Context Drives Diversification of Monocytes and Neutrophils in Orchestrating the Tumor Microenvironment

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Recent preclinical/clinical studies have underscored the significant impact of tumor microenvironment (TME) on tumor progression in diverse scenarios. Highly heterogeneous and complex, the tumor microenvironment is composed of malignant cancer cells and non-malignant cells including endothelial cells, fibroblasts, and diverse immune cells. Since immune compartments play pivotal roles in regulating tumor progression via various mechanisms, understanding of their multifaceted functions is crucial to developing effective cancer therapies. While roles of lymphoid cells in tumors have been systematically studied for a long time, the complex functions of myeloid cells have been relatively underexplored. However, constant findings on tumor-associated myeloid cells are drawing attention, highlighting the primary effects of innate immune cells such as monocytes and neutrophils in disease progression. This review focuses on hitherto identified contextual developments and functions of monocytes and neutrophils with a special interest in solid tumors. Moreover, ongoing clinical applications are discussed at the end of the review.

Keywords: monocytes, neutrophils, tumor microenvironment, myeloid cell heterogeneity, innate immunity, cancer immunology

MONOCYTES: FROM DEVELOPMENT TO DEPLOYMENT

Monocyte Development

Monocytes originally stem from the bone marrow and constitute 10% of leukocytes in human blood and 4% of leukocytes in mouse blood, respectively (1). The development of blood monocytes is dependent on colony-stimulating factor 1 receptor, CSF-1R (also known as M-CSFR; macrophage colony-stimulating factor receptor) (1, 2). CSF-1R is a hematopoietic growth factor receptor expressed on monocytes, macrophages, dendritic cells and their progenitors (1, 2). CSF-1R interacts with its ligands CSF-1 (M-CSF) and IL-34 to regulate the development of monocytes in the bone marrow (1, 2). In mice deficient in CSF-1R and CSF-1, monocyte development is inhibited, and therefore the number of monocytes in blood is remarkably reduced (1, 2).

With knowledge of CSF-1R, it is possible to navigate the development process of monocytes. From the bone marrow, hematopoietic stem cells (HSCs) give rise to heterogeneous multipotent progenitors (MPPs) generating common myeloid progenitors (CMPs) or common lymphoid progenitors (CLPs) in a CSF-1 dependent manner (3). While lymphoid cells such as T

lymphocytes, B lymphocytes, and natural killer cells are derived from CLPs, CMPs generate megakaryocyte and erythrocyte progenitors (MEPs) or granulocyte and macrophage progenitors (GMPs). Generated GMPs further go through a series of differentiation, firstly into macrophage, and DC progenitors (MDPs), then into common monocyte progenitors (cMoPs), and finally into monocytes (3). Differentiated monocytes can be divided into two main subpopulations defined as $Ly6C^{hi}CX_3CR1^{low}$ and $Ly6C^{low}CX_3CR1^{hi}$ cells in mice and as $CD14^{hi}CD16^{+/-}$ and $CD14^{low}CD16^{hi}$ cells in humans (4–8).

$Ly6C^{hi}CX_3CR1^{low}$ populations (hereinafter referred to as $Ly6C^{hi}$ monocytes) are named "classical" or "inflammatory" monocytes, whereas $Ly6C^{low}CX_3CR1^{hi}$ populations (hereinafter referred to as $Ly6C^{lo}$ monocytes) are named "non-classical" or "patrolling" monocytes for their preferential patrolling behavior while circulating the blood stream (9, 10). Development of $Ly6C^{hi}$ monocytes occurs during the cMoP stage, dependent on GM-CSF, c-FLIP, IRF8, and KLF4 (10). The widely accepted hypothesis on $Ly6C^{lo}$ monocyte differentiation is that after generation of $Ly6C^{hi}$ monocytes from the bone marrow, a proportion of them differentiate into $Ly6C^{lo}$ monocytes as downregulation of Ly6C and upregulation of Nr4a1, C/EBPβ, CSF-1R, and CX_3CR1 (4, 10, 11). However, this was questioned for some time in that deletion of transcription factors KLF4 and IRF8 hinders the development of $Ly6C^{hi}$ monocytes but not $Ly6C^{lo}$ monocytes (12–14). This finding led to controversy on whether $Ly6C^{lo}$ monocytes originate from $Ly6C^{hi}$ monocytes or not. The latter argues that $Ly6C^{lo}$ monocytes might have a distinct differentiation lineage in a $Ly6C^{hi}$ monocyte-independent way, namely direct differentiation from cMoPs.

Single-cell RNA sequencing provided an additional clue, reasserting that $Ly6C^{hi}$ monocyte population is the source of $Ly6C^{lo}$ monocytes (15). Application of such advanced technology revealed that steady-state $Ly6C^{hi}$ and $Ly6C^{lo}$ monocytes are homogenous populations, and C/EBPβ regulates the differentiation of $Ly6C^{hi}$ monocytes into $Ly6C^{lo}$ monocytes (15). This is also in line with remarkable expression/function of Nr4a1 on $Ly6C^{lo}$ monocyte development (16), as it was found that regulation of Nr4a1 is mediated by the expression of C/EBPβ and also KLF2 assisting conversion of $Ly6C^{hi}$ monocytes to $Ly6C^{lo}$ monocytes (12, 15–17). Besides Nr4a1, Ten-Eleven-Translation-3 (TET3), a target of hsa-miR-150, regulates differentiation of classical monocytes into non-classical monocytes in K562 human chronic myeloid leukemia and U937 human lymphoma (18). Upregulation of TET3 expression in classical monocytes following downregulation of hsa-miR150 rarely generates non-classical monocytes, but does not affect the survival of non-classical monocytes (18). Recently, single-cell RNA-seq has also led to the identification of two additional monocyte populations and their distinct relationships with other immune cells in human blood, highlighting the heterogeneity of myeloid cells (19). High-dimensional mass cytometry has further revealed heterogeneity within human non-classical monocytes, and has allowed distinguishing between two different non-classical monocyte subsets, $Slan^+$ and $Slan^-$, with functional differences based on Slan expression (20).

Some developed monocytes can enter non-lymphoid organs such as skin and lung without differentiation and orchestrate the physiological condition, while some portion of developed monocytes undergoes differentiation into macrophages or dendritic cells (21–24). Of note, differentiated macrophages are conventionally classified into pro-inflammatory M1 type and anti-inflammatory (pro-tumoral) M2 type, and these macrophages differentially regulate tumor progressions and metastases (25). However, this binary classification of macrophages is insufficient to represent their multifaceted and plastic functions (25). On the other hand, monocyte-derived dendritic cells have been mainly regarded as immune activators in the tumor microenvironment, recruiting and stimulating immune effector cells (26). Nevertheless, dendritic cells are also highly heterogeneous, and cancer cells can recruit the immunosuppressive subset of dendritic cells and/or suppress their anti-tumoral functions (26). All this flexibility appears in a context-dependent manner. Likewise, differing individual functions of monocytes might result from different contexts of development. While it is well-accepted that the bone marrow is the primary source of production and supply of monocytes in physiological condition (1), there is substantial controversy whether the bone marrow serves the same role in cancer-derived pathological conditions. Splenic progenitor cells are reinforced to generate monocytes during KP lung carcinoma progression, which suggests that the spleen could be a critical organ to produce and amplify monocytes (27). The pivotal role of spleen as a source of monocytes has also been highlighted in a different inflammatory condition (28). Angiotensin II plays a central role in amplifying $Ly6C^{hi}$ monocytes and their precursors in the spleen red pulp of KP lung carcinoma-bearing mice as well as releasing monocytes from their splenic reservoir (28, 29). However, a conflicting view has been suggested in a different lung tumor model. During the development of Lewis lung carcinoma (LLC), the bone marrow primarily promotes monocyte production while the spleen plays a minor role in monocyte production (30). Monocytes produced from the bone marrow are more favored to migrate into and to be accumulated in the tumor region than those from the spleen (30). Although an increased accumulation of monocytes in the spleen is also detected in the LLC model, it is because the bone marrow primarily accelerates monocyte production and transfers the newly formed monocytes to the spleen; the spleen is not the primary source (30). As such, different context might have yielded the controversy on tumor monocyte development. Therefore, further studies need to be conducted in as many types of tumors as possible (31).

Monocytes: Pro-tumoral vs. Anti-tumoral Functions in Solid Tumors

Other than the well-known feature as precursors of macrophage and dendritic cell populations, monocytes play a significant role *per se* in orchestrating the immune system not only in homeostatic condition (21), but also in tumor progression (7, 8, 32–35). Generally, high rate of monocyte infiltration into the tumor milieu indicates poor clinical prognosis of cancers (36, 37).

Since each subset of monocytes has different functions in tumor progression depending on the context, it is momentous to decide which subset of monocytes should be targeted in each tumor. Distinct functions of Ly6C^{hi} monocytes and Ly6C^{lo} monocytes in solid tumors have been explored (**Table 1**; **Figure 1**). These monocytes play pro-tumoral or anti-tumoral roles, regulating diverse mechanisms ranging from angiogenesis to immune modulation in a context-dependent manner (**Table 1**; **Figure 1**).

