogeneous population of myeloid-derived suppressor cells (MDSCs) and Foxp3⁺ regulatory T cells (Tregs) counteract tumor immunity. These cells are attracted and expanded by the tumor and its stroma to both control the present effector lymphocytes and to hamper the novo activation of tumor-reactive lymphocytes. Tumor cells evade the immune cells by a plethora mechanisms such as downregulation or loss of tumor antigens, releasing immunosuppressive extracellular vesicles including exosomes, releasing immunosuppressive molecules including IL-10 and transforming growth factor β (TGF- β), shedding soluble major histocompatibility complex

(MHC)-I, loss of adhesion molecules such as ICAMI, developing resistance to apoptosis by upregulation of BCL-2 and other anti-apoptosis molecules, and overexpressing programmed death ligand 1 (PD-L1) as well as Fas ligand and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). Tumor-released molecules shape the TME and induce immunosuppression that debilitate robust antitumor immune responses.

Dendritic cells (DCs) are key regulators of the immune system and orchestrate the immune reactions in the tumor but other innate immune cells play important roles as well. For example, M1 macrophages conduct tumor killing by releasing lytic enzymes, TNF- α , oxygen and nitrogen intermediates, as well as mediating antibody-dependent cell-mediated cytotoxicity (ADCC) (2–4). Basophils promote the infiltration of CD8⁺ T cells in the inflamed tumor tissue by secreting chemokines such as CCL3 and CCL4 (5). Eosinophils recruit in solid tumor due to the molecules released by tumor cells (6). Eosinophils not only regulate T-cell activation but also exert anti-tumor responses through degranulation and secretion of granzyme A as well as TNF- α via their natural killer group 2 member D (NKG2D) binding to NKG2D ligands (NKG2DLs) on tumor cells (7, 8). Finally, the N1 type of neutrophils can participate to activate anti-tumor T cell responses, induce tumor cell death by TRAIL, releasing reactive oxygen species (ROS), and participating in ADCC. **Figure 1** shows an overview of the complexity in the TME.

Such a complex condition challenges effective therapies against the established cancer. While traditional therapies such as chemo- and radiotherapy target the tumor directly, novel options such as the wide range of immunotherapies, generally target the microenvironment and specifically the immune system. Hence, immunotherapeutics indirectly kill tumor cells via modulation of the TME and/or effector immune cells. Mapping the cell types and molecules present in the inimical tumor milieu will support the development of more effective treatments and teach us how to combine current available options.

DENDRITIC CELLS

Tumor immunity is formed in the same manner as immunity against virally-infected cells but with the lack of virus-mediated Toll-like receptor (TLR) activation. In brief, immature DCs in the TME effectively engulf and process tumor antigens. Damage-associated molecule patterns (DAMPs) elicit DC maturation and activation in which they reduce the ability to capture antigens and gain capacity to present them to lymphocytes. Mature DCs migrate to the lymph nodes to activate CD4⁺ and CD8⁺ T cells via antigen presentation on MHC class II and I molecules, respectively. They also provide co-stimulatory molecules such as B7 and TNF family members as well as cytokines for full T cell activation. Mature DCs can also increase the activation status of NK cells by their release of cytokines such as IL-12. Very recently, it was elucidated that infiltrating conventional DCs in the TME contribute to antitumor immune responses by selectively release of IFN- λ 1 that stimulates Th1

differentiation and activation and effector CD8⁺ T-cell activation together with enhanced IFN- γ production through IL-12p70 production which as a result increases overall survival in human cancers (9).

Despite the capacity to mount anti-tumor immunity, the immune system fails to control the growing tumor. Tumor immune escape is as complex as immune activation. A crucial event is the lack of proper DC stimulation. If the DCs patrolling the TME are not properly matured, they will present tumor antigen in a tolerogenic fashion resulting in anergic/tolerant T cells. In the TME, regulatory or tolerogenic DCs (tDCs) display low expression of costimulatory molecules such as CD80 and CD86 as immature DCs, but they simultaneously have high expression of inhibitory molecules such as PD-L1 and cytotoxic T lymphocyte associated antigen-4 (CTLA-4). In fact, tDCs express various immunomodulatory factors (e.g., TRAIL, PD-L1, DC-SIGN, and galactin-1) and immunosuppressive molecules (e.g., TGF- β , IL-10, IL-27, NO, and IDO) (10). The tDCs can be induced by products of pathogens, VEGF, tumor-released cytokines (e.g., TGF- β and IL-10), and IL-10-secreting Tregs. Moreover, immature DCs that uptake apoptotic/necrotic DCs convert into tDCs with enhanced TGF- β secretion (11). Besides hampering activation of cytotoxic and T helper cells, IL-10-releasing tDCs elicit the differentiation of type 1 Tregs (Tr1) through immunoglobulin-like transcript 4 (ILT4)/HLA-G signaling pathway as well as inducing Foxp3⁺ Tregs from naïve CD4⁺ T cells (12).

TUMOR-INFILTRATING LYMPHOCYTES

Tumor-infiltrating lymphocytes (TILs) are certain lymphocytes infiltrating tumor site in response to molecular signals. The subsets include different T cell subsets including Tregs, innate lymphoid cells (ILCs) such as NK cells and NKT cells. The effector T cells and NK cells are frequently evoked and recruited to the TME for eliminating cancer cells through targeting tumor antigens and membrane ligands. The extravasation of TILs into the tissue is facilitated by selectins, integrins, and chemokines. Chemokines released by tumor cells and its stroma influence the immune cell infiltration, tumor cell proliferation, and metastasis (13). For example, CXCL16 and its receptor, CXCR6, enhance the infiltration of CD4 and CD8 T cells in colorectal cancer (14). CCL22 mediates Treg accumulation in the TME as shown in breast and ovarian cancer (15). Nevertheless, penetration of TILs into the tumor parenchyma considerably diminishes due to dysregulated vasculature that has down-regulated the receptors important for TIL attachment, rolling, and transmigration. Further, the chemokine profile can be reverted due to M2 macrophages and other immunosuppressive immune cells that together contribute to attract additional immunosuppressive cells while blocking TILs (16). TILs are present in the TME to defy the transformed cells. It is corroborated that a high level of CD3⁺, CD4⁺, and CD8⁺ TILs is associated with improved overall survival rates in cancer patients (17) while high levels

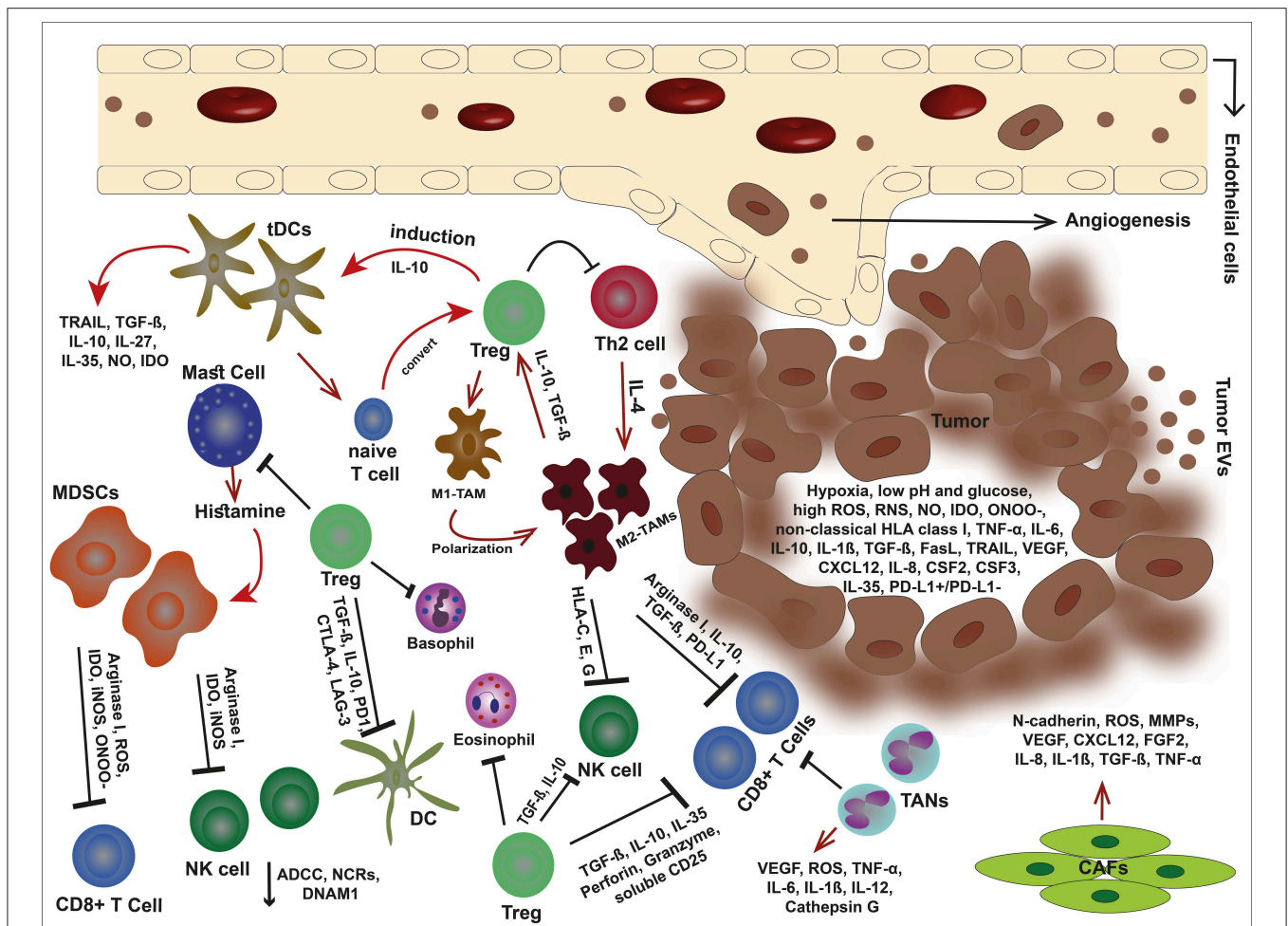


FIGURE 1 | An overview of tumor microenvironment. In the immunosuppressive TME, malignant cells debilitate the antitumor immune responses through secretion of offensive and detrimental molecules, collaboration with cancer-associated stromal cells, and exploit immune escape mechanisms to outwit the immune cells. Tumor cells alter their milieu by lowering pH and glucose but high production of VEGF, non-classical HLA class I, death ligands such as FasL and TRAIL, anti-inflammatory cytokines, and metabolites such as IDO, ROS, RNS, ONOO⁻, and NO. These molecules can not only inhibit the immune cells but also elicit the stroma cells and facilitate tumor development. The cancer-associated stroma cells favor tumor cells by suppressing the immune responses and even induce each other. Tregs are capable to inhibit effector immune cells, eosinophils, basophils, and mast cells. Mast cells themselves induce MDSCs by releasing histamine. Tregs also stimulate tDC via IL-10 and impose M1-TAM polarization into M2-type. In turn, M2-TAMs eliminate effector cells via non-classical HLA class I, arginase I, IL-10, TGF-β, and PD-L1. In addition, MDSCs hinder effector cells by releasing arginase I, and metabolites such as IDO, ROS, ONOO⁻, and iNOS. TANs are other players that eliminate CD8⁺ T cells. The condition becomes more complicated with CAFs that promote angiogenesis, tumor growth, and invasion.

of FoxP3⁺ TILs are negatively associated with overall survival (18). Both anti-tumor-reactive and tumor-supportive TILs are discussed below.

