DOI: 10.1002/JLB.3RI0718-282R

JLB JOURNAL OF LEUKOCYTE BIOLOGY

REVIEW

Getting TANned: How the tumor microenvironment drives neutrophil recruitment

Shuvasree SenGupta[1] | Bhagawat C. Subramanian[2] | Carole A. Parent[1]

[1]Department of Pharmacology, University of Michigan, Ann Arbor, Michigan, USA

[2]Laboratory of Cellular and Molecular Biology, Center for Cancer Research, NCI, NIH, Bethesda, Maryland, USA

Correspondence
Carole A. Parent, Department of Pharmacology, Michigan Medicine, Life Sciences Institute, University of Michigan, 210 Washtenaw Ave, Office 4437, Ann Arbor, MI 48109-2216, USA.
Email: parentc@umich.edu

Abstract

The directed migration of neutrophils to sites of injury or infection is mediated by complex networks of chemoattractant-receptor signaling cascades. The recent appreciation of neutrophils as active participants in tumor progression and metastasis has drawn attention to a number of chemokine-receptor systems that may drive their recruitment to tumors. However, the dynamic nature of the tumor microenvironment (TME) along with the phenotypic diversity among tumor-associated neutrophils (TANs) call for a more comprehensive approach to understand neutrophil trafficking to tumors. Here, we review recent advances in understanding how guidance cues underlie neutrophil migration to primary and secondary tumor sites. We also discuss how the presence of other myeloid cells, such as functionally diverse subsets of tumor-associated macrophages (TAMs), can further influence neutrophil accumulation in tumors. Finally, we highlight the importance of hypoxia sensing in localizing TAMs and TANs in the tumor niche and provide a cohesive view on how both myeloid cell types shape TME-associated extracellular matrix organization, which in turn contribute to tumor progression.

KEYWORDS

neutrophils, monocytes/macrophages, chemokines, chemotaxis, signaling cascade, signal transduction, leukocyte tumor interactions, cancer

1 | INTRODUCTION

The cellular makeup of the tumor niche or tumor microenvironment (TME) is highly heterogeneous. In addition to cancer cells, tumors are composed of stromal cells such as cancer-associated fibroblasts (CAFs), endothelial cells, pericytes, adipocytes, fibroblasts, and bone-marrow mesenchymal stromal cells (MSCs) along with an ensemble of local tissue-resident and infiltrated immune cells.[1] The major innate immune cells of myeloid lineage that compose the TME include tumor-associated macrophages (TAMs), polymorphonuclear neutrophils (tumor-associated neutrophils or TANs) and myeloid-derived suppressor cells (MDSCs).[2] Although the role of TAMs in tumor establishment and progression is well established,[3] the role of TANs in these events is only beginning to be appreciated. In fact, recent advances in in vitro and in vivo imaging reveal several novel tumor-associated functions of TANs that seem to complement TAM functions in the course of tumor cell metastasis (see Table 1).

Neutrophils are the first immune cells to be recruited in response to infection or tissue injury to protect the host from harmful agents. At the inflammation site, neutrophils display a variety of functional responses ranging from phagocytosis and respiratory burst to the extracellular release of their granule contents, which includes proteases and other microbicidal molecules as well as granule protein embedded DNA traps (NETosis).[4] However, growing evidence suggests a crucial regulatory role for neutrophils in tumor establishment and progression.[5–17] Recent studies also document the morphological and functional heterogeneity among TANs and their association with protumor or antitumor responses depending on the TME they are part of.[18–22] The mechanisms by which TANs promote or hinder tumorigenesis and metastatic spread, have been reviewed elsewhere.[22–24] However, limited information is available on the mechanisms

Abbreviations: ADCA, adenocarcinoma; CAF, cancer-associated fibroblasts; CSF-1, colony stimulating factor 1; CSF1R, colony stimulating factor 1 receptor; ECM, extracellular matrix; ESCC, esophageal squamous cell carcinoma; G-CSF, granulocyte-colony stimulating factor; G-MDSC, granulocytic-myeloid-derived suppressor cell; HDN, high-density neutrophils; HIF, hypoxia-inducible factor; LDN, low-density neutrophils; LD-PMN, low-density polymorphonuclear leukocyte; LOX-1, lectin-type oxidized LDL receptor 1; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; MSC, mesenchymal stromal cell; NET, neutrophil extracellular trap; NSCLC, non-small cell lung carcinoma; OPN, osteopontin; PDAC, pancreatic ductal adenocarcinoma; PSC, pancreatic stellate cells; RCC, renal cell carcinoma; rTEM, reverse transendothelial migration; SCCA, squamous cell carcinoma; SPARC, secreted protein acidic and rich in cysteine; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; TME, tumor microenvironment; TRM, tumor-resident macrophages; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2

Received: 20 July 2018 | Revised: 20 October 2018 | Accepted: 16 October 2018

TABLE 1 Potential influence of TAMs and TANs on the metastasis cascade

Metastasis cascade	Mechanism	Techniques/model	References
Tumor cell intravasation into the vasculature	TAMs migrate in association with streaming tumor cell clusters in the TME	Multiphoton intravital microscopy in lung metastasis model in mouse	28
	TAMs remain sessile and perivascular, associate with the vasculature signal via VEGF-A to promote transient vascular permeability	Multiphoton intravital microscopy in breast cancer model in mouse	29
	Monocytes are recruited in a CCR2-dependent manner, become motile TAMs, respond to TGF-β in the TME by upregulating CXCR4, migrate toward CXCL12 producing CAFs along the vasculature, become sessile, perivascular TAMs	Multiphoton intravital microscopy in breast cancer model in mouse	30
Premetastatic niche formation	Neutrophils migrate to lung preceding tumor cells, release leukotrienes, which boost expansion of tumor cells with highly metastatic potential	Bioluminescence based in vivo imaging system in breast cancer model in mouse	5
	Neutrophils preinfiltrate lung and release IL-16 that promotes successful tumor cell engraftment in the lung	Bioluminescence based in vivo imaging system in breast cancer model in mouse	31
Adhesion of circulating tumor cells (CTC) to distant organs	Neutrophil extracellular traps (NETs) entrap CTCs in the mesh-like projections of decondensed chromatin, enhance tumor cell adhesion to hepatic sinusoid, promote liver micrometastases	Intravital microscopy using murine sepsis model and mouse lung carcinoma cells	32
Extravasation of tumor cells	Neutrophil derived chemokine IL-8 enhances the extravasation rate of tumor cells	Confocal microscopy using on-chip model of human microvasculature	33
Successful colonization in distant tissue	Cancer cells induce neutrophils to release NETs, which promote cancer cell migration and invasion	Intravital microscopy in breast cancer model in mouse In vitro migration and invasion assay	6

driving neutrophil trafficking to the primary tumor niche and migration to distant organs in some cases, preceding the metastasized tumor cells.[14,17,25–27]