Recruitment of Classical Monocytes and Their Functions in Solid Tumors

Ly6C[hi] classical monocytes have been mostly reported to play pro-tumoral functions once recruited to the tumor microenvironment (**Table 1**; **Figure 1**). Ly6C^{hi} monocytes express high levels of CCR2 on their surface (32). CCR2 mediates the migration of Ly6C^{hi} monocytes from the bone marrow to CCL2-secreting tumor milieu in PyMT spontaneous breast carcinoma, KCKO pancreatic carcinoma, and MC38 colorectal carcinoma (29, 45, 46). These recruited classical monocytes release VEGFA (a major stimulator of angiogenesis) to facilitate tumor cell extravasation and lung metastasis (32, 47). In human pancreatic tumor as well as murine pancreatic lesion model, the tumor microenvironment releases CCL2 and thereby actively recruits CCR2-expressing CD14+CD16− classical monocytes from bone marrow to blood stream, which is a prognostic factor of worse outcome (45). In contrast, CCR2 inhibition attenuates the mobilization and thus leads to forming an anti-tumoral immune environment in KCKO pancreatic carcinoma and MC38 colorectal carcinoma (45, 46). In human RCC patients and xenograft models, the IL-1β/IL-1R interaction activates the MyD88-NF-kB signaling pathway, and thereby enables classical monocytes with pro-tumoral phenotypes to upregulate pro-tumoral genes such as VEGF, MMP-10, IL-8, TNF-α, and PTGS2 (38). Ly6C^{hi} monocytes/CD14+CD16− monocytes also facilitate cancer cell invasion and metastases via expressing F13a1 to promote fibrin cross-linking not only in murine KLN205 lung squamous cell carcinoma but also in human lung cancer, implicating poor survivals (39). As such, in hepatocellular carcinoma (HCC), Gr-1+ myeloid cells which contain Ly6C^{hi} monocyte population play pro-tumoral function supporting tumor fibrosis by secreting platelet-derived growth factor-beta (PDGF-β), a pro-fibrotic growth factor (40).

Moreover, classical monocytes play a major role in establishing a cancer therapy-resistant microenvironment (34, 48, 49). Doxorubicin treatment on MMTV-PyMT breast carcinoma, for induction of necrotic cell death, triggers the enhanced infiltration of CCR2-expressing monocytes. At later stages of cancer, this backfires; these monocytes have been revealed responsible for resistance against doxorubicin, promoting tumor relapse after treatment (48). In 4T1 and MMTV-PyMT breast carcinoma, paclitaxel treatment induces the secretion of tumor-derived extracellular vesicles (EVs), and these EVs upregulate pulmonary CCL2 expression to elicit classical monocyte expansion establishing a lung pre-metastatic niche (34). Applying radiotherapy on KPC pancreatic carcinoma also leads to a significant increase in CCL2 production by tumor cells. Subsequent recruitment of classical monocytes thereby endows

the tumor with resistance against the cancer treatment (49). Use of anti-CCL2 antibodies selectively restrains radiotherapy-dependent recruitment of classical monocytes, impeding tumor progression when combined with radiotherapy (49).

Based on these findings, treatment with anti-CCL2 antibody might sound attractive for tumor regression. However, the following study has proposed a caution for anti-CCL2 mono-treatment. During anti-CCL2 treatment in 4T1, J110, and Met-1 mammary carcinoma, a large population of the classical monocytes is retained within the bloodstream, and their homing to the primary tumor or to the metastatic site is attenuated (50). However, after anti-CCL2 treatment cessation, monocytes initiate their migration to the lungs, and the level of IL-6 rises within the lungs. The increased level of IL-6 augments pro-angiogenic VEGF-A expression in classical monocytes, and thereby accelerates tumor metastasis (50). IL-6RA is largely expressed in Ly6C^{hi} monocytes, and anti-IL-6R antibodies effectively target Ly6C^{hi} monocytes (51, 52). Notably, IL-6-IL-6R interaction not only promotes VEGF-A secretion from classical monocytes but also activates the STAT3 signaling pathway in cancer cells, which enhances tumor cell proliferation in pancreatic ductal adenocarcinoma (PDAC) (50–53). IL-6 is also strongly induced in adipocytes and tumor-infiltrated myeloid cells after anti-VEGF treatment on overweight breast cancer patients. The upregulated IL-6 mediates resistance to anti-VEGF therapy, leading to the proliferation of cancer cells and dysfunctional angiogenesis (54). IL-6 inhibition increases the tumor microenvironment's sensitivity to chemotherapy and anti-angiogenic therapy and promotes tumor cell death (52, 54).

Contrary to the pro-tumoral properties of Ly6C^{hi}/CD14+CD16− monocytes explicated above, it has also been reported that these classical monocytes play anti-tumoral functions in certain treatments (**Table 1**; **Figure 1**). Tumor fibrosis promotes tumor progression by increasing collagen deposition, reducing T cell infiltration, and inducing pro-tumoral macrophage polarization (33, 55–58). A distinct class of Mac1+F4/80−Msr1+ceacam1+Ly6C^{lo} monocytes has been recently discovered to promote fibrosis in C/EBPβ dependent manner (59). Meanwhile, Ly6C^{hi} monocyte infiltration into KPC pancreatic adenocarcinoma via IFN-γ and CCL2 following anti-CD40 treatment has been reported to facilitate degradation of tumor fibrosis, increasing the efficacy of the chemotherapy on PDAC while Ly6C^{hi} monocyte-containing Gr-1+ myeloid cells in HCC play pro-fibrotic roles (33, 40, 55).

Recruitment of Non-classical Monocytes and Their Functions in Solid Tumors

On the other hand, Ly6C^{lo}/CD14−CD16+ non-classical monocytes have independent mechanisms for infiltration to tumors, and their functions are context-dependent. In models of colorectal cancer, Jung et al. have firstly revealed immunosuppressive functions of non-classical monocytes in any context, including cancers (7, 8). Anti-angiogenic therapy leads to non-classical monocyte influx to CX$_3$CL1-secreting tumor milieu. Then these recruited non-classical monocytes secrete CXCL5, and mediate a massive infiltration of CXCR2-expressing neutrophils through the highly specific chemokine axis (7). This

TABLE 1 | Context-derived heterogeneous functions of monocyte subsets.

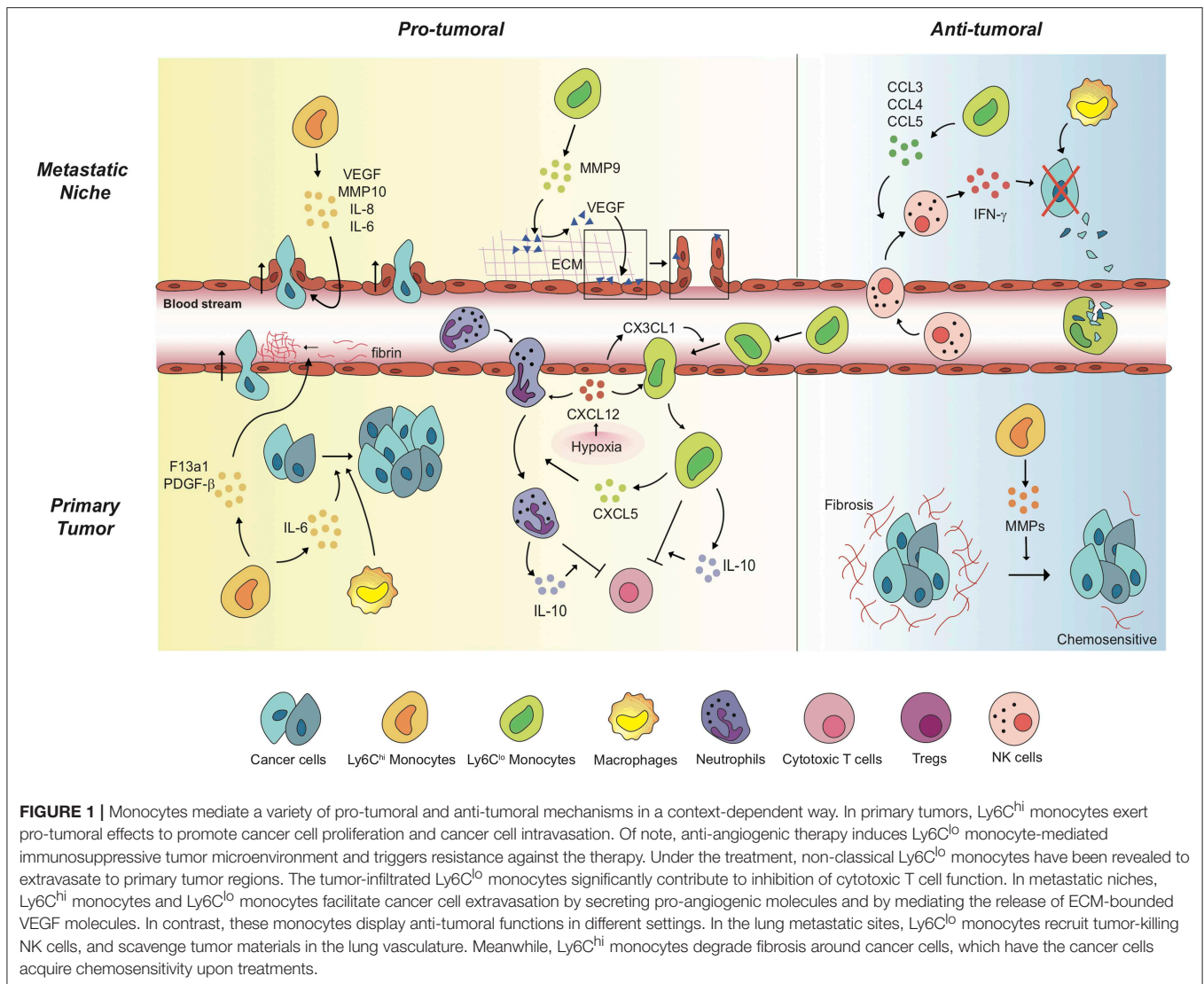| Type of monocytes		Function	Factor	Model	Cancer type/treatment	References
Classical monocytes	Protumoral	Metastasis; Tumor cell extravasation	VEGFA	Mouse	MMTV-PyMT breast cancer	(32)
			VEGF, MMP-10, IL-8, TNF-α, PTGS2	Human	Renal cell carcinoma	(38)
		Metastasis; Cancer cell invasion	F13a1	Mouse	KLN205 lung squamous cell carcinoma	(39)
				Human	Lung cancer	
		Tumor fibrosis	PDGF-β	Mouse	Hepatocellular carcinoma	(40)
	Antitumoral	Degradation of tumor fibrosis	MMPs	Mouse	KPC pancreatic adenocarcinoma w/anti-CD40 treatment	(33)
Non-classical monocytes	Protumoral	Immunosuppression	CXCL5, IL-10	Mouse	CT26, SL4 colorectal cancer w/anti-VEGFR2 therapy	(7, 8)
		Angiogenesis	MMP-9	Human cancer cell xenograft	DLD1, HCT116 human colorectal carcinoma	(41)
	Antitumoral	NK cell recruitment	CCL3, CCL4, CCL5	Mouse, Human cancer cell xenograft Human	B16F10 melanoma, A375 human melanoma, MMTV-PyMT breast cancer, Human lung cancer specimen (early stage)	(35, 42, 43)
		NK cell activation	IL-15	Mouse	B16F10, B16F0 melanoma	(44)