CD8⁺ T CELLS

Naïve CD8⁺ cells are activated into cytotoxic T cells (CTLs) by mature DCs loaded with tumor antigens and trafficking to the tumor-draining lymph nodes as discussed previously. Infiltration into the TME is a result of several signaling molecules such as chemokines (e.g., CCL3, CCL4, CCL5, CCL20, CXCL9, CXCL10, CXCL11, and CXCL16) and their receptors (19). CCL5 released by the tumor and CXCL9 released by APCs in response to IFN-γ seems crucial since

CCL5^{hi} CXCL9^{hi} tumors are highly infiltrated with TILs and respond to checkpoint blockade inhibition (CPI) therapy (20). CTLs target tumor cells via T cell receptor interaction with MHC class I and kill tumor cells through apoptosis induction. Apoptosis can be initiated via death receptor ligation, FasL and TRAIL, but also by secreting perforin and granzyme B. To avoid CTL-mediated killing, tumor cells down-regulate MHC class I but also undergo mutations that reduces the antigen processing and presenting capacity. Further, the tumor cells commonly upregulate anti-apoptotic molecules such as BCL-2 and/or have dysregulated expression of death receptors such as Fas. As a self-regulatory mechanism, CTLs upregulate checkpoint receptors including PD-1, CTLA-4, LAG-3, and TIM3 that makes them susceptible of inhibitory signaling since

binding of checkpoint receptors induces CTL exhaustion and anergy (21).

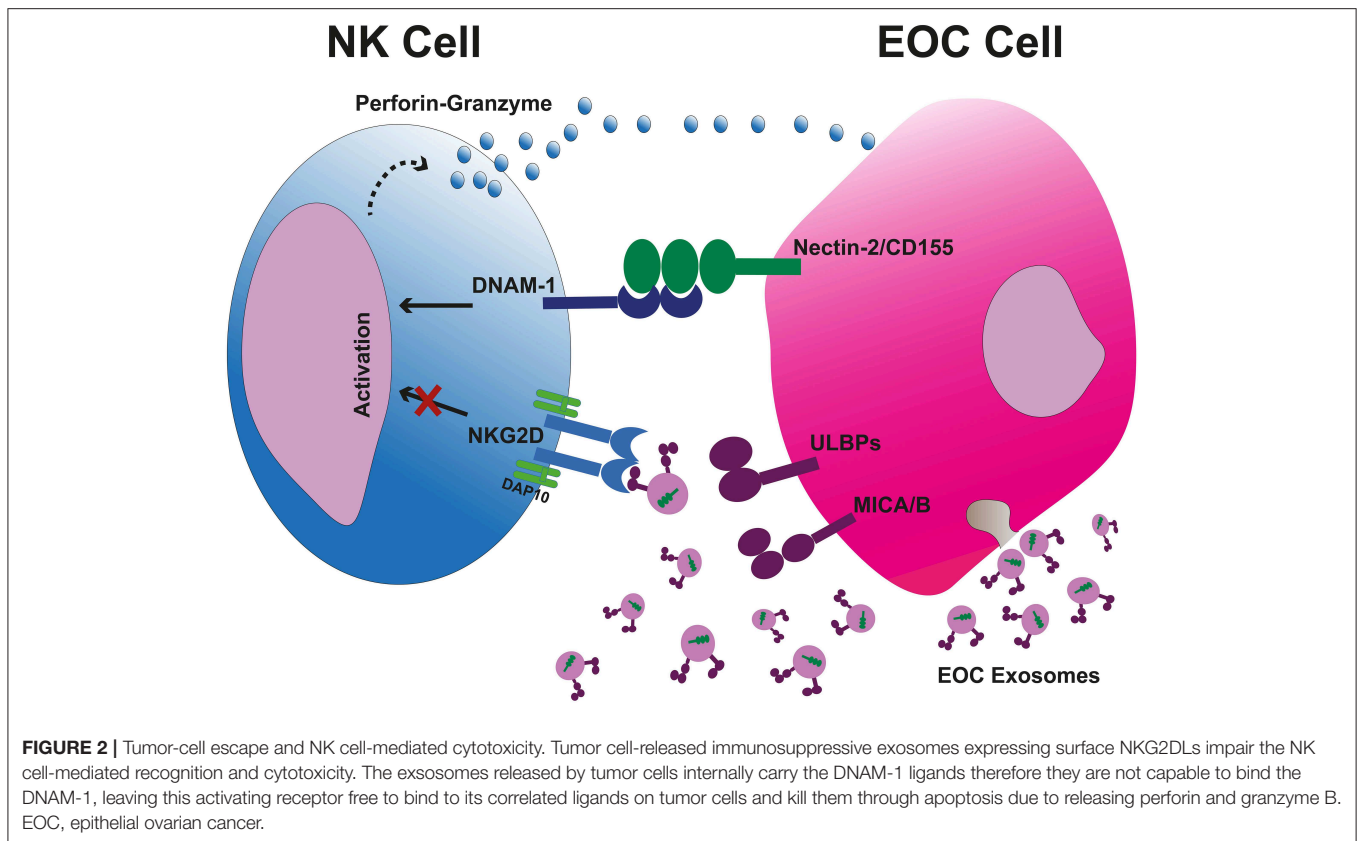
CD4⁺ T CELLS

Naïve CD4⁺ T cells encountering tumor antigens presented by APCs can differentiate into different cell subsets including Th1, Th2, Th9, Th17, Th22, and Tregs. Th1 cells secrete IL-2 and IFN- γ to provide help to CTL activation. However, IFN- γ also suppresses tumor growth, stimulate MHC upregulation and can suppress angiogenesis (22, 23). Th2 cells release cytokines such as IL-4 and IL-13 that can induce eosinophil recruitment into the TME which may participate in the antitumor response (24). However, CD4⁺ Th2-mediated responses may promote tumor growth via increasing angiogenesis and hampering Th1 cell-mediated immunity. Th17 cells exhibit dual roles in the TME. This cell subset elicit angiogenesis as well as tumor growth via pro-inflammatory IL-17A secretion and hamper antitumor responses by releasing immunosuppressive adenosine (25). Moreover, Th17 cells may convert into Treg cells due to the microenvironment to support immunosuppression in the tumor (25). Furthermore, abundant of Th17 cells in the tumor associate with poor prognosis (25). Nevertheless, it has been illustrated that the elevated number of Th17 cell population in ovarian tumors correlated to better survival rates (25). In fact, the association of chronic inflammation with tumor progression has been shown a long time ago (26). Both Th2 and Th17 contribute to chronic inflammation which promotes both tumor transformation and progression. Th22 cells secrete IL-22 that share similar structure with IL-10. The expression of this cytokine is not restricted to Th22 cells. Th17 subset and NK cells also produce IL-22 (27). Moreover, $\gamma\delta$ T cells co-express IL-22 and IL-17A (27). IL-22 promotes tumor cell proliferation through signal transducer and activator of transcription (STAT) 3 (27). STAT3 activation favors immunosuppressive activities in many immune cells such as DCs and macrophages. Hence, the enhanced number of tumor-infiltrating Th22 cells is associated with tumor progression. The nature of Th9 cells is still controversial. It was described that they are a distinct CD4⁺ T cell subset (28) while a contradictory study posed that Th9 cells are merely a subpopulation of IL-9⁺ Th2 cells (29). IL-9 is not restricted to Th9 cells but is expressed by subsets of Th2, Th17, ILC2 cells, Tregs, NKT cells, and mast cells (30). IL-9 can stimulate STAT1, 3, and 5 which are involved in many biological processes. Murine tumor-infiltrating CD4⁺ Th9 cells were shown to kill advanced tumors because of their ability for expressing cytolytic granzymes (28). In addition, Th9 cells significantly enhance CD8⁺ T cell trafficking into the TME *in vivo* (28).