Given the massive neutrophil infiltration in primary as well as secondary tumor sites, the TME most likely provides a beneficial environment to support the progressive accumulation of neutrophils.[34–39] Both tumor and stromal cells secrete chemokines, cytokines, and growth factors that are thought to contribute to the establishment of gradients that facilitate neutrophil mobilization from the bone marrow to the tumor niche.[15,40–43] In this review, we go over recent findings on the association of neutrophils with cancer outcome, discuss the cellular and molecular network that control neutrophil migration to the TME and influence their functional diversity (summarized as a schematic in Fig. 1). In addition, we dissect the pathways that mediate neutrophil trafficking to primary and metastatic tumor sites, present the current knowledge of their role once they reach the tumor niche and integrate it with the present understanding on TAMs in the context of tumor progression and metastasis.

2 | NEUTROPHIL DEVELOPMENT AND HOMEOSTASIS

Neutrophils are the most abundant leukocytes in human peripheral blood. Mature, terminally differentiated neutrophils arise in the bone marrow through a process called myelopoiesis.[22,44] As only mature neutrophils are released in the peripheral blood under basal conditions, increased demand during severe infection or tissue injury enhances the egress of both immature and mature subsets from the bone marrow.[45] The balance between neutrophil production and retention in the bone marrow versus their release into the circulation maintains cellular homeostasis.

Circulating neutrophils, under steady state, undergo rapid spontaneous apoptosis and are cleared by macrophages in the spleen, liver, or bone marrow.[46] Neutrophils recruited to injured or infected tissues, engage in diverse host defense mechanisms to eventually succumb to apoptotic death and are removed by tissue macrophages. Because activated neutrophils unleash several non-specific cytotoxic mediators, efficient removal of dying neutrophils helps to minimize their off-target effects on the host tissue and favors inflammation resolution.[47] However, death at injury sites may not be their sole fate as neutrophil apoptosis can be delayed in the presence of a number of cytokines, chemokines, and growth factors commonly found at inflammatory sites.[48] In fact, tumor-derived factors, which potentially have a number of inflammatory mediators, are reported to extend neutrophil longevity in vitro.[49] Interestingly, once finished with their part at the wound site, neutrophils can also migrate and re-enter the circulation from the damaged site via healthy tissue by a process termed reverse migration or reverse transendothelial migration (rTEM).[50] Wang et al.,[51] for example, recently showed that neutrophils recruited at sterile tissue injury sites migrate back into the vasculature. Remarkably, these "reverse migrated" neutrophils get trafficked to the lung before heading back to the bone marrow to undergo apoptosis.[51] Although the mechanisms driving rTEM of neutrophils and their subsequent fate requires further studies, the idea that TANs in the