Summarizes diverse protumoral and antitumoral functions of monocyte subsets (classical and non-classical monocytes) and their related factors in each model.

finding echoes a previous finding also showing that non-classical monocytes recruit neutrophils, albeit mediated by CXCL1—not CXCL5—in a different disease condition outside oncology (60). These tumor-infiltrating non-classical monocytes and neutrophils release immunosuppressive cytokines including IL-10 which inhibits infiltration and activity of cytotoxic T lymphocytes in tumors (7, 8) (**Table 1**; **Figure 1**). Jung et al. also successfully developed several therapeutic strategies targeting these non-classical monocyte-mediated cascades by blocking their infiltration and activity (7, 8). Through a series of *in silico* and *in vitro* screening, novel siRNA sequences against CX$_3$CL1 with potent knock-down efficacy were identified. The siRNA was formulated with nanoparticles particularly designed for endothelial cell-specific delivery, which resulted in inhibiting Ly6C^{lo} monocyte infiltration and subsequently reduced tumor growth (7). Notably, CXCR4 was discovered to be a critical chemokine receptor expressed on non-classical monocytes and neutrophils (8). CXCL12/CXCR4 axis in these cells mediates restrained cytotoxic T cell infiltration and builds up immunosuppressive tumor microenvironment in CT26, SL4 colorectal carcinoma, and E0771, MCa-M3C mammary carcinoma (8, 61). Supporting this finding, AMD3100 which is a potent CXCR4 inhibitor, also known as plerixafor, efficiently hinders the recruitment of non-classical monocytes, improving the treatment efficacy of anti-VEGFR2 therapy. This suggests the potential of rapid clinical translation, since AMD3100 is already an FDA-approved CXCR4 blocker being used in the clinic for other uses (8, 61).

Despite the several pro-tumoral features of Ly6C^{lo}/CD14$^-$CD16$^+$ non-classical monocytes, these monocytes also display anti-tumoral properties in different tumor/treatment conditions (**Table 1**; **Figure 1**). In B16F10

melanoma and MMTV-PyMT spontaneous mammary carcinoma, non-classical monocytes play a pivotal role in engulfing tumor material in the lung and attenuating tumor metastasis and activating NK cells (17, 35). In B16F10 and B16F0 melanoma, non-classical monocytes also activate NK cells by releasing IL-15, which is a determinant cytokine for NK cells' homeostasis, activation and effector function, preventing lung metastases in primary tumor-bearing mice (44). In B16F10 melanoma and A375 human melanoma xenograft models, exosomes secreted from non-metastatic cancer cells promoted the expansion of non-classical monocytes in the bone marrow (42). The expanded population of the non-classical monocytes leads to recruiting NK cells which function in cancer cell clearance at the pre-metastatic niche (42). This NK cell-recruiting function of non-classical monocytes have been reconfirmed in early stage lung cancer patients (43). Based on these findings, reduced CD16$^+$ non-classical monocytes might be correlated with NK cell paucity in this lung tumor lesions (43). According to *ex vivo* study of patients with stage IV cutaneous melanoma, CD14$^-$CD16$^+$ non-classical monocytes kill regulatory T lymphocytes (Tregs) by assisting ipilimumab, anti-cytotoxic T lymphocyte associated antigen 4 (CTLA4) monoclonal antibody, -mediated ADCC (antibody-dependent cell-mediated cytotoxicity) (62).

Importantly, it had been widely believed that non-classical monocytes are not able to extravasate out of blood vessels. Instead, they were known to stay inside vasculature and patrol the endothelium, which gave these monocytes the nickname "patrolling monocytes" (9). However, recent studies strongly suggest that they do have the capability of transmigration and actively infiltrate into tissues, proven by state-of-the-art *in vivo* imaging techniques (7, 8). Supporting this, in DLD1

FIGURE 1 | Monocytes mediate a variety of pro-tumoral and anti-tumoral mechanisms in a context-dependent way. In primary tumors, Ly6C^{hi} monocytes exert pro-tumoral effects to promote cancer cell proliferation and cancer cell intravasation. Of note, anti-angiogenic therapy induces Ly6C^{lo} monocyte-mediated immunosuppressive tumor microenvironment and triggers resistance against the therapy. Under the treatment, non-classical Ly6C^{lo} monocytes have been revealed to extravasate to primary tumor regions. The tumor-infiltrated Ly6C^{lo} monocytes significantly contribute to inhibition of cytotoxic T cell function. In metastatic niches, Ly6C^{hi} monocytes and Ly6C^{lo} monocytes facilitate cancer cell extravasation by secreting pro-angiogenic molecules and by mediating the release of ECM-bounded VEGF molecules. In contrast, these monocytes display anti-tumoral functions in different settings. In the lung metastatic sites, Ly6C^{lo} monocytes recruit tumor-killing NK cells, and scavenge tumor materials in the lung vasculature. Meanwhile, Ly6C^{hi} monocytes degrade fibrosis around cancer cells, which have the cancer cells acquire chemosensitivity upon treatments.

and HCT116 human colorectal carcinoma, recruited human patrolling monocytes in tumors secrete matrix metalloproteinase 9 (MMP9), a proteolytic enzyme fostering angiogenesis, triggering a release of matrix-bound VEGFA. This accelerates the extravasation and accumulation of these pro-angiogenic patrolling monocytes, promoting tumor progression (41). This also validates the first finding of non-classical monocyte extravasation directly visualized by intravital microscopic imaging (7, 8).

Tie2-Expressing Monocytes

Other than the traditional classification of monocytes by Ly6C expression level, another classification method by Tie2 (angiopoietin receptor) expression exists. Tie2-expressing monocytes (TEMs) are a monocyte population present in both human and mouse peripheral blood and tumor, and are localized in perivascular spaces but not incorporated with vascular endothelial cells (63, 64). Angiopoietin-1 (Ang-1),

a Tie2 ligand, is likely to promote the recruitment of TEMs to tumor vasculature before the turn-on of the angiogenic switch in early stages of N202 breast carcinoma, Rip1-Tag2 pancreatic insulinoma and U87 human glioma (63, 65, 66). In a following study, it was also elucidated that Angiopoietin-2 (Ang-2), another Tie2 ligand upregulated in tumor hypoxia, can also recruit TEMs. The TEMs are then reprogrammed to show proangiogenic phenotypes (67, 68). Meanwhile, Collagen triple-helix repeat-containing 1 (CTHRC1) secreted by several malignant tumors has been reported to recruit TEMs to the tumor microenvironment through upregulation of Ang-2 in endothelial cells and promote metastasis in human MiaPaCa-2, CFPAC-1, and Panc-1 pancreatic cancers (69). Recruited TEMs promote angiogenesis via secretion of a proangiogenic molecule, basic fibroblast growth factor (bFGF) (63–66). Also, Ang-2 and hypoxia cause TEM influx into the tumor microenvironment, and the TEMs mediate downregulation of TNF-α supporting cancer cell survival and causing metastasis of the primary tumor

(63, 67, 70). Blockade of Ang-2 impedes tumor angiogenesis in MMTV-PyMT breast carcinoma and Rip1-Tag2 pancreatic insulinoma through downregulation of Tie2 in TEMs (71).