NK CELLS

Like CTLs, NK cells kill tumor cells via induction of apoptosis which is primarily done by releasing cytolytic granules containing perforin and granzymes. NK cells are not MHC dependent. Instead, they have a range of activating and inhibiting receptors that regulate their killing capacity. Inhibiting receptors

recognize for example MHC-I which restricts their killing of normal, healthy cells while activating receptors trigger cytolytic function. Activating receptors transduce signals through immunoreceptor tyrosine-based activation motif (ITAM) located in their cytoplasmic tail. These activating receptors include NKG2D, DNAX accessory molecule 1 (DNAM-1), NKp30, NKp44, and NKp46. NKG2D is also expressed on other cell types such as NKT cells, CD8⁺ $\alpha\beta$ T cells, and $\gamma\delta$ T cells (31). NKG2D ligands in human belong to two families; the MHC class I chain-related antigens A (MICA) and B (MICB) as well as the cytomegalovirus UL-16-binding proteins (ULBP) 1-6. These ligands are expressed on infected cells and on DNA damaged or transformed cells but in exiguous levels on different healthy cells (32). Upon NKG2D receptor-ligand binding, signal transduction culminates in degranulation of NK cells to eliminate tumor cells. NK cells are important in tumor control as a low activity of NK cells has been associated with increment of cancer risk (33). However, tumor cells downregulate their surface ligands to hamper the anti-tumor recognition to escape NK cell-mediated immune surveillance. The ligand downregulation is promoted by TGF- β , IFN- γ , STAT3, hypoxia, proteolytic shedding, and forming soluble ligands, as well as certain micro RNAs (i.e., miRNA-20a, miRNA-106b, miRNA-93, miRNA-373, and miRNA-520d) (34–38). Cancer cells also release immunosuppressive microvesicles including exosomes expressing surface NKG2DLs to obstacle the NKG2D receptors and block the tumor recognition (39). Nevertheless, NK cells exert DNAM-1 (CD226)-mediated tumor recognition if the tumor cell expresses DNAM-1 ligands to overcome the NKG2D blockade. DNAM-1-mediated killing is very effective since there are no soluble or vesicle-bound DNAM-1 ligands. The DNAM-1 ligands are internally packed into tumor-derived exosomes and are not exposed to NK cells (Figure 2) (39). Nevertheless, tumor-infiltrating NK cells (TINKs) are also affected by the TME and display: (1) altered polarization and phenotype, (2) increased expression of angiogenic factors such as VEGF, (3) reduced IFN- γ , (4) malfunction of degranulation and cytotoxic ability, (5) down-modulated CD16, NKG2D, and DNAM-1 (40, 41). It has been described that CD11b^{high} CD27^{high} NK cells can be converted into MDSCs in the TME due to GM-CSF (42). Although NK cells as cytotoxic innate lymphoid cells (ILCs) have a pivotal role in eliminating tumor cells, other subpopulations of ILCs show dual roles. These cells present mostly in the mucosae and mucosal-correlated lymphoid tissues. Non-cytotoxic ILCs fall into three groups comprising T-bet⁺ ILC1 (releasing TNF- α and IFN- γ), GATA3⁺ ILC2 (secreting IL-4, IL-5, IL-9, and IL-13), and ROR γ t⁺ ILC3 (CCR6⁺ cells releasing IL-17A, IL-22, GM-CSF, and CCR6⁻ cells secreting TNF- α , IFN- γ , IL-22, and GM-CSF) (43). Interestingly, ILC2 and ILC3 subsets may transdifferentiate into ILC1 cells and vice versa (44). Therefore, they can acquire or lose certain types of cytokines. It has been indicated that an enhanced number of ROR γ t⁺ ILC3 cells is associated with increased lymph node metastasis (45). In contrast, NKp46⁺ ILC3 cells indicated supportive antitumor response in a mouse melanoma (B16) model in an IL-12-mediated fashion (44). Nevertheless, TGF- β -releasing cancer



cells convert NK cells into ILC1 cells in the TME as an immune escape mechanism (46).

NKT CELLS

After development from lymphoid precursors, NKT cells mature in the thymus. In human, NKT cells are few and form about 0.2% of peripheral blood T cells (47). The number of NKT cells becomes even lower in advanced cancer patients (47). Regardless of the low number, NKT cells may have an important function to eliminate tumor cells if they can infiltrate into tumor since the survival rate was higher in cancer patients with infiltrated NKT cells compared to those with no infiltration (48). The recruitment of NKT cells to the tumor might be due to the presence of lipid antigens that are essential for NKT-cell development; however, the role of such antigens in this regard is not clear (49). Nevertheless, it was shown that the recruitment of NKT cells can be mediated by CCR2, CXCR6, and leukocyte function associated antigen-1 (LFA-1) expressed on these cells (49). NKT cells not only show NK cell features such as expression of CD56, Fc receptor CD16, and granzyme production, but also display $\alpha\beta$ -TCR to recognize both endogenous and exogenous lipid antigens presented on CD1d, a non-classical antigen-presenting molecule resembling MHC class I. CD1d is expressed on APCs, epidermal keratinocytes, hepatocytes, adipocytes, thymocytes, and is downregulated in tumor cells (50). Upon induction and activation through $\alpha\beta$ -TCR and CD1d interaction, NKT cells

release cytokines of both Th1 and Th2 type. Examples of the cytokines released by NKT cells include IFN- γ , TNF- α , IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17, IL-21, TGF- β , and granulocyte monocyte-colony stimulating factor (GM-CSF) (51). Unlike type II subsets that have immunoregulatory function as for example seen in B-cell lymphoma mice to support MDSCs (52), type I NKT cells have antitumor activity through CD1d-dependent or—independent manner. The antitumor activity of type I NKT cells includes the regulation of effector cells via cytokines and tumor lysis via FasL, TRAIL, perforin, and granzymes (47). In addition, NKT cells expressing NKG2D can eliminate NKG2DL-expressing tumor cells. Nevertheless, interaction between type I NKT cells and Tregs showed the inhibition of NKT cells by Tregs via direct contact and providing help to Tregs by NKT cells via IL-12 secretion (53).

TUMOR-ASSOCIATED MACROPHAGES

Among tumor-associated immune stroma, tumor-associated macrophages (TAMs) have crucial functions and promote tumor progression. There is a correlation between TAM high densities with poor overall survival rate in patients with several cancer types except colorectal cancer (54, 55). Circulating monocytes migrate into the TME in response to different molecules such as growth factors [vascular endothelial growth factor (VEGF), colony-stimulating factor 1 (CSF-1), and

granulocyte/macrophage CSF (GM-CSF)], cytokines (IL-6, IL-10 and TGF- β), and chemokines (e.g., CCL2, CCL3, CCL4, CCL7, and CCL8) (56–59). These molecules are released by different cell types including tumor cells in the TME. After residing, monocytes differentiate into TAMs depending on certain signaling molecules in the TME such as IL-6 and leukemia inhibitory factor (60). The induction of TAM differentiation is rendered by pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and hypoxia. TAMs are classified into two phenotypes, pro-inflammatory M1 type and anti-inflammatory M2 type, with tumor suppressive and tumor supportive function, respectively. The M2 macrophages undergo further polarization to become a few more subtypes including M2a, M2b, M2c, and M2d. This division is based on a variety of stimuli (61). Strikingly, M2 TAMs undergo metabolic reprogramming such as glycolysis activation, fatty acid synthesis, modification of tricarboxylic acid and nitrogen cycle metabolism (62). In addition to low expression of MHC class II, the mannose receptor and stabilin-1, tumor-educated M2 TAMs acquire immunosuppressive functions and promote tumor progression by releasing VEGF, MMP-7, MMP-9, IL-12, high levels of IL-10, TGF- β , hepatocyte growth factor and basic fibroblast growth factor (bFGF), adrenomedullin, urokinase-type plasminogen activator (uPA), thymidine phosphorylase (TP), prostaglandin E2 (PGE2), and semaphoring 4D (59, 63, 64). Unlike M1 phenotype, M2 TAMs participate in immune suppression, tumor migration, invasion and angiogenesis. Indeed, M2 TAMs induce angiogenesis by releasing VEGF, IL-1, TNF- α , TP, PGE2, adrenomedullin, and semaphoring 4D. They mediate the degradation of the ECM by uPA, MMP-7, and MMP-9. In the TME, M2 cells crosstalk to other cell types. They interact with tumor cells, MDSCs, Tregs, CAFs, Th2 cells, CD8⁺ T cells, and NK cells. In particular, tumor cells are involved in M2-like polarization by secreting lactic acid, a function interceded by hypoxia-inducible factor (HIF1 α) (65). In turn, the tumor growth is influenced by TAM-releasing arginase 1 (Arg-1) induced by the lactic acid (65). Indeed, L-arginine depletion by arginase is culminated in the downregulation of the TCR ζ chain (66). TAMs induce the suppression of CD3- ζ chain expression in tumor-infiltrating T cells through oxidative stress (67). Thus, the TCR becomes non-functional due to loss of the CD3- ζ chain. TAMs also promote tumor progression through releasing molecules such as VEGF, IL-8 and IL-6 (68, 69). TAMs influence on tumor cell invasiveness when tumor-released TNF- α elicits the expression and secretion of CCL8 from TAMs (70). This chemokine not only upregulates the expression of CSF-1 from tumor cells but also recruits the circulating monocytes that express CCR2 (70). Furthermore, TAM secrete cytokines such as CCL22 and CCL20 to recruit CCR4⁺ and CCR6⁺ natural Treg (nTreg) cells, respectively (71). However, CCL22-expressing TAMs and tumor cells can be targeted and perished by CCL22-specific T cells (72). Macrophages expressing HLA-C, -E, and -G in both soluble and membrane forms can eliminate NK cells expressing NKG2 as well as the inhibitory leukocyte immunoglobulin-like receptor LIT-2 that consequently inhibit IFN- γ secretion (71). Moreover, TGF- β -secreting cells such as TAMs and tumor cells suppress T cells, NK cells, decline DC

migration, and promote CD4⁺ T cell differentiation into Th2 and Tregs (73). TAMs also interact with CAFs in the TME which is described in this review later. Finally, M2 TAMs can upregulate the expression of both PD-1 and PD-L1. PD-1⁺ TAMs exhibit a declined phagocytosis due to the inhibitory role of PD-1 expression on TAMs compared to PD-1⁻ TAMs (74). Therefore, checkpoint blockade of PD-1/PD-L1 can improve the phagocytosis function of macrophages. PD-L1 expression on TAMs can participate to control T cell activation in the TME.