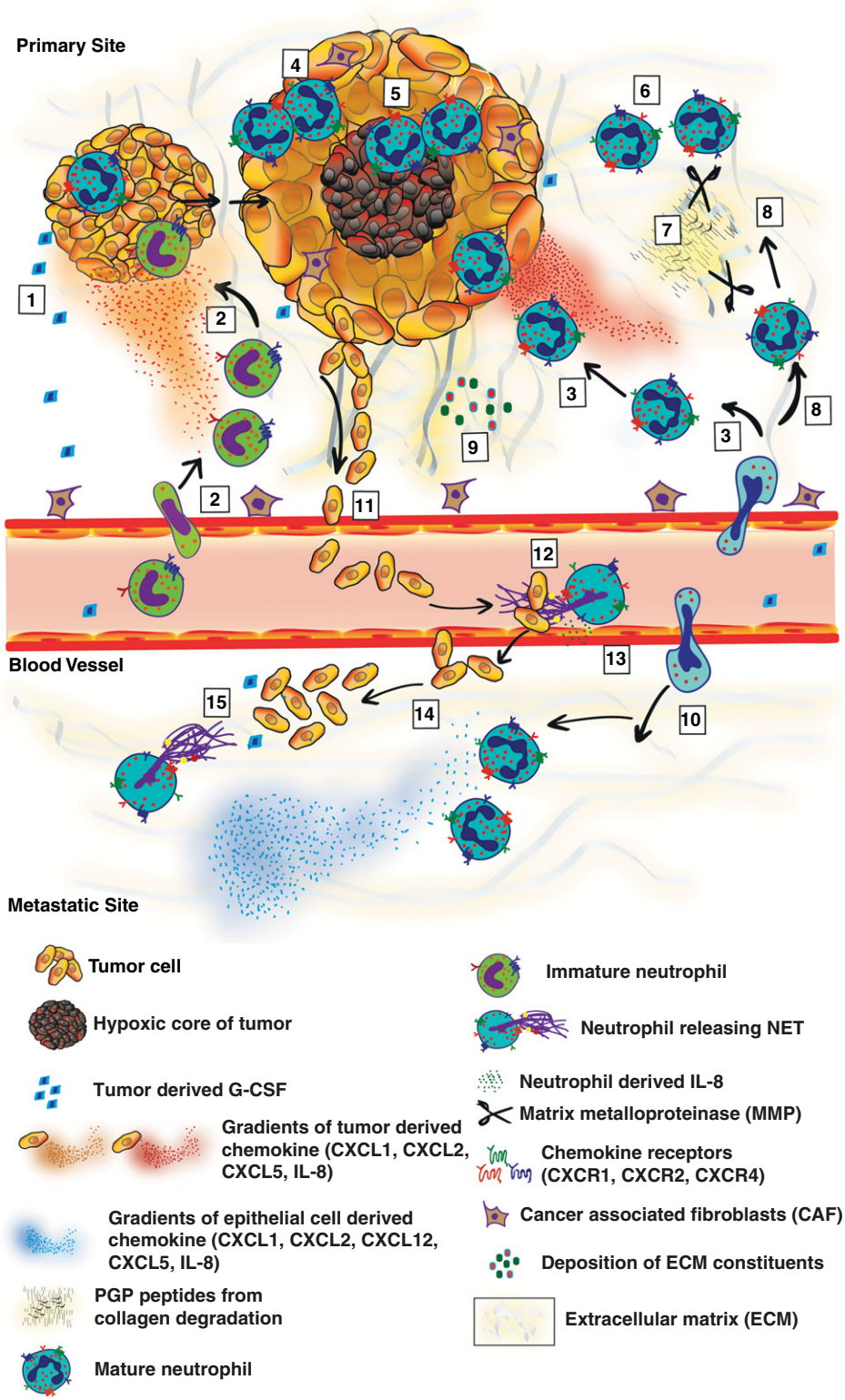

FIGURE 1 **Cartoon depicting the role of chemotactic signaling during the recruitment of neutrophils to the TME.** A schematic drawing showing the various mechanisms potentially involved in the recruitment of neutrophils to primary and metastatic tumor sites. (1) Tumor-derived soluble factors (e.g., G-CSF) induce the expansion and mobilization of neutrophils from the bone marrow into the circulation. (2) Immature and (3) mature neutrophils migrate toward tumor sites following gradients of chemokine(s) produced by tumor cells or tumor associated CAFs. Location of recruited neutrophils in peri-tumoral (4), intratumoral, (5) or stromal (6) regions of the TME. (7) Neutrophils release MMPs, which mediate ECM lysis and release of PGP fragments. (8) Chemotactic PGP peptides amplify neutrophil recruitment. (9) Recruited neutrophils contribute to desmoplasia. (10) Chemokines derived from epithelial cells at distant sites recruit neutrophils, which precede metastatic tumor cells and form a premetastatic niche. (11) Tumor cells intravasate into the circulation. (12) Neutrophil-derived NETs trap circulating tumor cells. (13) Neutrophil-derived IL-8 enhances extravasation of tumor cells. (14) Cancer cell-derived factors (e.g., G-CSF) induce neutrophils to release NETs (15), which in turn promote cancer cell migration and colonization in distant tissues

primary tumor sites survive beyond their limited lifespan and may reverse-migrate to establish a metastatic niche at secondary sites is an intriguing possibility to be explored.

3 | NEUTROPHIL ABUNDANCE AND CANCER PROGNOSIS

Numerous studies suggest that the peripheral neutrophil-to-lymphocyte ratio (NLR) represents an easily quantifiable biomarker with potential prognostic impact in patients with solid tumors. In general, a high blood NLR is associated with poor prognosis in many different types of cancers,[52] as elevated levels of circulating neutrophils are an indicator of cancer-related systemic inflammation.[52,53] Even immature neutrophils are found to accumulate in the circulation of cancer patients, suggesting tumor-driven alterations of myelopoiesis and a derailed balance of neutrophil retention and release from the bone marrow.[54–57]

TANs have been detected as $CD66b^+$- or $CD15^+$- or myeloperoxidase (MPO^+)-positive cell subsets in the TME of human colon,[58] lung,[59,60] hepatocellular,[61,62] renal,[34] esophageal,[63] melanoma,[64] head and neck squamous cell,[38] as well as pancreatic[65] carcinoma. An overwhelming number of studies report a correlation between the increased presence of TANs and poor prognosis in terms of patient survival[15,34,60–62,64–68] In contrast, some reports suggest an association of TANs with survival benefits.[10,38,69] Blaisdell et al.,[69] for instance, provided a positive correlation between elevated expression of genes encoding neutrophil specific chemokines and improved survival outcome in human endometrioid cancer (EC) and certain subtypes of brain, breast, colorectal, and ovarian cancers. Another study reported an association between the greater presence of TANs with a favorable prognosis in patients with esophageal squamous cell carcinoma (ESCC).[63] Such variability in the disease outcome with respect to the presence of TANs suggests that other factors are involved in regulating TAN responses. One such contributing factor may be the location of neutrophils in the tumor niche. TANs have been detected within the tumor nest (intratumoral TANs), adjacent to the tumor nest (peritumoral TANs) or in the stroma with no adjacent tumor (stromal TANs)[60,66,70,71] A meta-analysis conducted on the data from different malignant tumors reported an elevated intratumoral but not peritumoral or stromal neutrophils as an independent prognostic indicator for short survival.[66] Figuring out how neutrophils migrate to different locations within the TME will ultimately help manipulate their recruitment and the ensuing impact on disease progression. Interestingly, the capacity of neutrophils to act as a prognostic marker can also vary in different histologic subtypes of tumor. Non-small cell lung carcinoma (NSCLC) is one example where the presence of TANs at high density is associated with a favorable survival outcome in patients with the squamous cell carcinoma (SCCA) subtype, whereas an adverse outcome in patients with the adenocarcinoma (ADCA) subtype has been reported.[60] It will be interesting to investigate in depth the tumor milieu among different histologic subtypes of NSCLC that may differentially regulate the functional status of TANs and hence the survival outcome.

4 | NEUTROPHIL SUBSETS IN CANCER

Although mature neutrophils are terminally differentiated cells, it has recently been proposed that neutrophils exhibit phenotypic switching. Indeed, neutrophil subpopulations with seemingly opposite functions were identified in the circulation of cancer patients as well as in primary tumors and in the circulation of tumor bearing mice.[18,19,54,55] In this section, we discuss the primary neutrophil subsets along with their distinct tumor-associated functions.

4.1 | N1 and N2 TANs

Functional plasticity is commonly observed in mononuclear leukocytes. Based on their activation status, macrophages, for example, are categorized into the classically activated, proinflammatory M1 type and the alternatively activated, anti-inflammatory or immunosuppressive M2 type.[3,72] In the context of tumors, TAMs potentially undergo a transition from an antitumoral (M1) to a protumoral (M2) activation mode that is instructed by the cytokine milieu in the TME.[3] Similar to TAMs, in two studies[18,19] involving mouse tumor models, neutrophils with different activation modes have been observed: the tumor-regressive N1 and the tumor-promoting N2 TANs.

Fridlender's group first identified TGF-β, which is overexpressed by many tumors, as a determinant of N1 versus N2 phenotype in murine mesothelioma and lung cancer models.[18] Inhibiting TGF-β signaling promoted the immunostimulatory activities in TANs (N1), which included cytotoxic $CD8^+$ T lymphocyte (CTL) activation, reactive oxygen species-dependent direct killing effects on tumors and high expression of proinflammatory cytokines, including TNF-α, CCL3, and the costimulatory molecule ICAM-1 as well as low expression of the immunosuppressive enzyme arginase. Conversely, in the presence of intact TGF-β signaling, TANs exhibited the immunosuppressive N2 phenotype that favored tumor growth. Another study by Andzinski et al.[19] showed that IFN-β promotes the N1 phenotype of TANs. In tumor bearing *Ifnb*$^{-/-}$ mice, TANs displayed N2 characteristics, with a reduced expression of ICAM-1 and TNF-α, and a reduced capacity to directly kill tumor cells—a process that could be reversed by adding exogenous recombinant IFN-β. Clearly, much like TAM activation, the TAN activation profile is guided by cues present in the tumor milieu, such as immunosuppressive cytokine TGF-β[18] and immunostimulatory type I IFN, IFN-β.[19] To what extent the soluble factors in the local tumor niche influence N1 to N2 shift and how it contrasts to or integrates with the phenotypic switching in TAMs to impact tumor initiation and metastasis are interesting avenues for future research.