NEUTROPHILS: FROM DEVELOPMENT TO DEPLOYMENT

Neutrophil Development

Neutrophils are another myeloid compartment which plays critical roles both in homeostatic condition and tumor context. There is a train of precursors to be passed through to generate mature neutrophils in the bone marrow (72). Hematopoietic stem cells (HSCs) give rise to multipotent progenitors (MPPs), lymphoid primed multipotent progenitors (LMPPs), and granulocyte/macrophage progenitors (GMPs) in this very order (72). There are several more stages to go to be differentiated to neutrophils, namely a series of myeloblasts, promyelocytes, myelocytes, metamyelocytes, band cells, and finally neutrophils (72). These steps for neutrophil generation occur under major regulation by the granulocyte-colony stimulating factor (G-CSF), granulocyte–macrophage-colony stimulating factor (GM-CSF) and also minor regulation by other molecules such as IL-6 and KIT ligand (KITL) (73). Differentiating neutrophils express the G-CSF receptor (G-CSFR) throughout the myeloid lineage (73). During development in the bone marrow, neutrophils acquire three types of granules sequentially; azurophil (primary) granules which retain myeloperoxidase regulated by transcription factors C/EBPα and Gfi-1, specific (secondary) granules which contain lactoferrin mostly regulated by C/EBPε, and gelatinase (tertiary) granules which contain MMP9 regulated by C/EBPβ, C/EBPδ, C/EBPγ, and PU.1 (74, 75). Of note, mass cytometry has recently found new proliferative precursors of neutrophils after GMP stage which further differentiate to immature neutrophils and mature neutrophils with regulation of C/EBPε (76). Although the bone marrow is primarily responsible for the neutrophil formation, the spleen can be an alternative source of neutrophils during emergency granulopoiesis derived from cancer progression (73). In KP lung adenocarcinoma, splenic hematopoietic stem cells, and progenitor cells produce neutrophils during tumor progression (27). Presence of cancer cells upregulates the expression of several factors accelerating neutrophil development. The expression of CXCL1, CXCL2, CXCL5, and CXCL8, which are CXCR2 ligands, and the expression of KITL and GM-CSF are strongly enhanced by KRAS signaling in cancer cells and tumor-derived hypoxia (73). Moreover, IL-1β-producing macrophages and IL-17-producing γδ T cells secrete G-CSF to promote neutrophil development in the tumor (73). Cancer cells accelerate secretion of these cytokines and chemokines to instigate overactive granulopoiesis and neutrophilia (73). The accelerated secretion of these factors promotes the release of immature neutrophils to the blood stream, resulting in an increased number of circulating neutrophils (73). In 4T07, 4T1 mammary carcinoma, LLC, and Kras-driven pancreatic carcinoma, G-CSF production is also facilitated via RAS/MEK/ERK pathway in cancer cells,

promoting recruitment of neutrophils (77). Meanwhile, type I IFNs from tumor trigger differentiation of neutrophils to achieve an anti-tumoral phenotype, reducing not only CXCR4 expression in neutrophils which mediates tumor-homing, but also VEGF and MMP9 expression (78, 79). Moreover, type I IFNs suppress G-CSF signaling pathways in neutrophils, thereby reducing expression of Bv8, S100A8, S100A9, and MMP9 so that they can attenuate the formation of the pre-metastatic niche (78, 79). Inhibition of type I IFNs impairs cytotoxicity of neutrophils and promotes metastasis of B16F10 melanoma, MCA205 fibrosarcoma, 4T1 mammary carcinoma and LLC mediated by neutrophils (78, 79).

Neutrophils: Pro-tumoral vs. Anti-tumoral Functions in Solid Tumors

Functions of neutrophils in the tumor microenvironment vary by context including types of tumor, stages of tumor progression, and different therapies (**Table 2**; **Figure 2**).

Recruitment of Neutrophils to Tumor Milieu

As mentioned above, tumors promote the early release of neutrophils yet with immature phenotypes from the bone marrow. There are several ligand-receptor axes studied for neutrophil recruitment into the tumor. Upon research on diverse tumor models, it has been revealed that CXCR2 is a pressing chemokine receptor which recruits neutrophils to the tumor (7, 8, 119, 120). In KPC pancreatic carcinoma and inflammation-driven and spontaneous intestinal adenocarcinoma, the migration of myeloid cells, especially neutrophils, to the tumor microenvironment is impaired when CXCR2 signaling is suppressed (119, 120). This enhances tumor cell apoptosis and restrains tumorigenesis, resulting in a failure to set up a metastatic niche (119, 120). Moreover, activated neutrophils also express CCR7 on their membrane, which pushes those cells to tumor sites in response to CCL19, CCL21, and GM-CSF secretion (121). IL-17 also triggers neutrophil recruitment to tumor sites in 4T1 breast carcinoma (122), KRAS mutated lung carcinoma (123), and ovarian carcinoma (124). The recruited neutrophils present high expression of tumor-promoting genes such as TNF-α, CXCL1, MMP9, and VEGF (122). In zebrafish larvae model of glioblastoma initiation, neutrophils are actively recruited to KRAS-transformed cells very early in oncogenesis via the CXCL8-CXCR1 signaling axis, and this recruitment contributes to the proliferation of tumor-initiating cells (125). Gastrin-releasing peptide (GRP)-GRP receptor (GRPR) axis can also induce neutrophil migration in the tumor (126, 127). In A375 and M24met human melanoma, CXCL5 overexpression by tumor cells enhances neutrophil recruitment and infiltration into primary tumors and tumor lymphatic vessels (128). It triggers the proximal interaction between neutrophils and cancer cells near the lymphatic endothelial cells in order to help trans-endothelial migration of the cancer cells (128). In SL4 and CT26 colorectal cancer, tumor-infiltrated Ly6C^{lo} monocytes induced by anti-VEGF therapy can also recruit CXCR2-expressing neutrophils to the tumor site via the CXCL5-CXCR2 and CXCL12-CXCR4 axes (7, 8). Albeit in a different disease setting, it has been

TABLE 2 | Context-dependent multifaceted functions of neutrophils.

		Function	Factor	Model	Cancer type/treatment	References
Neutrophils	Protumoral	Tumor initiation	Neutrophil elastase	Mouse	Kras mutant	(80)
			ROS, RNS	Mouse	Colon cancer	(81)
		Cancer cell proliferation	NETs (Neutrophil elastase traps), HMGB-1	Mouse	MC38 colorectal cancer w/ischemia and reperfusion injury	(82)
			Neutrophil elastase	Mouse	A549 lung adenocarcinoma	(83)
			IL-6, IL-1β	Mouse	4T1 breast cancer	(84)
			Transferrin	Mouse	4T1 breast cancer	(85)
		Cancer cell colonization; Differentiation from monocytes to fibrocytes	MMP-9	Mouse	CMT93 colon carcinoma	(86, 87)
		Fibrosis	MAP kinase pathway	Mouse	HCA-1 hepatocellular carcinoma w/Sorafenib treatment	(88)
			IL-1β	Mouse	AK4.4, Pan02, KPC, iKRAS pancreatic adenocarcinoma	(58)
		Macrophage recruitment	MAP kinase pathway	Mouse	TRAMP-C1 prostate cancer, E0771 breast cancer w/VEGF blockade	(89)
		T cell suppression	IL-10	Mouse, Human cancer cell xenograft	CT26, SL4 colorectal cancer w/anti-VEGFR2 therapy/LS174T human colorectal cancer	(7, 8, 90)
			PD-L1	Mouse	H22-generated hepatoma	(91)
			IL-10, LGALS9, ARG1, MFGE8	Mouse	KP lung carcinoma	(92)
			Nos2	Mouse	KEP breast carcinoma, AB12 mesothelioma, LKRM lung carcinoma, LLC	(93, 94)
			ARG1	Human	Non-small cell lung cancer	(95)
		Regulatory T cell attraction	CCL17	Mouse	LLC, AB12 mesothelioma	(96)
		Angiogenesis	Bv8	Mouse	Rip-Tag pancreatic insulinoma	(97, 98)
			MMP-9	Mouse, Human cancer cell xenograft	Rip1-Tag2 pancreatic insulinoma, L929 fibrosarcoma, B16-F10 melanoma, LLC, HPV-15-induced squamouse carcinoma, HT-1080 fibrosarcoma/PC-3 human prostate carcinoma	(99–102)
			VEGF	Human	Oral cavity cancer	(103)
			FGF2	Mouse, Human cancer cell xenograft	Pan02, KPC pancreatic carcinoma/HT29, HCT-116, Lovo human colon cancer	(104)
		Metastasis; Tumor cell extravasation	IL-1β, Leukotriene, IL-8	Mouse, Human cancer cell xenograft	4T1, D2A1 breast cancer/Human MDA-MB-231 breast cancer, human A375-MA2, WM35, C8161.C19, UACC903 melanoma	(105–107)
		Metastasis; Epithelial-mesenchymal transition (EMT)	IL-17α	*In vitro* human cancer cell	(*In vitro*) Human MKN45, MKN74 gastric cancer	(108)
		Metastasis; Bridge between ICAM-1-expressing cancer cells and endothelial cells	MAC-1	Mouse, Human cancer cell xenograft	H50 Lewis Lung carcinoma/Human A549 lung carcinoma	(109)
		Cancer cell retention	NETs (Neutrophil elastase traps)	Mouse	H59 Lewis lung carcinoma w/cecal ligation and puncture/MC38 colorectal cancer w/ischemia and reperfusion injury	(82, 110)
		Activation of dormant cancer cell		Mouse	D2.0R breast cancer	(111)

(Continued)

TABLE 2 | Continued

	Function	Factor	Model	Cancer type/treatment	References
Antitumoral	Tumor cell death	TNF-α, NO, H_2O_2	Mouse	LLC, AB12 mesothelioma	(112)
		Granzyme B		CT26 colon cancer	(113)
		H_2O_2		AT3, 4T1, MMTV-PyMT breast cancer	(114, 115)
	IL-17+ $\gamma\delta$ T cell suppression	ROS	Mouse	B16F10 melanoma, Hepa1-6 hepatoma	(116)
	Impairment of tumor cell proliferation	Tsp-1	Human cancer cell xenograft	Human PC3 prostate cancer, human MDA- MB-231 breast cancer	(117)
	Stimulation of T cell response	CD54, CD86, OX40L, and 4-1BBL	Human	Lung cancer (early stage)	(118)

Summarizes multiple protumoral and antitumoral functions of neutrophils and their responsible factors in each different context.

also reported that monocytes recruit neutrophils in a TLR7-dependent manner through CXCL1 (60), different from the case of cancer context where non-classical monocytes-derived CXCL5 was newly discovered as the key chemokine attracting neutrophils (7, 8).