GRANULOCYTES

Basophils

Basophils differentiate and mature in the bone marrow and leave it to circulate in the blood. They participate in allergic diseases, autoimmunity, parasitic infection and Th2 cell differentiation (75, 76). Basophils mediate B cell proliferation, plasma cell survival, and immunoglobulin production (77). By releasing IL-4, they effect different cell types such as fibroblasts, monocytes, macrophages, B and T cells. However, the role of basophils in cancer and the TME is not entirely understood. Basophils interact with other immune cells; for example, they recruit CD8⁺ T cells to the TME by producing CCL3 and CCL4 (5). They also activate these cells through antigen presentation by MHC class I and costimulatory molecule CD86 (75). Interestingly, basophils may require peptide-MHC class II complexes from dendritic cells via trogocytosis which may explain their involvement in CD4 Th2 response activation (78). It was recently shown that regulatory T cells modulate basophil activation through IL-3 and STAT5 however the activation status of basophils in the TME remains obscure (79). Besides interaction with immune cells basophils may indirectly influence tumor growth by VEGF-A production, a proangiogenic factor (80). They may also worsen symptoms due to ascites in patients with ovarian cancer as VEGF-A increases capillary permeabilization. Indeed, ovarian cancer has an enhanced number of basophils in their ascites while their blood level remained low and similar to that of healthy individuals (81). However, in patients with lung cancer, the basophil density in the TME was low compared with other immune cells (82). The presence of basophils in tumor-draining lymph nodes of patients with pancreatic ductal adenocarcinoma and their low number in the blood of colorectal cancer patients was associated with poor prognosis (83). Nevertheless, data is still scarce on the potential role of basophils in cancer. Studies are warranted to show how basophils directly or indirectly help tumor progression and/or tumor immunity.

Eosinophils

Originated and matured in the bone marrow, eosinophils migrate into the blood circulation to reach the body tissues and to pursue their functions which involves controlling asthma and other allergic diseases, vasodilation, metabolic homeostasis, tissue repair, anti-bacterial, and anti-parasite activity (84–87). Eosinophils release different products such as reactive oxygen species, growth factors (e.g., VEGF and TGF- β), several cytokines (e.g., IL-2 and TNF- α) and lipid mediators (e.g., eicosanoids) (88–90). Eosinophils contribute to antigen presentation to helper

T cells and Th2 polarization (91). Therefore, they can promote T cell proliferation by presenting antigens from bacteria, parasites and viruses. Moreover, eosinophils cross-talk to DCs to regulate them. For example, they provide chemotactic abilities for DCs by releasing neurotoxin and induce DC maturation (92, 93). Furthermore, eosinophils regulate other cell types such as basophils, neutrophils and mast cells (94, 95). Eosinophils are commonly seen in the TME (96, 97). In colorectal cancer, their presence was inversely correlated with tumor stage (98). Further, it was shown *in vitro* that eosinophils activated by IFN- γ were capable of killing colorectal cancer cell lines due to their increased cytotoxicity (98). However, regulatory eosinophils, like other regulatory immune cells, promote tumor progression, and suppress immune cells. For example, eosinophils upregulate PD-L1 expression in an IFN- γ -dependent fashion leading to suppression of Th1 responses (99). They can also suppress T cells via galectin-10 by yet not fully understood mechanisms (100). Further by releasing cationic proteins they can hinder antibody production of B cells, inhibit T cell proliferation and elicit mast cell degranulation (101). Eosinophilia has been associated with either poor or better prognosis in different cancers (96, 97). Clearly, characterization of tumor-associated eosinophils and the conditions that promote either pro- or anti-tumorigenic activities is needed to better understand their role in cancer.

Tumor-Associated Neutrophils

There are two phenotypes of neutrophils known as TANs; anti-tumorigenic N1 neutrophils and pro-tumorigenic N2 neutrophils. Neutrophils type N1 are the first leukocytes to be recruited in the injured/inflamed sites. It was demonstrated that bone marrow neutrophils in mice had higher migration to the inflammation sites in early-stage cancer unlike those from late stage (102). This pattern is mediated by autocrine ATP signaling (102); however, Myh9 has also role in the migration process (103). N1 TANs educate effector T cells to reject tumors, elicit apoptosis of malignant cells by TRAIL, produce and secrete reaction oxygen species (ROS) to lyse cancer cells, participate in ADCC, and release matrix metalloproteinase 8 (MMP-8) to degrade the extracellular matrix (ECM) which is beneficial for tumor metastasis. Nevertheless, N2 TANs have a critical function in the immunosuppression of the TME as the tumor evolves. Blood neutrophilia is noted in patients with advanced cancer. Neutrophilia is stimulated by cytokines such as IL-1 β , IL-6, G-CSF, and VEGF secreted by stroma cells and tumors (104). In fact, neutrophils extravasate the blood and recruit into tumor tissue in response to cytokines (IFN- γ and TNF- α), chemokines (KC/CXCL1 and MIP2 α /CXCL2 in mice) and cell adhesion molecules (i.e., CD11b) (104). Once infiltrated, TGF- β -stimulated N2 TANs secrete different molecules that shape the TME. They promote angiogenesis via VEGF, promote tumor development and metastasis through cathepsin G and ROS, induce chronic inflammation by releasing TNF- α , IL-1 β , IL-6, and IL-12, and inhibit effector CD8⁺ T cells (105). It has been shown that in mice with depleted CD11b⁺/Ly6G⁺ TANs had reduced tumor growth and increased activation of CD8⁺ T cells (106). Moreover, neutrophil-derived leukotrienes selectively expand tumor cells (107). N2 TANs also indirectly

preserve tumor cells through recruiting macrophages and Treg cells into the TME (108). Furthermore, TANs were showed to be associated with poor prognosis in several cancer types (109, 110). In addition, enhanced number of CCL2⁺ or CCL17⁺ TANs was correlated with shorter survival rate in patients with hepatocellular carcinoma (108). Due to the abundance of neutrophils in blood and their immediate reaction to tissue injury or inflammation, they are likely important to set the tone of the immune milieu that favors Th2, M2, and N2 responses over Th1-mediated anti-tumor immunity.

MYELOID-DERIVED SUPPRESSOR CELLS

MDSCs are a heterogeneous and commonly immature population of myeloid cells originating from the bone marrow. They share morphology with granulocytes or monocytes. Therefore, the major two MDSC populations are called granulocytic MDSCs (G-MDSC) and monocytic MDSCs (M-MDSC), respectively. MDSCs include myeloid progenitor cells and immature myeloid cells (IMCs). The latest population normally differentiates into mature macrophages, DCs and granulocytes in the peripheral organs in response to growth factors and cytokines (111). Although IMCs in healthy individuals lack suppressive properties, they gain immunosuppressive capability in patients with either cancer or non-neoplastic diseases after activation (111). In the TME, MDSCs are activated by various molecular factors such as VEGF, GM-CSF, C5a, MMP9, IFN- γ , TGF- β , IL-1 β , IL-6, IL-10, IL-12, IL-13, CCL2, CXCL5, CXCL12, gangliosides, and prostaglandins (111). As a result of this activation, MDSCs upregulate immune-inhibiting molecules such as Arg1 and inducible nitric oxide synthase (iNOS) (111). The iNOS in turn generates nitric oxide (NO), a product that reduces ADCC in NK cells. Tumor cells secrete CXCL1 to recruit MDSCs into the TME in which they suppress T cell infiltration (16). They highly express indoleamine 2, 3-dioxygenase (IDO) in the TME which results in the stimulation of apoptosis in T cells which was shown in patients with breast cancer (112). IDO can also downmodulates NCRs and DNAM-1 which reduce NK cell-mediated tumor killing. MDSCs also overexpress peroxynitrite (ONOO⁻) which is a strong oxidant that inhibits T cell function (111). G-MDSCs inhibit CD8⁺ T cells through releasing ROS while M-MDSCs do it through secreting Arg1, iNOS, and ROS culminating in the impairment of ζ -chain expression, suppression of MHC class II expression, eliciting T-cell apoptosis, inhibition of Janus kinase (JAK) 3, and STAT5 in which all promote tumor progression (113). Additionally, MDSCs stimulate CD4⁺ T-cell tolerance in a MHC class II-dependent manner (113). Moreover, MDSCs also inhibit immune cell responsiveness to IFNs via reduction of STAT1 phosphorylation in tumor-bearing mice (114). MDSCs may regulate B cell responses since it was shown that the reduction of B cells in the bone marrow of mice with lung cancer was associated with MDSCs infiltration into the TME. Such a regulation was mediated through IL-7 and enhanced activation of STAT5 (115). Furthermore, Tregs are stimulated by MDSCs in a process that requires tumor-associated

antigens, IL-10, IFN- γ , and CTLA-4 (111). MDSCs can also be controlled and modulated by tumor cells and other immune cells. For example, tumor cells release exosomes expressing TGF- β and prostaglandin E2 (PGE2) to elicit MDSCs for promoting tumor progression (116). In this *in vivo* experiment, tumor-derived exosomes injected in mice recruited CD11b⁺ Gr-1⁺ cells to tumor and were taken up by them (116). It has been indicated that Tregs in inflammatory conditions enhance MDSC differentiation and function via releasing TGF- β leading to the enhancement of inhibitory functions of MDSCs (117). This cytokine enhances the inhibitory function of the MDSCs. Moreover, mast cells in the TME release histamine that binds to the histamine receptor 1 on MDSCs culminating in the elevation of Arg1 and iNOS and, thus, inhibition of T-cell proliferation (118). MDSCs are multi-potent immunosuppressive cells and, hence, one of the major obstacles to activate and maintain anti-tumor immunity.