Interestingly, the impact of soluble factors from tumors on neutrophil phenotype is not necessarily restricted to the tumor niche. For example, Casbon et al.[73] identified tumor-derived G-CSF (granulocyte-colony stimulating factor) as a key factor involved in the reprogramming of myeloid differentiation in the bone-marrow, thereby favoring the expansion of T cell suppressive neutrophils in the peripheral tissues during the onset of malignant conversion in an oncogene-driven murine breast cancer model. The role of these T cell suppressive neutrophils during tumor progression and metastasis needs to be evaluated. Of interest, in this model, very few neutrophils accumulated in

the primary tumor sites, warranting future studies to determine the mechanisms underlying their biased localization in peripheral tissues and further exploration across different cancer types.

4.2 | Immature versus mature neutrophils

Immature neutrophil subsets are characterized by their banded, ring-shaped, or non-segmented nuclear morphology compared with the segmented nuclei typically observed in mature neutrophils. Indeed, the murine N2 TANs identified by Fridlender et al.[18] exhibit a ring-like nuclear morphology similar to immature neutrophils, whereas the N1 subsets show mature hyper-segmented nuclei. The accumulation of immature neutrophils was also detected in the blood of tumor bearing *Ifnb*$^{-/-}$ mice.[19] Recently, Coffelt et al.[56] reported the expansion of immature neutrophils in the circulation, primary tumor, and distant organs of mammary tumor-bearing mice, which had metastasis promoting effects through the suppression of CD8$^+$ T cell proliferation and activation. Functionally, these immature neutrophil subsets are therefore similar to MDSCs of granulocytic origin (G-MDSCs) that have characteristic immunosuppressive effects on cytotoxic CD8$^+$ T cell responses.[74] A number of studies show that G-MDSCs expand in the spleen of tumor bearing mice and also accumulate at the tumor site.[74–76] Both G-MDSCs and protumoral N2 TANs have common surface markers and morphologic features. It is therefore possible that they represent the same functional subset of neutrophils. But controversy surrounds identifying N2 TANs as G-MDSCs. Transcriptomic profile comparison in tumor-bearing mice by Fridlender et al.[77] for instance, suggests that TANs represent a discrete population from splenic G-MDSCs. However, this does not necessarily rule out the possibility that G-MDSCs transition into TANs when exposed to the TME. Of note, immunosuppressive G-MDSC accumulation into tumor sites can also rely on host-derived factors. Ban et al.,[78] for example, showed that the altered neutrophil maturation and G-MDSC accumulation in the primary tumors of 4T1 and PyMT murine breast tumors are controlled by myeloid CCR5 and an autocrine CCR5-CCL5 axis, but not by tumor-derived CCL5. In the absence of host CCL5, neutrophils similar to N1 TANs are recruited into tumors.[78] Taken together, these studies show that functional plasticity exists among neutrophils along their course of maturation during tumorigenesis and tumor progression.

Immature neutrophil enrichment has also been reported in the blood of cancer patients. For instance, Brandau et al.[55] reported expansion of a mixture of immature and mature neutrophils, designated as low-density polymorphonuclear leukocytes (LD-PMNs), during the course of cancer progression in the peripheral blood of patients with head and neck, lung, and urologic cancers. Unlike the normal granulocytes that are high in density, LD-PMNs sediment in the low-density mononuclear fraction during density gradient centrifugation of blood and represent different developmental stages of neutrophils as identified by nuclear morphology and the differential expression of surface markers.[55,79] In line with this finding, Sagiv et al.[54] identified a distinct mixture of low-density neutrophils (LDNs) in the blood of patients with advanced stage lung and breast cancer, as well as in different mouse models of breast, mesothelioma, and lung cancer. LDNs comprised of both an immature subset with ring- or band-shaped nuclei and a mature subset with segmented nuclei in patient blood and therefore essentially represent the same cell population as LD-PMNs. Although the high-density mature neutrophils (HDN) had antitumor N1-like functionalities, LD-PMN (aka LDN) function similarly to N2 TANs with immunosuppressive effects on T cell proliferation, activation, and function.[54,55] Similar to cancer patients, tumor-bearing mice also had circulating LD-PMN that accumulated during the course of cancer progression.[54]

The immature LD-PMNs might in fact represent the T cell suppressive G-MDSCs or PMN-MDSCs that are known to increase in the blood of late stage cancer patients.[79,80] However, due to the lack of specific surface marker, LD-PMNs as a whole are generally known as PMN-MDSCs. A recent study has detected lectin-type oxidized LDL receptor 1 (LOX-1) as a surface marker specifically associated with LD-PMNs/PMN-MDSCs, but not with HDNs in both the peripheral blood and tumor tissues of cancer patients.[81] Given the existing heterogeneity in TANs, identification of LOX-1 as distinct marker opens an avenue to further study the way LD-PMNs are trafficked into tumor tissues and how their accumulation associates with survival in different cancer types. However, LOX-1 is not associated with LD-PMNs in tumor-bearing mice.[81] In vivo tracking of LD-PMNs and selective targeting to determine what specific role they may have during tumor progression remains to be determined. Recently, LDNs were constitutively detected in the circulation of normal non-human primates.[82] Notably, only the mature CD33$^+$ LDN subset possessed a T cell suppressive phenotype and identified as PMN-MDSCs.[82] Similarly, a mature CD10$^+$ LDN subset with suppressive properties was also identified in healthy humans receiving G-CSF.[57] In fact, in this study CD10 was used as a phenotypic marker to discriminate mature CD10$^+$ neutrophils from their immature CD10$^-$ counterpart in the LDN fraction from cancer patients. This provides great opportunity for selectively purifying mature or immature LDN subsets based on CD10 expression to define what immunoregulatory role they may play during the course of cancer progression in humans.

A reduced ability to migrate toward tumor conditioned medium has been noted using in vitro cell migration assays with LD-PMNs from cancer patients and tumor-bearing mice compared with mature HDN. This was most likely due to the inadequate expression of chemokine receptors CXCR1 and CXCR2 on the surface of the LD-PMNs.[54,55] How such migration defects influence the overall recruitment of these neutrophil subsets to tumor sites during the course of cancer progression and metastasis needs to be further addressed. In murine systems, interestingly, TGF-β was identified as the prime factor to drive the HDN to LD-PMN transition.[54] Equally interesting will be to identify the molecular signals that drive the switch from regular neutrophils to LDNs and their presence in the circulation of cancer patients.