Pro-tumoral Functions of Neutrophils in Solid Tumors

Recruited neutrophils from the blood stream have potent influences on various components of tumor progression and metastasis, including tumor initiation, cancer cell survival/proliferation, immune modulation, angiogenesis, and intra/extravasation of cancer cells (**Table 2**; **Figure 2**).

Neutrophils and tumor initiation

In Kras mutant mice, airway inflammation induces secretion of IL-8 by lung keratinocytes, recruiting CXCR2-expressing neutrophils to the inflammation site (80). Neutrophil elastase (NE), a powerful serine protease exclusively found in primary granules of neutrophils, facilitates inflammation-mediated tumor initiation in the lung (80). H. hepaicus-induced colitis triggers tissue infiltration of MPO$^+$ neutrophils and macrophages into the infected sites (81). These neutrophils and macrophages generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) which subsequently cause molecular damage, promoting tumorigenesis (81). Transcriptional analysis reveals that genes involved in repairing DNA damage were downregulated, but genes associated with reactive chemical species generation were upregulated in infected colons (with no impact on cell proliferation) (81).

Neutrophils and cancer cell survival/proliferation

Beyond tumorigenic functions of neutrophils, their roles in cancer cell survival and proliferation have been also elucidated well. Overexpression of insulin receptor substrate 1 (IRS-1) is known to reduce tumor growth (83). In A549 lung adenocarcinoma, neutrophil elastase (NE) degrades IRS-1 in cancer cells, thereby causing tumor cell proliferation. PI3K signaling pathway alteration mediates this process by fostering the interaction with PDGF-receptor (83). In 4T1 breast carcinoma, transferrin, an iron-transporting protein secreted by neutrophils, binds to its receptor expressed on cancer cells (85).

Then the transferrin supplies iron to the cancer cells for their proliferation (85). As tumor growth and metastasis are promoted, transferrin secretion by neutrophils increases (85). Sorafenib, a rapidly accelerated fibrosarcoma (RAF) inhibitor, is commonly used as treatment for HCC. However, the use of sorafenib causes side effects and resistance such as tumor desmoplasia. Gr-1$^+$ myeloid cells including neutrophils have been revealed to be responsible for the resistance (40, 88, 129, 130). Sorafenib treatment induces tumor hypoxia, which upregulates CXCL12 expression in HCA-1 cancer cells and stromal cells. Then, CXCR4-expressing Gr-1$^+$ myeloid cells are promoted to infiltrate to CXCL12-secreting tumor sites, and the infiltrated cells support differentiation and activation of hepatic stellate cells via the MAP kinase pathway and fibrosis in HCC (40). Of note, CXCL12-CXCR4 axis triggers increased infiltration of Tregs and M2 type macrophages and upregulation of intratumoral PD-L1 in HCA-1 HCC (88). Moreover, in Ak4.4, Pan02, KPC, and iKRAS pancreatic adenocarcinoma, adipocytes of obese population secrete increased levels of IL-1β to recruit neutrophils to the tumor along with enhancing Treg infiltration, and hindrance of CD8$^+$ T cell infiltration (58). The recruited neutrophils then activate pancreatic stellate cells via IL-1β secretion to accelerate fibrosis, which promotes tumor growth and reduces sensitivity to chemotherapy (58).

Neutrophils and immune modulation

Neutrophils play essential roles in tumor growth and metastasis not only to regulate cancer cell proliferation and survival, but also to modulate innate and adaptive immunity. The recruited neutrophils via CXCL12-CXCR4 axis secrete IL-10 that suppresses cytotoxic T cell function on tumor cells, which then causes anti-VEGF therapy resistance in SL4 and CT26 colorectal carcinoma (7, 8). Similar findings have been recently reported in LS174T human colorectal carcinoma (90), which confirms the previous observations in preclinical murine models (7, 8). The CXCL12-CXCR4 axis in myeloid cells including neutrophils is also responsible for NK cell apoptosis and inactivation by enhancing the Fas signaling pathway and restraining IL-18 production in neutrophils, respectively, in metastatic B16F0 melanoma, PyMT breast carcinoma, and YAC-1 lymphoma
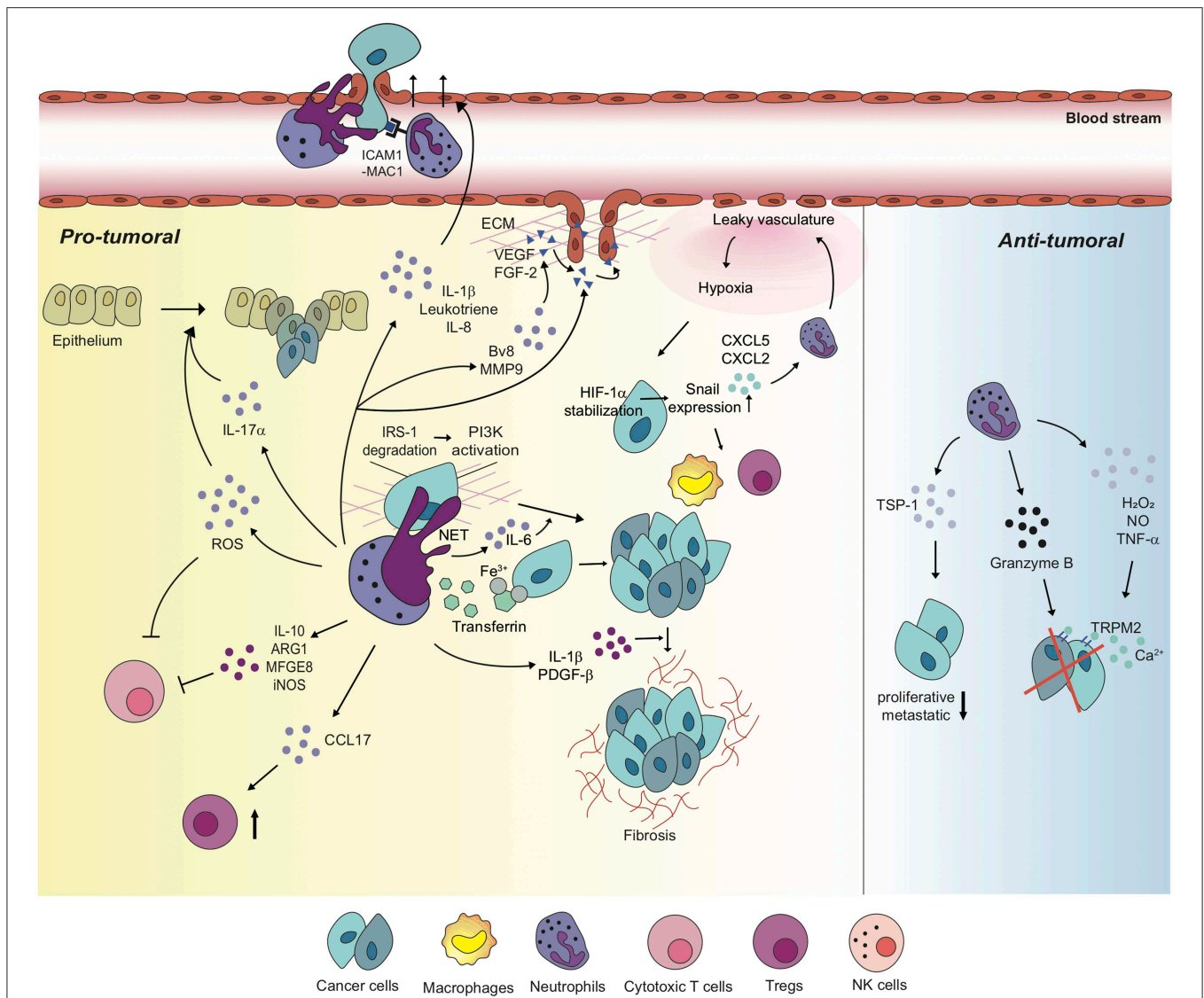
FIGURE 2 | Neutrophils differentially regulate tumor microenvironment with diverse mechanisms. Neutrophils perform pro-tumoral roles in most tumor settings, promoting tumorigenesis, and cancer cell proliferation via diverse mechanisms. Moreover, neutrophils regulate the functions of other immune cells including cytotoxic T cells and Tregs in order to build up tumor-favorable tumor microenvironment. On the one hand, neutrophils stimulate tumor angiogenesis via inducing the release of VEGF and FGF-2 from ECM or secreting pro-angiogenic molecules, themselves. Furthermore, metastatic competence of cancer cells can be achieved by physical interaction with neutrophils and neutrophil-derived secretory molecules, facilitated to extravasate to secondary tumor sites. Neutrophils also create a positive feedback loop with cancer cells toward the formation of the tumor-supportive microenvironment, developing dysfunctional vasculature around the tumor, leading to hypoxia which recruits more neutrophils and pro-tumoral immune cells into tumor milieu. On the other hand, a couple of studies indicate anti-tumoral functions of neutrophils in different contexts. In these contexts, neutrophils perform a cytotoxic function on cancer cells, and have cancer cells lose proliferative and metastatic properties.