T REGULATORY CELLS

T regulatory cells significantly infiltrate the tumor and are associated with a poor survival rate (119). Tregs commonly differentiate from naïve CD4⁺ T cells in response to IDO, IL-10, and TGF- β differentiate into CD25⁺ Foxp3⁺ Tregs (called natural Tregs) in the thymus, and into CD25⁻ Tregs including IL-10⁺ Tr1, TGF- β ⁺ Th3, and Foxp3⁺ cells (called inducible Tregs or iTregs) in the periphery (120). Thymic mature Tregs originate from CD25⁺ Treg cell progenitors and Foxp3^{lo} Treg cell progenitors (121). Tregs such as NKT Tregs, Qa-1-specific CD8⁺ Tregs, $\gamma\delta$ -TCR Tregs, CD8⁺CD28⁻ Tregs, and CD8⁺CD25⁺ Tregs may originate from CD8⁺ T cells as well (122). Resting Tregs are not immunosuppressive unless they become activated through TCR engagement and signaling molecules (123, 124). Tregs exhibit either positive or negative functions depending on conditions such as autoimmunity, infection, pregnancy, inflammation, and cancer. Their positive roles include participation in the immune tolerance, preventing autoimmune diseases, inhibition of tissue damage, and controlling inflammation after infection while their negative role is to hamper cancer immunity. Tregs infiltrate into the TME by signaling molecules such as chemokines and their receptor (e.g., CCL1-CCR8, CCL5-CCR5, CCL22-CCR4, CCL28-CCR10, and CXCL12-CXCR4) (125). After recruitment, Tregs promote tumor growth and metastasis. Tregs release immunosuppressive cytokines including TGF- β , IL-10, and IL-35 which are involved in many of the Treg suppressive functions. For example, DCs maturation is inhibited by TGF- β and IL-10 which can also differentiate them into tDCs (126). Further, it was recently indicated that Tregs debilitate DCs capacity as APCs by removing their peptide-MHC class II complex (127). Hence, Tregs indirectly hamper T cell activation by reducing the number of matured DCs and converting the DCs into tolerogenic partners. However, Tregs directly inhibit T cell activation and reduce their proliferative property by releasing soluble CD25 that sequester IL-2, as well as producing IL-10 and TGF- β (128). The T cell metabolism is further interrupted with adenosine and cAMP

by CD39- and CD73-expressing Tregs (120). Treg-released IL-35 influence the expression enhancement of several inhibitory receptors such as PD1, LAG3, and TIM3; thus, promoting intratumoral TIL exhaustion (129). In a similar manner to T cell inhibition, Tregs can directly and indirectly suppress NK cell function. It was elucidated that CD4⁺ CD25⁺ Tregs hinder the NKG2D-mediated NK cell cytotoxicity in a TGF- β -dependent manner, regardless of IL-10 (130). Tregs suppress other immune cells such as basophils, eosinophils, and mast cells as well as B-cell activation and proliferation (120, 131). Finally, it has been demonstrated that Tregs release perforin and granzymes upon T cell receptor engagement which can target cell lysis of T cells and APCs (132).

CANCER-ASSOCIATED ADIPOCYTES

Adipocytes and other cell types such as pericytes, endothelial cells, monocytes, macrophages, and pluripotent stem cells make up adipose tissue consisting of two main types white and brown (133). Cancer-associated adipocytes (CAA), or so called fat cells or lipocytes, store energy as triacylglycerol and support cancer cells by providing lipids. Moreover, these cells have pivotal roles in tumorigenesis, tumor growth and metastasis by overexpression of pro-inflammatory cytokines (e.g., IL-1 β , IL-6, and IL-10), matrix metalloproteinases (e.g., MMP-11), and insulin-like growth factor binding protein-2 (133–135). Additionally, they release molecules that recruit myeloid cells into the TME, to weak their differentiation status toward M2/MDSC, and promote angiogenesis. These molecules include IL-8, CCL2, VEGF, TGF- β , TNF- α , hepatocyte growth factor, plasminogen activator inhibitor-1, cathepsin S, and monocyte chemoattractant protein-1 (MCP1) (136, 137). Thus, CAAs certainly participate to maintain the deleterious immune milieu in the TME that hinder anti-tumor responses and promote tumor progression.

TUMOR-ASSOCIATED ENDOTHELIAL CELLS

Tumor-associated endothelial cells (TAEC) line blood vessels in the TME. They possess critical functions such as angiogenesis, permeability, regulating blood fluidity, transportation of the immune cells, intravasation and extravasation of tumor cells during metastasis. Cell adhesion molecules such as intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) as well as E- and P-selectin expressed on the endothelial cells mediate leukocyte attachment, rolling, and transmigration into tissue. However, these receptors are commonly dysregulated in blood vessels in the TME reducing the TIL infiltration to the tumor bed. Endothelial cells express not only MHC class I but also MHC class II, therefore they can act as non-professional APCs to elicit antigen-experienced T cells but not naïve T cells due to lack of CD80 and CD86 expression (138). In cancer, endothelial cells undergo abnormalities through overexpression of VEGF and aberrant expression of transcription factors (139,

140). Endothelial cells express TRAIL, FASL, PD-L1, and PD-L2 that can support tumor evasion but also inducible T-cell costimulatory ligand (ICOSL), OX40L, CD40 that may otherwise affect immunity (141). It has been indicated in a mouse model of melanoma that T cell infiltration is enhanced by 18-fold in melanoma cells through inhibition of VEGF and consequently overexpression of CXCL10 and CXCL11 in tumor vessels (142). TAECs play a crucial role in tumor progression to support their required nutrients, and the fragile and dysregulated status of TAEC reduces anti-tumor immunity via dysregulated receptors for TIL migration into tissues.

TUMOR-ASSOCIATED PERICYTES

Tumor-associated pericytes (TAPs) wrap around vascular endothelial cells and closely interact each other, both physically (e.g., gap junctions) and paracrine signaling (143). Together with endothelial cells, pericytes effectively participate in angiogenesis. In addition, they maintain blood flow, modulate ECM remodeling, and contribute to the blood-brain barrier in the brain (143). In cancer, pericytes are abnormal with different marker expression and with cytoplasmic projections into the tumor. Aberrant signaling between pericytes and endothelial cells affect angiogenesis and metastasis in cancer which is reviewed elsewhere (144). Pericytes can participate in immunomodulation of the TME. For instance, in response to IFN- γ , they can upregulate MHC class II and a range of cytokines and chemokines to attract lymphocytes (CXCL10) and granulocytes (CXCL8 and CXCL1) to the site of inflammation (145). However, it has been shown that pericytes upregulate PD-L1 during interaction with tumor cells (146). In line with these findings, pericytes activated by murine glioblastoma diminished T cell activation in the TME (147). Thus, TAPs promote tumor progression and may participate to disrupt anti-tumor T cell responses.

CANCER-ASSOCIATED FIBROBLASTS

Fibroblasts are heterogeneous cells that originate from different cell types such as mesenchyme, epithelial cells, mesothelial cells, fibrocytes, endothelial-mesenchymal transition (EndMT) and epithelial-mesenchymal transition (EMT) (148, 149). They have tissue-specific functions and interact with other stromal cells and immune cells via various signaling molecules. In addition to fibrosis and wound healing, fibroblasts participate in the structure of the ECM through synthesizing collagen, fibronectin, and other constituents (148). They also regulate inflammation and the recruitment of the immune cells through growth factors, cytokines, and chemokines. Cancer-associated fibroblasts (CAFs) are derived from different origins: (1) TGF- β -activated resident fibroblasts; (2) EMT; (3) EndMT; (4) smooth muscle actin-upregulated stellate cells; (5) CAAs; or (6) mesenchymal stem cells with TGF- β overexpression or via CXCL16-CXCR6 (150). There is a reciprocal interaction between tumor cells and CAFs in the TME. Cancer cells stimulate the expression of hepatocyte growth factor (HGF) in CAFs through secreting platelet-derived

growth factor, IL-1 and basic fibroblast growth factor (151). HGF plays a critical role in anti-apoptosis, anti-inflammation, and angiogenesis which influences the invasive growth of the tumor (152). Furthermore, CAFs support tumor invasion and dissemination by releasing MMPs (e.g., MMP-2 and MMP-9) and help cancer cell proliferation by releasing exosomes, SDF-1, FGF, IL-6, TGF- β , and osteopontin (150, 153). Indeed, it was recently indicated that CAF-derived exosomes highly express miRNA-21, miRNA-143, and miRNA-378e compared with those from normal fibroblasts (154). After exosome uptake, the tumor cells display EMT and an aggressive phenotype (154). It has also been demonstrated that CD10⁺ GPR77⁺ CAFs can induce the enrichment of cancer stem cells and chemoresistance by secreting pro-inflammatory cytokines IL-6 and IL-8 (155). Besides the interaction with tumor cells, CAFs support angiogenesis by secreting VEGF, FGF2, and FGF7 (150). Compared with normal fibroblasts, CAFs highly express adrenomedullin, a vasodilator peptide hormone, which promote angiogenesis and increase cell tolerance to oxidative stress (156).

Interaction between CAFs and other immune cells has been extensively studied. By secreting TGF- β and IL-10, CAFs convert T cells into iTregs, induce tolerogenic DCs, eliminate APCs by reducing signal activity, inhibit T cell and NK cell function, and activate M2 macrophages. Further, monocytes undergo M2 polarization by M-CSF-secreting CAFs (157, 158). In fact, the number of TAMs significantly correlates with a high grade of CAFs (157). CAFs comprise up to 80% of the tumor mass in pancreatic and breast cancers that are both connected to high level of myeloid cell infiltration (159). A subset of CAFs classified as CAF-S1 provides an immunosuppressive microenvironment in the TME by recruiting CD4⁺ CD25⁺ T cells through OX40 ligand, JAM2, PD-L2, and CXCL12 (160). Moreover, CAF-S1 increases Treg differentiation and expresses B7H3, DPP4, and CD73 to enhance the infiltration of CD25⁺ FOXP3⁺ T lymphocytes (160). Hence, CAFs clearly participate to the immune hostile TME besides their major role to support tumor growth and invasion.