4.3 | Hybrid TANs

A unique subset of HLA-DR$^+$ TAN with antitumor capabilities was detected in the early stage of human lung cancer.[7,49] This subset had

characteristics of both granulocytes and APC such as dendritic cells and macrophages, hence aptly named "hybrid TAN," which efficiently induced tumor antigen specific and non-specific T cell responses. Remarkably, the number of such hybrid TANs declines in large tumors, seemingly due to the associated hypoxic TME.[7] Interestingly, hybrid TANs exhibited banded nuclei, indicating their likely derivation from immature neutrophils possibly through the action of inflammatory factors from the TME such as GM-CSF and IFN-γ.[7] Our current understanding on such APC-like hybrid TANs suggests that they have a distinct and potentially opposite role compared with immature neutrophils with immunosuppressive functions. Perhaps, exposure to specific cytokines drives such polarized/hybrid states of immature neutrophils in the TME.

5 | FACTORS REGULATING NEUTROPHIL RECRUITMENT AT TUMOR SITES

Tumors, unlike infection or wound induced inflammation, are characterized by a chronic, unresolved type of inflammation that assists tumor growth and metastatic spread.[83] In response to infection, chemokines and their receptors are upregulated to rapidly recruit leukocytes, which are terminated upon the resolution of the infection. However, persistent inflammatory responses lead to chronic inflammation.[84] The cytokines and chemokines secreted by the TME and tumor-associated immune cells therefore promote a sustained and non-resolving tumor-associated inflammation.[83] The directed migration of neutrophils toward sites of inflammation is mediated by chemoattractants, which are diverse in nature ranging from lipid metabolites, proteolytic fragments and small peptides to a large family of chemokine peptides derived from various cellular sources. Interestingly, most chemoattractants bind and activate their cognate receptors that belong to the G protein-coupled receptor family to mediate their effects.[85] Chemokine family members are in general small proteins (8–14 kDa) that are sub-grouped into CC, CXC, CX3C, and C depending on the positions of the first two conserved cysteine residues near the N-terminus, where X represents any other amino acid. Based on the presence or absence of an N-terminal tripeptide motif composed of glutamic acid-leucine-arginine (ELR) and preceding the first cysteine residue, CXC chemokines can be further categorized into ELR^+ or ELR^- chemokines. ELR^+ chemokines are involved in driving the tissue recruitment of neutrophils during chronic inflammatory disease such as ulcerative colitis, ischemia and pulmonary disorders.[86] Besides their primary role in controlling leukocyte trafficking, the chemokine ligand-receptor network has also gained attention for its contributions to tumor development, angiogenesis and metastatic spread.[86,87] A number of studies have shown that chemokines including CXCL1, CXCL2, IL-8 (CXCL8), CXCL5, CXCL12, CCL2, and MIP-1α (CCL3) as well as lipid mediators like leukotriene B_4 (LTB_4), all promote neutrophil infiltration to a variety of tumors.[15,40–43,88,89] Chemokine receptors including CXCR1, CXCR2, as well as LTB_4 receptors are highly expressed on the surface of human peripheral blood neutrophil,[90,91] whereas constitutive expression of most of the CC chemokine receptors are marginal to none.[92] Yet, neutrophils recruited into inflamed tissues from patients with chronic obstructive lung disease and rheumatoid arthritis do express CCR1-3 and CCR5, mostly in response to inflammatory cytokines and respond chemotactically to their respective chemokine ligands in vitro.[92]

During inflammation chemokines can undergo posttranslational modifications, such as proteolytic cleavage, nitration of Tyr residues, citrullination of Arg residues, and glycosylation.[84] Matrix metalloproteinases (MMPs), for instance, have been shown to cleave CXCL5, potentiating its action in the recruitment of neutrophils in an in vivo peritonitis model.[93] However, the roles of such modifications in regulating neutrophil trafficking to the TME is not well established.[84] It has been reported that CCL2 nitration by reactive nitrogen species (RNS) produced in the TME can prevent CTL infiltration into tumors as detected in mouse models of different tumors[94] Interestingly, RNS-modified CCL2 also loses its efficacy to attract human CD8+ T cells and exhibits reduced potency for attracting human monocytes in vitro.[94] In addition to posttranslation modifications, chemokines such as CXCL8 or CXCL1 can reversibly exist as monomers and dimers.[95–97] But how such chemokine monomers-dimers impact neutrophil trafficking to the TME remains to be determined. Below, we discuss the contribution of key chemoattractants in mediating neutrophil recruitment to primary and metastatic sites and the functional consequence of neutrophil recruitment in tumor progression.

5.1 | Primary tumors

CXCR2 seems to play a pivotal role in neutrophil trafficking to tumor sites.[26,27,40,61,69,98–100] Chemokine ligands for CXCR2 include CXCL1-3, CCL5, CXCL7, and IL-8, all of which belong to the ELR^+ CXC chemokine family.[101] Fridlender et al.[18] have shown that the recruitment of N1 TANs increases when TGF-β signaling is blocked in mice bearing lung tumors, a process that is correlated with the enhanced tumoral mRNA expression of potent neutrophil chemokines such as CXCL2, 5 and CCL3.[18] Although the contribution of each chemokine to the discrete steps of neutrophil recruitment remains largely unexplored, it was observed that the recruited TANs had higher CCL3 mRNA expression, which may help amplify their own influx or bring in other immune cells.[102] Another study by Jablonska et al.,[26] in a mouse model of melanoma, showed the crucial involvement of CXCL1, 2, and 5 in neutrophil recruitment to tumor sites. CXCR2-dependent neutrophil trafficking was also shown in the uterus during the early stages of EC development, where the TANs induced tumor cell sloughing, thus impeding tumor progression. In this model, the hypoxic tumor niche resulted in the expression of CXCR2-specific ligands, CXCL1, 2, and 5.[103] Although CXCR2 ligands are traditionally thought to have overlapping chemotactic functions, recent studies suggest that they have distinct functions during neutrophil recruitment to sites of infection or inflammation.[104–106] CXCL5, for instance, can oppose CXCL1- and 2-mediated neutrophil recruitment to lung in a mouse model of *E. coli* pneumonia by disrupting concentration gradients of the latter in blood versus lung.[104] On the other hand, CXCL5 itself can be a potent neutrophil chemoattractant as its higher expression correlates with extensive intratumoral neutrophil infiltration.[41,61] Future studies

defining the precise role of each chemokine in the CXCR2–ligand axis and how they coordinate or oppose each other's actions during tumor progression will shed light into the complex mechanisms underlying neutrophil-tumor dynamics.