(131). Neutrophil-mediated modulation of NK cells has been confirmed in 4T1 and D2A1 metastatic mammary carcinoma context (D2A1 inoculation after 4T1 injection) as well (105). The expanded population of neutrophils near metastatic sites inhibits functional activation of NK cells, and thus the NK cells lose their ability to clear intraluminal tumor cells (105). This consequently comes across with a favorable environment for cancer cell survival and metastasis (105). CXCR4 depletion in those myeloid cells recovers the tumor-killing capacity of NK cells (131). In H22-generated hepatoma-bearing mice, PD-L1 is upregulated in

tumor-infiltrating neutrophils (TINs), induced by GM-CSF and TNF-α secretion from the tumor microenvironment (91). The overexpressed PD-L1 of neutrophils suppresses proliferation and activation of PD-1^{+} T cells, dampening anti-tumor immunity (91). In STK11/LKB1-deficient KP lung carcinoma, recruited neutrophils produce suppressive factors such as IL-10, LGALS9, Arginase1 (ARG1), and Milk fat globulin EGF factor (MFGE8) which are also involved in cytotoxic T cell suppression as well as the tumor-promoting cytokine IL-6 (92). In KEP breast carcinoma, Nos2, the gene encoding iNOS, is largely upregulated

in neutrophils (93). Then the neutrophils suppress CD8^{+} T cell activity via NO from iNOS, promoting lung metastasis (93). Effect of neutrophil NO production on CD8^{+} T cell apoptosis has been also confirmed in AB12 mesothelioma, LKRM lung carcinoma, and LLC (94). TNFα-mediated iNOS upregulation and NO secretion in neutrophils induce the apoptosis of non-activated CD8^{+} T cells via direct contact between cells in these tumor contexts (94). ARG I secretion by neutrophils has been also uncovered to affect T cell suppression by degrading extracellular arginine in non-small cell lung carcinoma patients (95). IL-8 and TNF-α secretions are enhanced in non-small cell lung carcinoma patients, and these cytokines induce ARG I release from exocytosis of granules in neutrophils (95). In 4T1 mammary carcinoma, SCF-c-kit signaling increases c-kit+ neutrophil frequency in the circulatory system. Even in nutrient-limited tumor microenvironments, these neutrophils exploit fatty acid metabolism to maintain mitochondrial function and support ROS production, resulting in T cell suppression (132). This also echoes the formal observations of immunosuppressive neutrophils in colon cancers (7, 8). Meanwhile, a research adopting mass cytometry and single-cell RNA sequencing has recently revealed that a unipotent precursor of neutrophils promotes B16F10 melanoma progression via inhibition of pro-inflammatory T cell activation, eliciting an immunosuppressive microenvironment around the tumor (133).

Neutrophils affect tumor progression by regulating other immune cells beyond NK cells and cytotoxic T cells. In LLC and AB12 mesothelioma, neutrophils attract Tregs via CCL17 secretion and thus scupper the formation of an anti-tumor immune microenvironment (96). Furthermore, blockade of VEGF in TRAMP-C1 prostate carcinoma and E0771 breast carcinoma triggers Gr-1^{+} myeloid cell recruitment which mediates macrophage recruitment to the tumor microenvironment via activation of p38 mitogen-activated protein kinase (MAPK) to promote lung metastasis (89).

Myeloid-derived suppressor cells (MDSCs) are immunophenotypically defined as CD11b^{+} Gr-1^{+} cells (in mice) and possess pro-tumorigenic functions including immune suppression (134). These MDSCs can be further classified into granulocytic (or polymorphonuclear) MDSCs [gMDSCs or PMN-MDSCs] (CD11b^{+} Ly6C^{−} Ly6G^{+} in mouse, CD11b^{+} CD14^{−} CD15^{+} in human) and monocytic MDSCs [mMDSCs] (CD11b^{+} Ly6C^{+} Ly6G^{−} in mouse, CD11b^{+} CD14^{+} HLA-DR^{low} CD15^{−} in human) (134). Although we do not doubt that MDSCs play important roles in regulating tumor progression, the definition of MDSCs is still under debate since it is difficult to clearly discriminate the heterogeneous myeloid cell mixtures only with markers being currently used (7, 8). Indeed, phenotypical and functional features of MDSCs are considerably overlapped with those of monocytes and neutrophils, which we discuss in depth throughout this review. Therefore, we would rather not go into details of MDSCs here.

Neutrophils and angiogenesis
Even though cancer cells and cancer-associated fibroblasts are considerably responsible for the source of angiogenic factors, tumor-infiltrated myeloid cells including neutrophils also exert potent properties in tumor angiogenesis over diverse tumor settings (135). Upregulation of Bv8 following STAT3 activation is responsible for neutrophil-mediated tumor angiogenesis in the early stages of Rip-Tag pancreatic insulinoma (97, 98). In Rip1-Tag2 pancreatic insulinoma, L929 fibrosarcoma, B16-F10 melanoma, LLC, HPV-15-induced squamous carcinoma, HT-1080 fibrosarcoma, and PC-3 human prostate carcinoma, neutrophils infiltrated to the tumors majorly secrete MMP9 remodeling the ECM to release VEGF and FGF-2, and activating them to trigger chronic angiogenesis and thereby promotes tumor progression (99–102, 135). Meanwhile, in oral cavity cancer patients, neutrophils actively secrete VEGF, and promote tumor angiogenesis and metastasis (103). By studying HT29, HCT-116, LoVo human colon carcinoma and Pan02, KPC murine pancreatic carcinoma, it has also been studied that neutrophils are the main source of FGF2 (104). Here, these neutrophils play a proangiogenic role to develop unsystematic tumor vasculature and prompt liver metastasis, facilitating endothelial cell proliferation and migration (104). Hence, inhibition of FGF2 delays tumor growth via normalizing the vasculature (104). In KP lung carcinoma, neutrophils alter angiogenesis around tumor tissue, causing a hypoxic environment (136). HIF1α stabilization induced by hypoxia increases expression of the Snail gene in cancer cells (136). The Snail-expressing cancer cells secrete increased levels of CXCL5 and CXCL2 to recruit more pro-tumoral neutrophils to the tumor, creating a positive amplifying loop to facilitate tumor growth (136, 137). Notably, it has been revealed that Snail has a pro-tumorigenic influence via recruiting pro-tumoral M2 macrophages as well in 4T1 breast cancer and LLC1 lung cancer (137). Snail also induces Treg differentiation and impairs the activity of dendritic cells in B16F10 melanoma (138).

Neutrophils and metastasis
While affecting primary tumor cell proliferation, modulation of the immune microenvironment, and angiogenesis, neutrophils also play significant roles in supervising tumor metastasis. It has been reported that neutrophil infiltration is essential to endow non-malignant BMT-11 fibrosarcoma cancer cells with malignant and metastatic phenotypes (139). The metastatic incidence is significantly reduced with the anti-Gr1 antibody-mediated neutrophil depletion in blood circulation or integrin β2 knockout mice lacking in neutrophil extravasation (139). Of note, integrin β2 mediates neutrophil adhesion on activated endothelium with high affinity, which leads to transmigration of neutrophils across the endothelium (140, 141). A number of subsequent researches have endeavored to illuminate diverse factors and mechanisms which can explain functions of neutrophils on tumor metastasis. In 4T1 and D2A1 metastatic mammary carcinoma (D2A1 injection after 4T1 injection), recruited neutrophils activate endothelial cells via secretion of IL-1β. This, in turn, facilitates trans-endothelial migration of intraluminal tumor cells, forming small protrusions of the cell bodies across the endothelial layer (105, 106). In 4T1 breast carcinoma and human MDA-MB-231 breast carcinoma, leukotrienes derived from neutrophils transform cancer

cell populations to acquire highly metastatic competence in lung pre-metastatic sites (106). The metastatic competence of cancer cells can be also acquired by IL-17α secretion of neutrophils in gastric cancer (108). Of note, epithelial-mesenchymal transition (EMT) endows cancer cells with invasive properties and high-grade malignancy (108, 142). Coculture of gastric cancer patient-derived neutrophils and human MKN45, MKN74 gastric cancer cells has proven that IL-17α activates JAK2/STAT3 axis in the cancer cells following by their acquisition of mesenchymal characteristics, and the IL-17α is mostly derived from tumor-associated neutrophils (TANs) (108).

Employment of a multiplexed microfluidic model of the human microvasculature has revealed that neutrophils also secrete IL-8 by themselves (143). The self-secreted IL-8 induces not only neutrophil sequestration in A375-MA2 human melanoma cells but the interference of endothelial barrier function, supporting cancer cell extravasation (143). In human WM35, A375, C8161.C19, and UACC903 melanoma, IL-8 secreted from entrapped melanoma cells attracts neutrophils and increases integrin β2, specifically MAC-1, on the neutrophils. This leads to the enhancement of neutrophil-melanoma cell interaction, facilitating lung metastasis (107). There is another research subsequently conducted which confirms the interaction between MAC-1 and ICAM1 (neutrophils and cancer cells, respectively) (109). In H59 Lewis lung carcinoma and A549 human lung carcinoma, MAC-1 on neutrophils acts as a bridge between ICAM-1-expressing cancer cells and endothelial cells in favor of liver metastasis (109). Even though both pieces of research by Huh et al. (107) and Spicer et al. (109) have elucidated the MAC-1-ICAM-1 interaction and highlighted the significant function of neutrophils on tumor metastasis, the finding of Spicer et al. (109), is incompatible with Huh et al. (107), in that neutrophils come first to metastatic sites, and then circulating tumor cells directly adhere to the arrested neutrophils in the early step of metastasis. Interaction between neutrophils and circulating tumor cells in the bloodstream has also been elucidated to be mediated via VCAM-1 in 4T1 breast carcinoma (84). Also, the neutrophils physically clustered with circulating 4T1 breast cancer cells support the cancer cell cycle progression, secreting IL-6 and IL-1β, and promote metastasis of cancer cells (84). In MCF-7 human ER+ breast cancer, estradiol alters the neutrophil phenotype to overexpress integrin LFA-1, promoting ER+ cancer cell dissemination by activating cell-cell interaction (144). Meanwhile, it has been recently studied that neutrophils regulate diurnal transcription profiles in the lung, and promote the migration of B16F1 melanoma cells to the lungs (145). In CCL9-expressing CMT93 colon carcinoma, CCR1+ neutrophils secrete MMP9 to foster cancer foci, and in late phases of tumor the neutrophils recruit fibrocytes or induce differentiation from monocytes to fibrocytes which secrete MMP2, accommodating tumor cell colonization (86, 87). In short, the collaborative work of CCR1, MMP9, and MMP2 at metastatic sites promotes cancer metastasis (86, 87).