TUMOR-ASSOCIATED EXTRACELLULAR VESICLES

Almost cells release extracellular vesicles (EVs) such as microvesicles (MVs) and exosomes to communicate with other cells in the body. EVs are taken up by other cells and therefore functioning as a messenger both at short and long distances. The uptake of EVs seems to be selective and depending on the interaction between EV surface proteins with plasma proteins of the recipient cells. For example, it was indicated that milk EVs expressing MUC1, a transmembrane glycoprotein, are taken up by DCs expressing DC-SIGN, a C-type lectin receptor, whereas EVs released by other sources that lack this protein are not internalized by the DCs (161). Herein, we focus on the exosomes that have gained much interest over the past few years. Indeed, exosomes are cup-shaped nanovesicles with a size range of 30–150 nm and an endosomal origin that cargo proteins, lipids, and RNA species (162). Tumor cells commonly

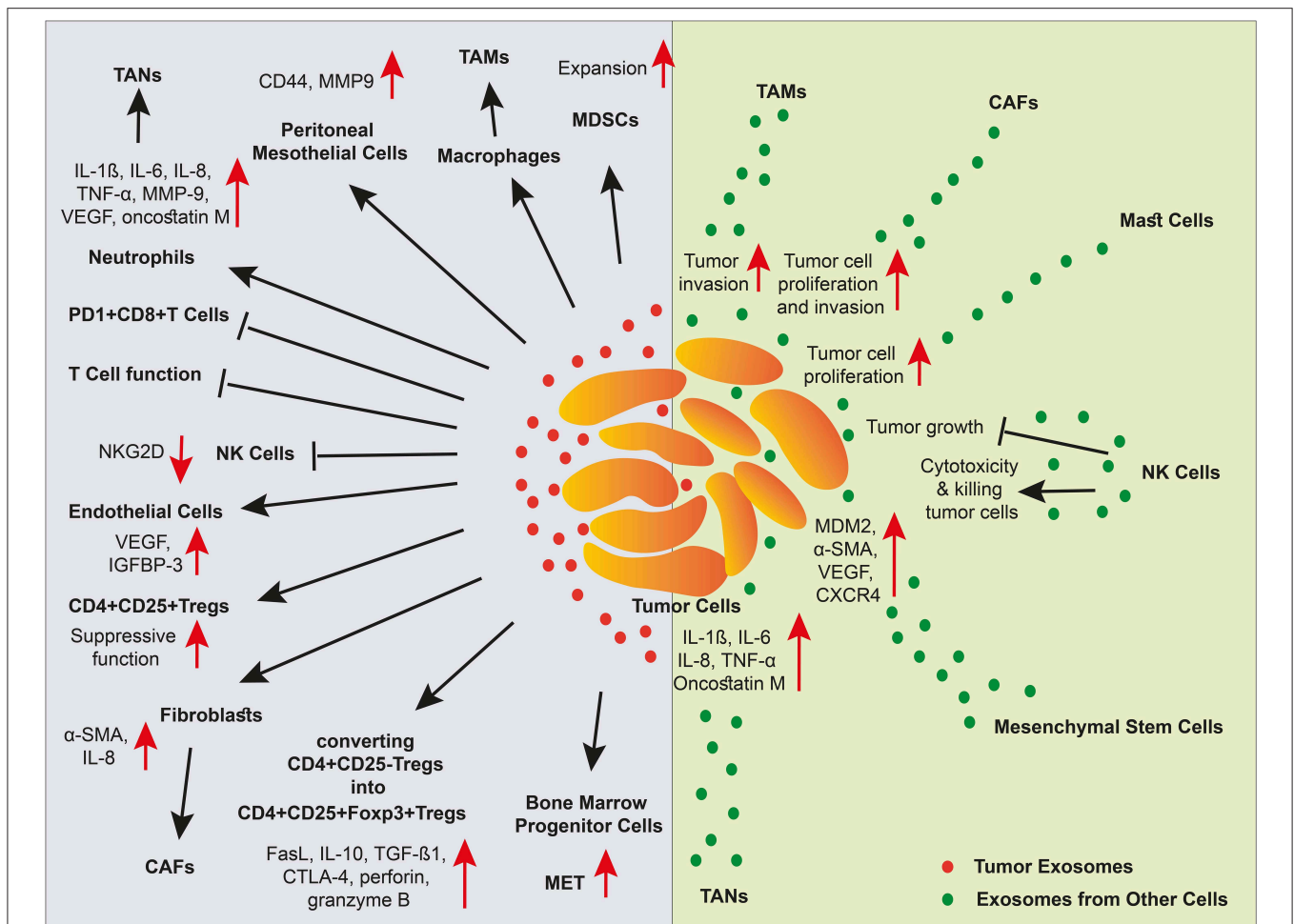


FIGURE 3 | The effect of exosomes in a niche of tumor microenvironment. Different cell types and cancer cells crosstalk in tumor via EVs. In the TME, cancer-associated stroma cells promote tumor progression via exosomes. Tumor cell-derived exosomes inhibit NK cells, and T cells, elicit MDSC expansion and Treg suppressive function, stimulate angiogenesis, and metastasis, and polarize macrophages and neutrophils into TAMs and TANS, respectively. Malignant cells also receive support via exosomes released by cancer-associated stromal cells. However, exosomes released by NK cells induce tumor cell apoptosis through cytotoxicity function.

release high amounts of exosomes. In cancer, exosomes are involved in most biological features including angiogenesis, immune suppression, metastasis, anti-inflammatory function, anti-apoptosis, drug resistance, and reprogramming the recipient cells via transferring RNA species such as miRNAs. The functions of tumor-derived exosomes on immune cells as well as the immune cell-derived exosomes on tumor cells are summarized in **Figure 3**.

Metastasis and tumor progression is a hallmark of cancer and can be partly induced by tumor exosomes. Tyrosine protein kinase Met is a hepatocyte growth factor receptor (HGFR) and its activation by HGF triggers the downstream pathways such as Ras, PI3K/STAT3, and β -catenin (163). This oncoprotein is overexpressed in bone marrow progenitor cells by exosomes from melanoma cells which in turn promotes tumor progression (164). Human peritoneal mesothelial cells (HPMCs) uptake exosomes containing CD44 that are released from

ovarian cancer cells; therefore, CD44 is elevated in the HPMCs and induced MMP9 secretion which promote tumor invasion (165). Furthermore, metastasis can be promoted by exosomes expressing CD151 and tetraspanin 8 released from rat pancreatic adenocarcinoma cells (166). Both CD151 and tetraspanin 8 contribute to matrix degradation since they are involved in integrin and protease interactions. In a recent study, the effect of tumor exosomes on fibroblasts was revealed. Exosomes released from both early and late stage of colorectal cancer differently activated fibroblasts which led to their conversion into distinct CAF phenotypes. One CAF phenotype formed after induction of early-stage cancer exosomes that had high pro-angiogenic and pro-proliferative properties, and another CAF phenotype formed after stimulation of last-stage cancer exosomes that displayed invasive features (167). Further, CAFs supported tumor cell proliferation and invasion by their exosomes through Wnt-planar cell mobility signaling (168). In chronic myeloid

leukemia, tumor cells stimulate bone marrow stromal cells by releasing exosomes (169). After induction, bone marrow stromal cells produce and secrete IL-8 in the TME; thus, leading to the activation of two chemokine receptors, CXCR1 and CXCR2, to promote tumor growth (169). Mesenchymal stem cell (MSC)-derived exosomes commonly lead to tumor growth inhibition (170). However, it has also been shown that exosomes released from MSCs transfer mRNA encoding *CXCR4*, *VEGF*, α -*SMA*, and *MDM2* to tumor cells, thus enhancing angiogenic activity and tumor growth (171). Furthermore, interaction of cholangiocarcinoma cell-derived EVs with MSCs induces the expression of α -smooth muscle actin, *CCL2*, *CXCL-1*, *IL-6* in the MSCs which in turn influence the proliferation of tumor cells via enhanced *STAT3* phosphorylation (172). Tumor exosomes promote angiogenesis by delivering angiogenic factors into endothelial cells or induction of stroma cells, to support tumor progression. For example, it was described that rat adenocarcinoma tetraspanin-8-expressing exosomes bind to and taken up by endothelial cells which induced the modulation of angiogenic genes in the recipient cells in an *VEGF*-independent manner (173). Further, treatment of mesenchymal stem cells with exosomes from prostate cancer cells generated myofibroblasts with pro-angiogenic function due to high levels of *VEGF-A* and pro-invasive property because of high production of *MMP-1*, *-3*, and *-13* (174). A more recently study showed that exosomes derived from head and neck squamous cell carcinoma were internalized by human umbilical vein endothelial cells (HUVECs) and enhanced vascularization (175). Compared to both pre- and non-malignant cells, exosomes from malignant breast cancer cells containing higher level of annexin II were shown to be very aggressive due to cell mobility (176). Incubation of these exosomes with HUVECs significantly provoked higher angiogenesis (176). In addition, tumor cell-derived EVs transfer membrane-bound epidermal growth factor receptor (EGFR) to endothelial cells. EGFR activates the autocrine vascular growth factor receptor (*VEGF*)/*VEGFR-2* pathway in endothelial cells that supports angiogenesis (177). MVs from other cell types can also contribute to the induction of angiogenesis. For example, exosomes released from bone marrow mesenchymal stem cells can be internalized in tumor cells and elicit angiogenesis through transferring *VEGF*, *CXCR4*, α -*SMA*, and *MDM2* mRNA transcripts (171).

Immune cells also release exosomes that can affect the tumor or the anti-tumor immune response. NK cell-derived exosomes contain FasL, granulysin, perforin, granzyme A and B therefore exerting cytotoxic activity against tumor cells (178–180). This property is likely true for tumor-targeting T cells as well. For example, it was recently shown that engineered chimeric antigen receptor (CAR) T cells release CAR⁺ exosomes that were cytotoxic to tumor cells (181). Further, it has been indicated that CTL-derived exosomes can provide additional stimulation of bystander CTLs that were previously activated by low affinity antigens (182). Nevertheless, immunosuppression and tumor evasion from the immune recognition in cancer patients are also orchestrated by tumor-derived exosomes and larger released vesicles. For example, it was elucidated that *NKG2DL*-expressing exosomes from ovarian cancer cells bind to the *NKG2D* receptor

on NK cells, culminating in the *NKG2D* downregulation as well as impairment of the NK cell-mediated degranulation and cytotoxicity (39). Other studies also showed that tumor exosomes diminish the expression of *NKG2D* and hamper NK cell cytolytic activity (183). Inhibition of NK cell function is also conducted by hypoxic tumor-derived MVs containing *TGF- β 1* following their uptake by NK cells and consequently the down-modulation of *NKG2D* (184). Tumor exosomes can also affect the function of effector CD8⁺ T cells. Hepatocellular cancer cell-derived exosomes can be internalized into the recruited T cells to deliver *14-3-3 ζ* . The subsequent upregulation of this protein in the T cells leads to the modulation of multiple cellular pathways and down-modulation of their anti-tumor activity (185). Another study showed the influence of phosphatidylserine-expressing EVs from tumor cells on the T cell impairment via the regulation of Akt phosphorylation (186). Moreover, exosomes displaying *CD39* and *CD73* released from tumor cells could generate adenosine to eliminate T cells (187). The other important role of tumor exosomes is to suppress the function of effector T cells by *PD-L1*/*PD-1* interaction. Tumor exosomes can display *PD-L1* and bind to *PD-1*-expressing effector T cells and suppress their activity even in the draining lymph node demonstrating the long distance communication capacity of tumor cells via the release of exosomes (188). Other effects on the immune system includes the ability of tumor exosomes to reduce ADCC by binding tumor-reactive antibodies that would otherwise target the tumor cells (189). Furthermore, exosomes released from gastric cancer cells induced N2 polarization of TANs and these cells promoted cancer cell migration (190). They can also elicit MDSC expansion by *STAT3* activation via membrane-associated *Hsp72*, and recruit Tregs expressing *CCL20* into the TME (191, 192). Tumor-derived exosomes were shown to mediate the conversion of CD4⁺ CD25⁻ T cells into CD4⁺ CD25^{high} FOXP3⁺ regulatory T cells with enhanced expression of Fas ligand, *IL-10*, *TGF- β 1*, *CTLA-4*, granzyme B, and perforin (193). Moreover, the effect of tumor exosomes in macrophage polarization has been shown in recent studies. Tumor-derived exosomes stimulate macrophage polarization into M2 phenotype following activation of *NF- κ B* pathway and enhanced gene expression of pro-inflammatory cytokines such as *IL-1 β* and *IL-8* (194). Such a macrophage polarization was also shown by hypoxic tumor exosomes enriched in *CCL2*, *CSF1*, *TGF- β* , macrophage migration inhibitory factor (*MIF*), ferritin heavy/light chain, endothelial monocyte-activating polypeptide 2 (*EMAP2*), and leukotriene A-4 hydrolase (195).