IL-8, a ligand for both CXCR1 and 2 and a potent neutrophil chemoattractant, is constitutively overexpressed in many human solid tumors.[107] Additionally, host T cells, such as γδT17 cells that infiltrate human colorectal cancers, produce IL-8, which may have a role in promoting PMN-MDSC recruitment to tumor tissues.[108] Rodents lack the gene encoding IL-8 and other CXC chemokines like CXCL1/KC and CXCL2/MIP2, apparently considered IL-8 mouse homologues, do not share complete functional redundancy with IL-8[109] and serve as effective ligands only for CXCR2.[110] Rather, mouse GCP-2 (aka CXCL-5/6) is a high affinity chemokine ligand for mouse CXCR1,[110] but whether GCP-2 functionally replaces IL-8 in the context of tumor-driven neutrophil recruitment in tumor-bearing mice is still an open question. As mice CXCR1 and 2 bind to IL-8,[110,111] alternative approaches have been used to uncover the role of IL-8 in TAN recruitment. For instance, using transgenic mice bearing IL-8 hBAC (human bacterial artificial chromosome), Asfaha et al.[109] showed that human IL-8 enhanced the mobilization of immature CD11b+Gr-1+ myeloid cells and, in turn, contributed to inflammation-induced colonic tumor initiation and progression. In contrast, Lee et al.[43] reported enhanced neutrophil recruitment into tumor in a mouse model xenografted with IL-8 overexpressing human ovarian cancer and its positive correlation with attenuated tumor growth. A very recent study used a hydrodynamic gene transfer approach to transiently express IL-8 in the liver of mice bearing HT9 tumor xenograft. Influx of both G-MDSCs and their monocytic counterparts, Mo-MDSCs, was detected in IL-8 expressing liver, in a CXCR1- and CXCR2-dependent manner[112] Finally, it has been reported that the amount of IL-8 progressively increases in the exhaled breath condensate of NSCLC patients as the cancer progresses to advanced stages, possibly contributing to neutrophil recruitment into lung tumors as detected in a number of studies.[59,60,113]

LTB_4, a chemotactic leukotriene, is mostly produced by immune cells of myeloid lineage including neutrophils and certain non-immune cells in response to proinflammatory stimuli.[85] Neutrophils express two distinct LTB_4 receptors BLT1 and BLT2, which exhibit high and a low LTB_4 affinity, respectively.[85] The role of BLT1 is well characterized in the context of neutrophil recruitment during autoimmune pathology or sterile inflammation.[114] The LTB_4-BLT1 signaling axis has been shown to dramatically amplify chemotactic responses of neutrophils to sites of sterile tissue injury.[115] LTB_4 released from chemotactic neutrophils acts in an autocrine and paracrine manner and relays the signal among neutrophils such that they efficiently migrate over long distances toward primary chemoattractants.[116,117] Increased amounts of LTB_4 have been detected in gastrointestinal cancer tissue samples[118,119] and in the exhaled breath condensate of patients with NSLC.[113] A recent study demonstrated that LTB_4 is also a crucial determinant of crystalline silica (CS)-induced neutrophil recruitment and tumor growth in a spontaneous lung tumor model.[89] LTB_4 was produced mainly from mast cells and alveolar macrophages in response to CS exposure and was shown to be critically involved in mediating neutrophil influx in the lung. Although other chemokines (CC/CXC family members) may be contributing to neutrophil recruitment in such a system, the LTB_4–BLT1 axis appeared to be the predominant effector in this context.[89] In the future, it will be interesting to address if the role of the LTB_4–BLT1 axis in mediating neutrophil influx into tumors is at the level of primary chemoattractant or as a relay signal such as in sterile tissue injury.

5.2 | Tumors at distant sites

Metastasis remains the prime determinant of cancer associated mortality. Over the past decade, the existence of neutrophils with either prometastatic or antimetastatic properties have been reported,[5,12,120–124] adding further complexity to role of TANs during cancer. Neutrophil infiltration was detected in the lungs of xenografted human renal cell carcinoma (RCC) cells in mice. However, neutrophils in this model prevented the metastatic seeding of tumor cells. Indeed, the metastatic capacity of RCC cells was inversely correlated with the expression of chemokines IL-8, CXCL2, 3, and 5, which were downregulated in highly metastatic cells accompanied by attenuated neutrophil influx in the lung.[120] In contrast, Tabariès et al.[12] reported that infiltration of CD11b+/Ly-6G+ neutrophils to liver favors the establishment and growth of hepatic metastasis using liver metastatic 4T1 breast cancer cells. Interestingly, 4T1 primary tumors exhibited limited neutrophil accumulation. Greater abundance of neutrophils in the secondary tumor sites correlated with higher levels of CXCL2 in the metastatic tumor.[12] A similar trend was observed in a MMTV-$PyMT^+$ breast cancer mouse model, where neutrophil infiltration in mammary tumor was minimal when compared with their extensive accumulation in distant organs (lung and liver).[5] In fact, neutrophils accumulated in the lung even before cancer cells infiltrated, as determined by histologic staining for neutrophils and $PyMT^+$ tumor cells in lung sections, suggesting that neutrophil recruitment precedes metastatic seeding and potentially promotes the establishment of a prometastatic niche for infiltrating metastatic tumor cells. Additionally, neutrophil-derived LTB_4 was reported to facilitate successful lung colonization by tumor cells.[5] Perhaps, LTB_4 also helps increase neutrophil influx in an autocrine-paracrine manner. However, what triggers neutrophil trafficking to the lung in the first place remains to be determined.

Primary tumor-derived factors have been reported to bring systemic changes and educate secondary sites to become permissive to tumor cell homing. For instance, Liu et al.[121] identified tumor-derived exosomal RNA (exoRNA) as a lung educating factor in a spontaneous metastatic mouse model. Tumor-derived exoRNA triggered chemokine (CXCL2, CXCL5, and CXCL12) production from the lung epithelial cells in a TLR3-dependent manner and induced neutrophil infiltration for premetastatic niche formation.[121] Another study with 4T1-related metastatic and non-metastatic cells revealed a critical role for tumor-derived G-CSF in inducing the expansion and mobilization of $Ly6G^+Ly6C^+$ neutrophils, which accumulate in the lung and promote lung metastasis.[122] Seubert et al.[123] reported similar metastasis promoting functions of neutrophils in a mouse colon cancer model, where hepatic premetastatic niche formation was dependent on prior

homing of neutrophils to the liver by the CXCL12–CXCR4 axis, whereas neutrophil depletion remarkably attenuated the metastatic burden. Conversely, a recent study by Hand et al.[124] revealed a protective role of neutrophils against the recurrence of colorectal liver metastasis. The presence of fewer neutrophils in the distal resected margins after curative hepatectomy was associated with relapse of liver metastasis. However, the presence of neutrophils was evaluated after surgical resection of already established hepatic metastases, which may be different from the ones required for initial seeding. Together, these studies call for a detailed evaluation of the different chemotactic factors involved in neutrophil recruitment in the distal organs during metastasis, while highlighting the need for careful consideration when assessing the prognostic value of TANs in different stages of tumor malignancy.