Neutrophil elastase traps (NETs) consist of extracellular decondensed DNA with granules and histones derived from neutrophils (146). Through a myriad of studies, it was explored that upon activation of neutrophils, neutrophil-derived NETs degrade virulence factors and trap bacteria within the vasculature, eventually killing them. Thus, NETs work as antimicrobial substances (146–148). It has also been elucidated that NETs play potent roles in tumor cell migration by trapping circulating cancer cells in vasculature and releasing secretory molecules by themselves (110). A study on the progression of H59 Lewis lung carcinoma after cecal ligation and puncture (CLP), represented as an alternative model of postsurgical infection, has proven that systemic sepsis induces neutrophil-derived NET formation in the hepatic sinusoid (110). Then the NETs enable stable retention of tumor cells and accelerate tumor growth within the liver (110). The link between trapped cancer cells by NETs and their proliferation in metastatic sites has been explicated in a metastatic MC38 tumor model followed by ischemia and reperfusion (I/R) injury, which is in an inevitable state after liver resection (82). Tumor hypoxia promotes NET formation within the metastatic site, and the NETs release the High mobility group box 1 (HMGB-1) protein (82). Secreted HMGB-1 activates TLR9, which encourages tumor progression via activation of related intracellular growth signaling pathways, involving phosphorylation of p38, Stat3, JNK and p65 of NF-kB (82). Moreover, it has been recently elucidated that NETs are involved in activation of dormant cancer cells in D2.0R mammary carcinoma (111). NET formation driven by LPS inflammation mediates laminin cleavage and thrombospondin-1 (Tsp-1) modulation by neutrophil elastase and NET-associated proteases (111, 149). This stimulates integrin α3β1 on dormant cancer cells and activates the FAK/ERK/MLCK/YAP signaling pathway to awaken cancer cells (111). Even in the absence of infection, 4T1 mammary cancer cells induce neutrophils to form NETs once they arrive at lung metastatic sites, promoting the expansion of disseminated cells (150). In ID8 ovarian cancer, the cancer cell-derived factors such as IL-8, GRO-a, GRO-b, and G-CSF enhance neutrophil influx to premetastatic omental niche and promote NET formation. In sequence, the NETs support tumor metastasis throughout trapping circulating ovarian cancer cells (151).

Meanwhile, the importance of considering cancer as a systemic disease has been highlighted again through its interaction with bones (152). In KP lung adenocarcinoma, the lung tumor activates Ocn+ osteoblasts via secretion of the soluble receptor for advanced glycation end products (sRAGE), which induces tumor infiltration of siglecF^high neutrophils and promotes tumor growth (152). These neutrophils represent a tumor-promoting transcriptional profile with upregulated expression of genes associated with angiogenesis (VEGFA, HIF1α, and SEMA4d), myeloid cell differentiation and recruitment (CSF1, CCL3, and MIF), extracellular matrix remodeling (ADAMDEC1, ADAM17, and many cathepsins), T cell suppression (PD-L1, FCGR2b, and HAVCR2), and tumor cell proliferation (TNF, TGFβ1, and IL-1α) (152). In contrast, the siglecF^high neutrophils downregulate genes involved in cytotoxicity (CD244, ITGAL, and Fas) (152). Furthermore, these neutrophils increase ROS production and foster monocyte differentiation into macrophages (152).

Anti-tumoral Functions of Neutrophils in Solid Tumors

The majority of the hitherto conducted researches indicate that neutrophils can only serve to promote tumor progression (**Table 2**; **Figure 2**). However, depending on the context, neutrophils suppress tumor metastasis by inhibiting malignant progression. In CT26 colon carcinoma, neutrophils inhibit the growth of G-CSF-producing cancer cells via contact-mediated cytostatic activity, but not G-CSF-nonproducing cancer cells (153). It has been recently revealed that the H_2O_2 secreted by neutrophils leads to tumor cell death, and TRPM2-mediated calcium influx acts as a go-between for this tumor killing process by neutrophils in AT3 and 4T1 breast cancer (114). In 4T1 mammary carcinoma and MMTV-PyMT spontaneous mammary carcinoma, entrained in the pre-metastatic lung prior to the arrival of metastatic cancer cells from primary sites, neutrophils play a cytotoxic function via physical contacts with cancer cells, secreting H_2O_2 and inhibiting the seeding of the cancer cells (115). Neutrophil-derived ROS secretion in B16F10 melanoma and Hepa1-6 hepatoma suppress IL-17$^+$ $\gamma\delta$ T cells which have pro-tumoral features, but not CD8$^+$ T cells, in tumor niches (116). Neutrophils also have cytotoxic activity against CT26 colon cancer cells via production of granzyme B (113). Meanwhile, in B16F10 melanoma, T241 fibrosarcoma, LLC, and MMTV-PyMT-derived lung adenocarcinoma, tumor-induced TNF-α stimulates the NF-kB signaling pathway to express proto-oncogene MET in neutrophils (154). This enables the hepatocyte growth factor (HGF), also driven by the tumor, to bind to MET (154). HGF/MET signaling promotes neutrophil extravasation, induces iNOS and NO production, and thereby supports tumoricidal neutrophil function (154). In human PC3 prostate cancer and MDA-MB-231 breast cancer, bone marrow-derived CD11b$^+$ Gr1$^+$ cells which contain neutrophil populations mainly induce thrombospondin-1 (Tsp-1) in lung premetastatic sites, impairing tumor cell proliferation at the sites (117). It has been also reported that tumor-infiltrated neutrophils undergo functional changes and acquire an anti-tumoral phenotype, supporting T cell responses against tumor in early stages of human lung cancer (118). Photodynamic therapy (PDT) augments anti-tumor immunity and tumor regression by regulating the anti-tumoral functions of neutrophils (155).

Furthermore, neutrophils regulate pro-tumoral or anti-tumoral mechanisms depending on tumor stage. In LLC and AB12 mesothelioma, TANs from the early tumors are more cytotoxic toward tumor cells and produce higher levels of TNF-α, NO, and H_2O_2, while these expressions are downregulated in late stages of tumors in which TANs acquire an enhanced pro-tumoral phenotype (112). Although depletion of neutrophils in the early stages of tumor has no effect on tumor growth, depletion of neutrophils in late stages of tumor dramatically decreases tumor growth (112).

Polarization of Tumor-Associated Neutrophils

According to a myriad of aforementioned studies on functions of neutrophils in diverse tumor circumstances, it has been well-established that TANs acquire pro-tumoral phenotype or anti-tumoral phenotype depending on related factors (147, 156). In AB12 mesothelioma and LKR lung carcinoma, TGF-β secreted by the tumor induces neutrophil polarization toward a pro-tumorigenic phenotype (156). Blockade of TGF-β attracts anti-tumorigenic neutrophils which release a large number of proinflammatory cytokines to infiltrate into the tumor microenvironment (156). Moreover, as the tumor develops, neutrophils display different functions regarding tumor growth through pro-tumoral or anti-tumoral mechanisms. IFN-β (type I IFN) differentiates neutrophils to achieve an anti-tumoral phenotype, reducing VEGF, and MMP9 expression (78, 79, 157). Inhibition of IFN-β endows TANs with pro-tumoral properties, and promotes growth and metastasis of B16F10 melanoma, MCA205 fibrosarcoma, 4T1 mammary carcinoma, CT26 colon carcinoma and Lewis lung carcinoma (78, 79, 157). *In vitro* study of BGC-823, MGC80-3, SGC-7901, and HGC-27 human gastric cancer cells has elucidated that interaction between HMGB1 secreted by the cancer cell-derived exosomes and toll-like receptor 4 (TLR4) on neutrophils fosters the formation of the autophagosome, inhibition of ROS production, and upregulation of MMP9 and VEGF in neutrophils, inducing polarization of neutrophils, promoting cancer cell migration (158).