The impact of tumor EVs on their targets is not limited to their protein content since they also convey genetic materials such as miRNA and lncRNA transcripts that can profoundly affect the targets. The EVs from other cell origins also contribute to tumorigenesis due to their RNA species. It has explicitly been elucidated that miRNAs possess diverse expression and play critical functions in the body. In general, miRNAs implicate in cell development, proliferation, differentiation, apoptosis, and diseases including cancer. Both endogenous and exogenous miRNAs are packed into the EVs. These miRNAs like other RNA species are preserved in exosomes from enzymatic degradation (196). Exosomal miRNAs are delivered into recipient cells to

execute functional roles in the targets and examples of these roles are described elsewhere (197). Briefly, such functions include: (1) contribution to chemotherapy resistance via miR-21 and miR-155 (198); (2) provoking angiogenesis in endothelial cells with miR-210 (199); (3) NK cell inhibition by miR-23a through targeting CD107a (184); (4) T cell elimination via miR-24-3p (200); (5) inducing polarization of macrophages into M2 phenotype via miR-222-3p, miR-940 and miR-301a (201–203); and (6) promoting tumor cell proliferation and metastasis via miR-148a and miR-423-5p (204, 205). However, exosomes released by stromal cells such as TAMs and CAFs can elicit tumor invasion and metastasis via miR-223, miR-21, miR-143, and miR-378e (154, 206). In addition to miRNA transcripts, lncRNAs carried by tumor-derived MVs and exosomes can be associated with tumor progression. Cancer cells release H19-containing exosomes that modulate endothelial cells to enhance angiogenesis (207). Hypoxic exosomes from bladder cancer cells carry urothelial cancer-associated 1 (UCA1) that augment tumor growth through epithelial-mesenchymal transition (208). Metastasis-associated lung adenocarcinoma transcript 1 (MALA1) is another lncRNA that can be sorted into exosomes of epithelial ovarian cancer cells and shuttled into HUVECs and increase angiogenesis (209). In a study, the induction of tumor invasion and migration was mediated by exosomal HOX transcript antisense RNA (HOTAIR) originated from bladder cancer cells (210).

METABOLITES

Metabolism is biochemical reactions in the organism, transforming one chemical into another via metabolic pathways. Metabolism is ensuring that the food we eat will be converted to sources of energy and building blocks for DNA-, protein-, and fat synthesis. During malignancy, the cell metabolism undergoes changes to support the progressing tumor and the malignant phenotype (211). For example, due to hypoxia and acidic conditions the tumor switches to glycolytic metabolism which further reduces pH due to lactic acid production by the tumor as reviewed elsewhere (212). The immune system is affected by the metabolic alterations in the TME. For example, TILs react to high acidic condition, the absence of oxygen and the unfavorable composition of metabolites in the TME. While a low pH can trigger maturation of DCs likely due to protons being recognized by DAMP, T cells will become less prone to cytolytic responses and IFN- γ production. However, the T cell anergy can be reversed if the T cells are exposed to normal conditions (213, 214). Hypoxia plays a crucial role in drug resistance, angiogenesis, stimulation of metastasis, and tumor invasion as well as immune suppression (215, 216). It has also been shown that hypoxia contributes to an immunosuppressive microenvironment through upregulation of checkpoint molecules including modulation of CD47, HLA-G, and PD-L1, and finally regulation of lactate and adenosine (216). A hypoxic microenvironment together with lactic acid reduces T cell activation, proliferation and lytic ability (215). Hypoxia also attracts TAMs, Treg cells, and MDSCs into the TME (215).

Different metabolites also affect the immune system in the TEM including arginase, adenosine, IDO, and NOS. Their actions are briefly discussed herein.

Arginine known as L-arginine (L-Arg) is an essential α -amino acid which is beneficial for the immune cells. For example, L-Arg is required for the expression of the CD3 ζ chain and therefore crucial for TCR signaling and cytotoxicity (217, 218). Nevertheless, L-Arg might be depleted by L-Arg-degrading enzymes such as arginases. Such a depletion is conducted by TAMs, N2 neutrophils and monocytic MDSCs that release high level of arginase in the TME which subsequently metabolizes L-Arg into urea and L-ornithine (219). It was shown *in vivo* that L-Arg depletion accumulates MDSCs and prevents T cell proliferation (220). Interestingly, arginase inhibitors partially eliminate MDSCs which produce high levels of arginase *ex vivo* and can impede myeloid cell-mediated suppression in the TME and enhance TIL function (221, 222).

The cellular metabolite adenosine is a nucleoside derived from ATP barely present in the extracellular space in healthy tissue. However, upon injury it is present due to an ATP dephosphorylation process leading to adenosine. In the extracellular space adenosine binds to its cellular receptors. Adenosine is important in diverse functions, reviewed elsewhere (223) but in the TME it is involved in immunosuppression. High adenosine concentration suppresses CTLs and NK cells by diminishing their cytotoxic capabilities because of a cAMP build-up which leads to protein kinase A (PKA) activity and thereby a negative effect on signaling pathways needed for T cell and NK cell function (223, 224). The NK cell maturation is also inhibited by adenosine (225). Blocking adenosine is illustrated elsewhere (223).

Three enzymes IDO1, IDO2, and tryptophan-2,3-dioxygenase (TDO) catabolize tryptophan (Trp), an essential amino acid for physiological processes such as immune regulation, into kynurenine (kyn). IDO1 is produced in mesenchymal stromal cells, endothelial cells, fibroblasts, tolerogenic DCs, TAMs, CAFs, MDSCs, and elevated in tumor cells and can be loaded into tumor-derived microvesicles (226–228). IDO1 elevation has pivotal roles in tumorigenesis and immunosuppression, resistance to chemotherapies and is correlated to shorter survival in cancer patients (229–231). The immunosuppressive IDO halts NK cell cytotoxicity, inhibits the infiltration and function of CD8⁺ T cells, recruits and activates MDSCs and Tregs in the TME, stimulates differentiation of CD4⁺ T cells into Treg phenotypes through activation of AhR by Kyn, induces tumor tolerance to apoptosis, impairs TCR via Vav1 elimination, elicits differentiation of immunogenic DCs into tolerogenic DCs, stimulates polarization of macrophages into TAMs, exerts resistance to checkpoint inhibitors, and suppresses CD19-CART cells (228, 232–238). Moreover, IDO influences PD-1 overexpression by Tregs and these cells in turn stimulate expression of PD-L1 and PD-L2 on DCs (235). Such a process negatively modulates T cells. However, blocking these ligands by monoclonal antibodies enhances T-cell proliferation and activation (239). Since IDO elevation is associated with carcinogenesis and tumor progression, there has been efforts to inhibit this enzyme clinically. Different strategies

have been applied for cancer patients regarding IDO pathway; such as inhibition of IDO1, AhR, Kyn, Trp-Kyn pathway and dual IDO1-TDO inhibition. Although IDO1 inhibitors showed promising results in clinical trials, a combination of Epcadostat, an IDO1 inhibitor, with pembrolizumab, a PD-1 checkpoint inhibitor, did not show a significant difference with placebo plus pembrolizumab (240).

NOS is an enzyme catalyzing the production of nitric oxide (NO) from the metabolite L-arginine. An inducible form, iNOS, is commonly expressed in cancer which leads to high NO levels which can cause DNA damage, oncogene activation, inhibit DNA repair enzymes and suppressor genes as reviewed elsewhere (241). However, NOS functions as either anti-tumor or pro-tumor, depending on its concentration (242). At high concentration, NO enhances the anti-tumor activity through broad DNA damage, cell apoptosis, cytotoxicity, oxidative, or nitrosative stress whereas at low concentration, it increases cell proliferation, apoptosis, angiogenesis, invasiveness, and metastasis (242). In addition to cancer cells; macrophages, neutrophils, hepatocytes, endothelial cells, cardiomyocytes, and chondrocytes can produce high levels of NO (219). Interaction between NO and reactive oxygen species forms nitrogen dioxide, reactive nitrogen species and peroxynitrite (ONOO-) in which the latest product stimulates carcinogenesis such as angiogenesis, metastasis, apoptosis elimination, DNA damage induction, and increasing cell proliferation; however, it may act as an antioxidant (242). Hence, this ion plays important roles under pathophysiological conditions. Of interest, peroxynitrite can nitrate both subunits α and β of the CD8 T cell receptor thus impairing antigen recognition (243). In addition, MDSC-released peroxynitrite inhibits the recruitment of effector CD8⁺ T cells via nitration of CCL2 (244). Further, iNOS upregulation is elicited by a cytokines secreted by Th1 cells (e.g., IL-1, TNF- α , and IFN- γ) as well as hypoxia and lipopolysaccharide (219). Hence, these events augment the deficiency of L-Arg which in turn provides immunosuppression and tumor progression (219). In terms of tumor eradication, NOS inhibitors have been used in colorectal cancer to eliminate the migration of malignant cells by interruption of angiogenesis pathway (245).