6 | TAMs IN CROSS-REGULATING TANs RECRUITMENT

TAMs are represented by tumor-resident macrophages (TRMs) and the ones derived from circulating classical monocytes (CCR2^{high}) after extravasation into tissues. Both are TME integral cell types that are well characterized and recognized for their contribution to the establishment and spread of cancer.[72,125] Although bone marrow is the major source of circulating monocytes, spleen can serve as an extramedullary reservoir of monocytic progenitors and supply monocytes into tumors as detected in tumor-bearing mice.[126,127] Monocyte recruitment into tumors is largely dependent on the CCL2–CCR2 chemokine-receptor axis,[72,128,129] however other factors, including chemokine CCL5 and CSF-1, assist during monocyte migration to the TME.[72,128,130] On the other hand, non-classical monocytes (CX3CR1^{high}) mostly patrol inside the blood vessel and scavenge tumor elements, thus resisting metastasis.[131]

As both TAMs and TANs are key components of the TME, studies depicting TAMs as crucial regulators of neutrophil recruitment into tumors are emerging. For example, when TAM recruitment to tumors is blocked by inhibiting CSF1 receptor (CSF1R), a compensatory and significant increase in TANs is observed.[132] Further, blocking TANs using CXCR2 antagonists in conjunction with CSF1R inhibition was shown to have strong antitumor effects. Also, in a mouse model of cervical carcinogenesis, the lack of CCR2 resulted in macrophage deficiency in the cervix and a concomitant increase in MMP9^{+} TANs, which is thought to provide alternative paracrine support for tumor angiogenesis and progression in the absence of TAMs.[133] The presence of TAMs in the TME therefore limits the extent to which TANs can be recruited. However, this cross-regulation may not apply to patrolling monocytes, as in the case of colorectal cancer, where vascular endothelial growth factor receptor 2 (VEGFR2) inhibition led to enhanced extravasation of patrolling monocytes that in turn enhanced the recruitment of TANs via CXCL5 to contribute to the overall immunosuppression and tumor resistance to VEGFR2-based therapy.[134] Therefore, dual targeting of TAMs and TANs is proving to be beneficial to overcome compensatory myeloid cell-type recruitment and resistance to individual therapy in multiple mouse models including pancreatic ductal adenocarcinoma (PDAC)[135] and others.[132] While the regulation of TANs recruitment is impacted by TAMs, the mechanisms driving this in individual model system remains unclear. Moreover, although most studies have focused on the blockade of TAMs and its consequences, little is known about the impact of depleting TANs in individual tumor models and the resulting impact on TAMs and tumor progression or resistance to therapy.

7 | TAMs, TANs, AND THE TME

In this section, we discuss our current understanding of the major TME physicochemical cues that are implicated in regulating TAMs and TANs recruitment and their tumor-associated functions.

7.1 | Tumor-associated hypoxia on TAM/TAN recruitment

Hypoxic environments in most solid tumors result from the rapid proliferation of cancer cells and the lack of sufficient oxygen carrying vasculature.[136] Reduced oxygen tension stabilizes and activates members of hypoxia-inducible factor (HIF) family. HIFs are transcription factors that regulate the expression of a number of hypoxia responsive genes to support a more oxygenated tumor niche, and cancer cell survival. HIFs can either directly induce angiogenic factors in the cancer cells[136] or indirectly recruit proangiogenic immune cells.[137,138] In a mouse model of lung carcinoma, a subset of TAMs with low MHC-II expression (MHC-II^{lo}), specifically localized in the hypoxic regions of tumors, are known to upregulate hypoxia-sensitive genes for proangiogenic factors such as VEGF, angiopoietin, and others.[137] However, what molecular guidance selectively positions the MHC-II^{lo} TAMs in the hypoxic areas is not clear. A role for the semaphorin 3A/neuropilin-1 axis has been shown to recruit and retain TAMs in hypoxic regions of tumors.[138] TAMs deficient in Neuropilin-1 localize to normoxic regions, thus ablating their proangiogenic and immunosuppressive functions, which is reflected by tumor growth inhibition and reduced metastasis in multiple mouse tumor models.[138]

Unlike TAMs, hypoxia driven regulations of TAN recruitment or their functional profiles are relatively under-studied, although new details are emerging. In a mouse model of colon carcinoma, for instance, HIF-2α expressed in the intestinal epithelial cells, drives intratumoral neutrophil recruitment through the activation of the CXCL1–CXCR2 signaling axis which, in turn, increases tumor burden.[139] Another study using a genetic mouse model of uterine cancer[69] shows localization of tumor invading neutrophils in severely hypoxic regions, positive for HIF-1α, suggesting a close association between hypoxic TME and neutrophil accumulation in the tumor. TANs, in this study, however, inhibited rather than fostered malignant progression, by inducing the detachment of tumor cells from the basement membrane. Massena et al.[140] identified a subset of CD49^{+}/VEGFR1^{high} neutrophils in the circulation that accumulated in transplanted hypoxic tissue, presumably in a VEGFA-dependent manner, and enhanced neovasculature formation.

If similar neutrophil subsets also invade the hypoxic tumor niche, which is frequently enriched with VEGF, remains unknown. Additionally, given the emerging role of neutrophils in tumor progression, it will be important to understand how tumor-associated hypoxia alters functional responses of neutrophils as observed during infections.[141]

7.2 | Regulation of tumor-associated extracellular matrix by TAM/TAN

Another prominent feature of solid tumors is the remodeling in the extracellular matrix (ECM), through degradation or deposition of ECM constituents, that dictates the degree of tumor growth, neovascularization, intravasation of metastatic tumor cells, and metastatic niche establishment.[142] Interestingly, both TAMs and TANs are critical in ECM remodeling.[142–150]