CLINICAL ASPECTS

Prognostic Biomarkers

There have been a number of trials to predict cancer prognosis, including the TNM staging system established by The American Joint Committee on Cancer/Union Internationale Contre Ie Cancer (AJCC/UICC) (159). Through the TNM staging system, tumor prognostic information can be provided depending on tumor burden, the presence of cancer cells in lymph nodes (N) and event of distant metastases (M). Nonetheless, TNM provides limited capacity for accurate prediction (159). Cancer is a multidimensional disease, beyond difficulties in cure and prediction, which incurs many systemic alternations to be considered for effective treatment (159). One of the alternations emanates from the immune microenvironment. Reflecting the considerable impact of the immune system on tumor progression, the application of the immune parameter (Immunescore) has been introduced in disease classification to overcome the limitations of the traditional TNM staging system (159). As described above, presence of monocytes and neutrophils can be a double-edged sword, pro-tumoral or anti-tumoral, depending on the characteristics of tumors and applied therapies. In lung cancer, increased amount of monocytes within the tumor is associated with a poor survival rate, represented by progression-free survival (PFS) and overall survival (OS) of patients (36). In patients with colorectal cancer, profound influx of CCR2$^+$ classical monocytes from the bone marrow to the circulatory system is correlated with worse clinical outcomes, showing accelerated liver metastasis (46). Reversely, in patients with melanoma, high frequency of classical monocytes allows us to predict favorable treatment response to anti-PD1 therapy and increased survival rates (160). Presence of TEMs and M2-polarized macrophages infiltrated in PDAC is associated with a high possibility of tumor recurrence and poor survival rates

(161). In hepatitis B virus related hepatocellular carcinoma, high percentage of TEMs in peripheral blood monocytes represent poor overall survival and a shorter time to disease recurrence after resection (162). Changes in abundance between TEMs before and at 1 month after initial therapy also could serve as a biomarker in order to predict overall survival of hepatocellular carcinoma patients treated with sorafenib, a multi-kinase inhibitor of tumor angiogenesis (163). In breast cancer, endometrial cancer, prostate cancer, bladder cancer, ovary cancer, and urothelial cancer patients, high density of tumor-associated monocytes/macrophages (TAMs) has been reported to correlate with poor overall survival rates, while high density of TAMs in colorectal cancer patients shows longer overall survival (164). High density of TAMs is also associated with advanced tumor stages (III+IV) rather than with early stages (I+II) in breast cancer, oral cancer, and bladder cancer patients (164). However, there was no observed relation between TAMs and disease free survival rate in this clinical study (164).

Despite the controversial functions of neutrophils, neutrophil lymphocyte ratio (NLR) could be a potential biomarker for clinical use in some cases. After surgical removal of colorectal cancer (CRC), esophageal squamous cell carcinoma (ESCC), and PDAC, patients with lower values of NLR have a greater survival rate and reduced disease progression compared to patients with high NLR (165–167). When using everolimus for treatment of metastatic renal cell carcinoma (RCC), patients with low NLR also represent increased levels of both overall survival and PFS (168). Meanwhile, NLR inversely correlates with prostate-specific antigen (PSA) responsiveness to abiraterone acetate (abiraterone), a medication for metastatic castration-resistant prostate cancer patients (169, 170). In hepatocellular patients, tumor-infiltrated neutrophils represent upregulated PD-L1 expression (91). The ratio of PD-L1[+] neutrophils to PD-1^{+} T cells helps better predict the disease-free survival of HCC patients (91). The NLR system is still under investigation across various cancer types, and it would be safe to be cautious to make an interpretation of disease prognosis with this system.

On one hand, counting TINs indicates controversial clinical outcomes. In RCC, presence of TINs has a negative impact on survival rates (171) and in melanoma patients, high amount of TINs mediated by activated pSTAT3 is linked to poor disease prognosis (172). Robust tumor infiltration of neutrophils also presents a negative disease progression of head and neck squamous cell carcinoma (HNSCC) (173). In the same manner, colorectal cancer patients with increased level of TINs are more likely to acquire a malignant phenotype of cancer and show adverse prognosis (174). Moreover, upon bevacizumab treatment (anti-VEGF therapy) for metastatic colorectal cancer patients, neutrophil infiltration engenders drastically low survival rates and represents a hostile clinical response against bevacizumab treatment (90). However, according to a couple of other clinical researches regarding influence of TINs on colorectal cancer prognosis, neutrophil infiltration to tumor tissue positively associates with favorable disease prognosis (175, 176) and with better responses to 5-FU-based chemotherapy (177). Interestingly, level of TINs may affect tumor prognosis differently depending on the sex of gastric cancer patients. Extensive amount of TINs reduces mortality risk of female patients while it does not affect male patients (178). Meanwhile, in non-small cell lung cancer (NSCLC) TINs do not represent any immediate impact on recurrence-free survival and overall survival (179).

Tests of functional single-nucleotide polymorphisms in genes regulating TAMs also enable us to predict clinical treatment outcomes (180). Through related trials, TBK1 rs7486100, CCL2 rs4586, CCL18 rs14304, and IRF3 rs2304205 have also been revealed to correlate with overall survival and progression free survival of metastatic colorectal cancer patients treated with bevacizumab (180).

Therapeutic Applications

The CCL2-CCR2 chemokine axis plays a major role in recruitment of TAMs, which renders the immunosuppressive tumor microenvironment immunosuppressive and thereby promotes tumor progression (46). Conversely, inhibition of this axis restores anti-tumor immunity (46). Combination therapy of CCR2 inhibitor PF-04136309 with FOLFIRINOX chemotherapy for PDAC restores the anti-tumor immune microenvironment, preventing CCR2+ monocytes from emerging from the bone marrow (181). Carlumab is a human immunoglobulin $G_{1\kappa}$ monoclonal antibody which specifically binds to human CCL2 with high affinity, leading to CCL2-CCR2 axis disruption (182–184). Clinical trials conducted for Carlumab in ovarian cancer, prostate cancer and other solid tumors with and without other chemotherapies such as docetaxel, gemcitabine, paclitaxel+carboplatin, or PLD has proven that Carlumab is well-tolerated but unfortunately fails to trigger significant tumor responses, since it could not sustain the long-term blockade of CCL2 (182–184). Since the CSF-1/CSF-1R axis is responsible for differentiation and survival of pro-tumoral TAMs, incessant efforts have been made to target CSF-1R to eliminate or repolarize TAMs (185). There are several CSF-1R inhibitors currently in clinical trials in many tumor types (186). Emactuzumab (RG7155) is a recombinant, humanized monoclonal antibody of IgG1 subclass, targeting CSF-1R expressed on macrophages (186). Clinical treatment of emactuzumab to patients with tenosynovial giant cell tumor shows durable tumor responses and functional improvement of patients with significant reduction of infiltrated macrophages in the tumor (186).

Another CSF-1R inhibitor is pexidartinib (PLX3397), a small-molecule inhibitor (187). As delineated above, preclinical studies in diverse solid tumors including mammary carcinoma, melanoma, lung carcinoma, pancreatic carcinoma, and glioma have proven that this molecule effectively blocks CSF-1R signaling, suppresses infiltration of macrophages into tumors, and accordingly restrains tumor progression (187–191). The dramatic tumor response to PLX3397 has provided a rationale to begin work on its clinical applications, currently ongoing in many solid tumors with and without combination with pembrolizumab, a monoclonal antibody targeting PD-1. According to a clinical case report, the progression of tenosynovial giant cell tumor was inhibited during non-surgical management with pexidartinib treatment (192).

In the context of tumor where neutrophils exert detrimental influence, the activation and homing of neutrophils need to be interrupted for better prognosis. Repertaxin is a small molecule inhibitor of CXCR1 and CXCR2 for blocking neutrophil trafficking (193). In patients with HER-2 negative metastatic breast cancer, treatment of repertaxin in combination with paclitaxel shows a durable tumor response with fine safety and tolerance. In this setting, an increased rate of neutropenia has not been observed, which needs to be evaluated further (194). Meanwhile, myeloid cell-derived IDO could be another attractive target for tumor regression since it shows suppressive activity on T cells (195). Preclinical research using MMTV-Neu breast tumor model has revealed that indoximod, a small molecule inhibitor of IDO, in combined use of paclitaxel, successfully induces tumor regression (195). As a clinical trial, targeting IDO with a peptide vaccine elicits long-lasting disease stabilization in lung cancer patients along with reduction of Treg frequency and increased cytotoxicity of CD8$^+$ T cells to kill cancer cells (196). Clinical application of indoximod is also ongoing in metastatic solid tumor patients (197). However, it may be asked whether IDO is an effective target, since phase III ECHO301 trial of epacadostat, another inhibitor of IDO, with pembrolizumab for melanoma as a combination therapy failed, missing the first primary endpoint of improving PFS vs. pembrolizumab alone (198).

CONCLUDING REMARKS

As thoroughly discussed in this review, tumor-associated monocytes and neutrophils are highly heterogeneous in a context dependent manner. Setting aside the need for the fine-tuning, we still have limited knowledge of their versatile functions in diverse tumor scenarios: cancer types, stages of disease, and applied therapies. In order to decipher these multifaceted roles of monocytes and neutrophils, there are several demands to be considered. First, we strongly suggest establishing orthotopic tumor models for preclinical studies. Ectopic tumor implantation has been conducted in many pieces of researches without consideration of organ settings. However, since the organ specific microenvironment, including different immune landscape, differently regulate tumor growth and progression, neglecting it undermines the validation of ectopic tumor models. Second, we also urge that researches be further progressed with development of applicable technologies such as single-cell RNA sequencing, intravital imaging, and mass cytometry. Application of advanced technological methods not only help in systemically understanding the heterogeneous and dynamic tumor microenvironment, but will also let us forecast disease prognosis and make therapeutic decisions with minimal side effects. Lastly, more clinical studies are required to validate prognostic markers and therapeutic agents.

With the fulfillment of these methodological and practical suggestions, we will be able to heighten our understanding of heterogeneous functions of monocytes and neutrophils in various tumor contexts, and further establish effective tumor therapies based on the comprehensive understanding.

AUTHOR CONTRIBUTIONS

KJ conceived the concept. JJ, YS, and KJ wrote the manuscript.

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