IMMUNOTHERAPY—THE CHALLENGES OF THE TME

Activation of the immune system to combat cancer has been an appealing treatment method that for many decades eluded success in the vast majority of cancer indications. However, immunotherapy in the form of bacillus Calmette-Guerin (BCG) has been standard-of-care for non-muscle-invasive urinary bladder cancer since the past 40 years (246). These patients have rather low tumor burden at the stages when BCG is successful, meaning that the immunosuppressive mechanisms are still not advanced. With the growing body-of-evidence pin-pointing the immune escape mechanisms used by the tumor and its microenvironment, it has become evident that these mechanisms need to be combated to fully activate antitumor immunity.

The first attempts were performed by Rosenberg et al., whom combined T cell therapy with so called preconditioning. The preconditioning consisted of cyclophosphamide and fludarabine given a few days prior to infusion of *ex vivo*-stimulated TILs to reduce regulatory T cells. This strategy, sometimes combined with whole body irradiation, finally resulted in clinical responses to immunotherapy in patients with melanoma (247). This was the first study demonstrating that immunotherapy could eradicate solid cancer in last stage patients and these pivotal steps built the basis for T cell therapy including the successful protocols using genetically engineered CAR T cells (248, 249). The increasing understanding of how T cells are regulated revealed that after activation, T cells upregulate checkpoint receptors that initiate self-regulation upon stimulation by counter receptors. Such checkpoints including PD-1 and CTLA-4 are important to restrict unwanted immunity as well as to contract responses when after an infection is eradicated. However, stimulation of checkpoint receptors in cancer is commonly chronic and lead to a premature abruption of the immune response. Antibodies blocking CTLA-4, PD-1, or the PD-L1 counter receptor are collectively called checkpoint inhibitors (CPI).

CPIs has changed the cancer therapeutic landscape over the past few years and have added immunotherapy to the cornerstones of cancer therapeutics (250). For many indications such as melanoma and lung cancer, a CPI is now the first line treatment over conventional chemotherapy and radiotherapy. Although CPI has shown impressive outcomes, especially in immune sensitive tumors, the majority of patients are refractory or become resistant (251). Such mechanisms of resistance were recently reviewed elsewhere (252). Briefly, unresponsive patients commonly have a poor TIL level in the tumor which may depend on few neoantigens leading to robust T cell responses. For example, it is known that patients with mismatch repair defects have a high level of mutations and consequently a wide range of antigens for TILs to target. These patients have a high response rate to CPI while those lacking mismatch repair defects commonly have a poor outcome (253). Another resistance mechanism in refractory patients is a defect antigen-presentation machinery. Such defects include β 2-microglobulin (B2M) deficiency leading to absence of MHC class-I on the cell surface or defects in the antigen processing machinery (254). Hence, these defected cells are invisible to T cell recognition. However, besides intrinsic tumor resistance, the TME including immunosuppressive immune cells certainly contribute to hamper immunotherapy such as CPI. Likely, any therapy aiming to reduce immunosuppression, like CPI, must be given simultaneously to other agents that activate immune reactions or counteract other suppressive arms such as the myeloid cell compartment.

Immune activating therapies include a wide range of different treatment modalities such as tumor-peptide-based vaccines, oncolytic viruses, agonistic antibodies and a variety of immune cell therapies based on T cells, NK cells, and dendritic cells reviewed elsewhere (255). Options to reduce the action of immunosuppressive cells or molecules are also being evaluated.

TABLE 1 | Clinical outcomes correlated with the cell density alteration of cancer patients after immunotherapies.

Cancer type	Treatment	Cell types	Clinical correlation	References
Prostate	Ipilimumab	CTL ↑ ($p < 0.05$) Memory T cells ↑ ($p < 0.05$)	OS ↑ PFS ↑	(262)
NSCLC	Nivolumab	CTL ↑ ($p < 0.05$)	OS ↑ PFS ↑ RR ↑	(263)
	Pembrolizumab	ND	ORR ↑ PFS ↑	(264)
Urothelial	Atezolizumab	CTL ↑ ($p = 0.02$)	OS ↑ ORR ↑	(265)
Melanoma	AdCD40L	CTL ↑ ($p = 0.0001$)	NS	(266)
	Pembrolizumab	CTL ↑ ($p < 0.0001$)	NS	(267)
	Nivolumab	ND	OS ↑ PFS ↑	(268)
	Nivolumab	Th9 cells ↑ ($p < 0.05$) Th2 ↑ ($p < 0.05$)	ND	(269)
	Pembrolizumab + T-VEC	Memory T ↑ ($p < 0.001$)	OS & PFS (NS)	(270)
		B cells ↑ ($p < 0.001$)	OS & PFS (NS)	
		CD4 ⁺ T ↑ ($p < 0.001$)	OS & PFS (NS)	
		CD68 ⁺ cells ↑ ($p < 0.001$)	OS & PFS (NS)	
		CTL ↑ ($p < 0.001$)	OS & PFS (NS)	
	Ipilimumab + ATRA	Treg ↓ ($p < 0.001$)	OS & PFS (NS)	(271)
MDSCs ↓ ($p < 0.05$)		ND		
Ipilimumab	CD4 ⁺ ICOS ⁺ T ↑ ($P < 0.0001$)	(272)	(272)	
	Treg ↓ ($p < 0.001$)			
	EM CD8 ⁺ T ↑			
	MoMDSCs ↓ ($p < 0.001$)			
Bladder	AdCD40L	Treg ↓ ($p = 0.008$)	ND	(273)
		CTL ↓ ($p = 0.02$)		
Renal	IL-2-based immunotherapy	Treg ↑ ($p < 0.001$)	Poor prognosis PR ↑ PR ↑ PR ↑	(274, 275)
		Neutrophils ↓ ($p = 0.026$)		
		CD3 ⁺ cells ↑ ($p = 0.002$)		
		CD8 ⁺ T ↑ ($p = 0.003$)		
		CD57 ⁺ NK ↑ ($p = 0.001$)		
Lung, hepatic	NK cell- immunotherapy	NK cells ↑ ($p < 0.01$)	PFS ↑	(276, 277)
		CD3 ⁺ T cells ($p < 0.01$)		
Gastric	RAM-containing chemotherapy	PD-1 ⁺ CD8 ⁺ T cells ↓	NS NS	(278)
		Treg ↓ $P = 0.034$		
Colorectal	Bevacizumab plus chemotherapy	Treg ↓ ($P = 0.0039$)	ND	(279)
ALL, lymphoma	CAR T cells	MDSCs ↓ ($p = ?$)	OS ↑	(280)
Glioblastoma	Bevacizumab	Treg ↓ ($p < 0.01$)	OS ↓	(281)
		Neutrophils ($p < 0.05$)	OS ↑	
		Monocytes ($p < 0.001$)	OS ↑	
HNC	Tadalafil + M/pICLC V	MDSCs ↓ blood ($p < 0.05$)	ND	(282)
		Treg ↓ blood ($p < 0.018$)	ND	
		MDSCs ↓ tissue, $p = 0.007$	ND	
		Treg ↓ tissue, $p = 0.007$	ND	
		CD8 ⁺ T ↑ tissue, $p = 0.023$	OS ↑	

Clinical correlations were statistically significant unless mentioned. NSCLC, non-small-cell lung cancer; HNC, head and neck carcinoma; ALL, acute lymphoblastic leukemia; T-VEC, Talimogene Laherparepvec; RAM, Ramucirumab-containing chemotherapy; M/pICLC V, MUC1/polyICLC vaccine; ATRA, all-trans retinoic acid; EM, effector memory; ND, not determined; OS, overall survival; ORR, objective response rate; SD, stable disease; PR, partial response; PFS, progression-free survival; ORR, overall response rate; CRR, complete response rate; RR, response rate; NS, not significant.

For example, antibodies that blocks chemokine receptors may prevent accumulation of suppressive myeloid cells such as M2 macrophages in the TME (256), the small-molecule mebendazole polarizes macrophages toward the anti-tumoral M1 phenotype (257) and common chemotherapeutics such as gemcitabine, or tyrosine kinase inhibitors, reduces both MDSCs and/or Tregs in patients (258–260). Such agents may be of high interest to combine with CPIs and activating immunotherapeutics. However, agents affecting angiogenesis

may be of interest since they not only limit blood supply to the tumors but also normalize the otherwise dysregulated blood vessels in the tumor tissue. Such a normalization may allow for better lymphocyte attachment, rolling, and transmigration into the tumor site (261). **Table 1** shows a selection of clinical trials that correlated clinical observations after treatment with different immunostimulatory/TME modulating agents to the level of immune cell findings in the tumor or blood.

CONCLUDING REMARKS

The TME is clearly complex and participates to promote tumor formation, progression, and metastasis while hampering anti-tumor immunity. It is important to consider the different aspects of the TME when designing new therapies and new treatment protocols for cancer patients. Many activating immunotherapies have failed to show clinical benefit over the years which is likely due to the previous lack of understanding of immunosuppression in the patients. As CPIs entered the stage, it is now possible to release the TME break of CTLs but CPI therapy is dependent on *de novo* T cell activation. Hence, combinations of an immune activating treatment and CPIs are appealing. However, active immune cells must be able to enter into the tumor parenchyma to exert their function. The next generation of immunotherapy

trials will likely involve drugs that can normalize tumor blood vessels. Moreover, considering the highly immunosuppressive role of fibroblasts and other stroma, novel treatments that may target the function of these cells should be considered together with immunotherapy. Finally, more efforts need to consider microbiota and lifestyle mechanisms in the efficacy evaluation of immunotherapy.

AUTHOR CONTRIBUTIONS

Conception of idea was from AL-M. All contributed to the writing of the review. AL-M the most and MA-M the least. AL-M prepared the figures and the table. AL supervised and edited the manuscript. All authors approved the final version of the manuscript.

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Conflict of Interest: AL declares the following conflict of interests: she is the CEO and board member of Lokon Pharma AB, the chairman of the board of Repos Pharma AB and Vivolux AB and a board member of Bioimics AB. She is an alternate board member and advisor to NEXTTOBE AB. She also holds a contract research agreement with Lokon Pharma AB and has royalty agreements with Lokon Pharma AB and Alligator Biosciences AB.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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