7.3 | ECM degradation

ECM degrading proteases, such as MMPs, ADAMs (A Disintegrin And Metalloproteinase), and related proteinases, are highly expressed in TAMs and TANs and known to regulate tumorigenesis, angiogenesis, and metastasis.[142] MMP-mediated cleavage of collagen generates proline-glycine-proline (PGP) tripeptides, which have been shown to increase the directionality of migrating neutrophils[151], suggesting a role for MMPs in regulating neutrophil accumulation in the TME. MMP9 expression, for instance, was shown to be sufficient to promote squamous carcinogenesis in a mouse model of HPV16-mediated skin cancer through its specific expression in hematopoietic cells.[143] MMP9 is released as an inactive pro-MMP9 form that must be processed to be catalytically active. Unlike TAMs, TANs release a unique form of pro-MMP9, which is free of the endogenous inhibitor, tissue inhibitor of metalloproteinases-1 (TIMP-1), and therefore readily available for subsequent activation.[144] In fact, a comparative study using mouse models of different tumor types showed that TANs but not TAMs are the major in vivo source of MMP9 and thus potentially the prime regulator of tumor angiogenesis and metastasis.[145] Of note, in this setting, TAMs skewed toward the M2 phenotype also acquired the ability to produce the neutrophil-like TIMP1-free MMP9, albeit in less amounts than TANs.[145] The importance of MMP9 released from hypoxia responsive TAMs has also been highlighted in a mouse model of glioblastoma, where MMP9 is required for VEGF release to promote tumor invasion.[152] Additionally, TAMs were shown to secrete TGF-ß to promote the expression of MMP9 in glioma stem-like cells, which was required for tumor invasiveness.[146] On the other hand, MMP12 expressed by TAMs restricts pulmonary tumor growth and metastasis by reducing tumor-associated microvessel density.[147] Tumor-defying and antimetastatic property has also been attributed to MMP8, most likely derived from TANs, in murine melanoma and lung cancer models where MMP8 increased adhesion of tumor cells to ECM proteins and reduced tumor invasiveness.[148] Collagen fragments, most likely derived from various MMP actions, are further degraded through endocytic pathways by a subset of TAMs, originating from CCR2+ monocytes in a mouse lung carcinoma model.[153] Therefore, the dynamics of TAM/TAN-mediated ECM remodeling dictate the fate of tumor progression in vivo.

7.4 | ECM deposition

The excessive growth of connective tissue, referred to as desmoplasia and a feature of many types of cancers, is known to be regulated by TAMs through the upregulation of matrix-related glycoproteins such as osteopontin (OPN), osteoactivin, fibronectin, and SPARC.[142,154,155] Interestingly, OPN has been reported to colocalize with TANs in human glioblastoma samples, suggesting TANs as a probable source of OPN.[156] Moreover, OPN promotes neutrophil migration in vitro through its integrin binding RGD (Arg-Gly-Asp) motif.[156] Collagen deposition is another key component that contributes to ECM remodeling in TME to promote tumorigenesis and TAMs have been shown to contribute to the production of collagen I, VI and XIV in an orthotopic colorectal cancer model.[154] Interestingly, TRMs, but not BMDMs, express profibrotic genes in PDAC models.[155] Apart from directly producing collagen and related ECM proteins, TAMs also regulate CAFs to promote collagen production.[142,154] Similarly, TANs have been reported to exhibit an indirect stimulatory effect on obesity-associated desmoplasia in a murine model of PDAC where a cross-talk between TANs, cancer associated adipocytes and pancreatic stellate cells (PSC), partly through soluble factors like IL-1β, activates PSC and leads to pronounced collagen-I deposition.[157] Although the ability of TAMs and TANs to detect and translate mechanical changes in the ECM into biochemical signals to regulate tumor growth has been proposed and investigated, more targeted studies are required to understand the specific contribution and the extent of physical modification of the TME by innate immune cells.

8 | CONCLUDING REMARKS

The presence of neutrophils has been acknowledged in the TME of a wide array of cancers. Yet, the mechanisms that regulate the recruitment of neutrophils to primary or distal tumors and the role of TANs in survival outcome remain unknown. Emerging findings point toward the importance of chemokine receptor–ligand networks in the trafficking of neutrophils to the tumor niche. Especially, CXCR2, and its multiple CXCL ligands, have gained much attention in mediating TAN recruitment in both primary and secondary tumor sites. However, our knowledge of the chemokine signaling pathways behind TAN recruitment is modest and a number of questions remain: (i) which chemokine ligands provide molecular guidance and at what stages of malignancy?; (ii) how do neutrophil subsets maneuver their way to the tumor in the presence of competing gradients of multiple ligands within the TME?; (iii) do chemokines undergo structural modification given the presence of MMPs and other neutrophil-derived proteases in the TME and how that would potentiate/dampen their activity to recruit additional neutrophils?; (iv) which cues dictate the spatial distribution of TANs in the tumor niche (intra- or peri- or stromal TAN)?; (v) are intercellular signals involved in recruiting neutrophils, much like what is observed during sterile injury or infection? Cross-species differences in chemokine

receptor or ligand should be carefully considered while extrapolating in vivo data to human in the pursuit of these endeavors. Furthermore, TAMs can hinder or facilitate TAN trafficking to tumors. More studies on the different subsets of TAM/TAN that populate tumor sites are needed. How do TAM/TAN impact each other's recruitment in a spatiotemporal fashion and complement or compensate their roles in tumor progression and metastasis? Finally, tumor-associated hypoxic conditions and cross-talk between ECM and TAM/TAN, give rise to soluble mediators that may influence trafficking and the functional fine tuning of both myeloid cell types. Given the constantly evolving nature of the TME and the dynamics of TME–TAN interactions, caution must be taken while targeting isolated mediators to manipulate TAN accumulation in tumors. In the development of better therapeutic intervention against cancer, future studies should analyze both cellular and molecular circuits in individual tumors that dictate TANs recruitment and behavior by using a combination of in vitro models of 3D tumor spheroids embedded in ECM matrices with in vivo tumor models with advanced imaging techniques.

AUTHORSHIP

S.S., B.C.S., and C.A.P. wrote and edited the manuscript.

ACKNOWLEDGMENTS

The authors wish to thank members of the Parent laboratory for useful discussions. This work was funded by the Intramural Research Program, National Cancer Institute, National Institutes of Health to B.C.S. and by internal funding from the University of Michigan to C.A.P.

DISCLOSURES

The authors declare no conflicts of interest.

ORCID

Bhagawat C. Subramanian http://orcid.org/0000-0002-6976-7512
Carole A. Parent http://orcid.org/0000-0002-1518-1452

REFERENCES

1. Bussard KM, Mutkus L, Stumpf K, Gomez-Manzano C, Marini FC. Tumor-associated stromal cells as key contributors to the tumor microenvironment. *Breast Cancer Res*. 2016;18:84.
2. Engblom C, Pfirschke C, Pittet MJ. The role of myeloid cells in cancer therapies. *Nat Rev Cancer*. 2016;16:447–462.
3. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. *J Hematol Oncol*. 2017;10:58.
4. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*. 2013;13:159–175.
5. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. *Nature*. 2015;528:413–417.
6. Park J, Wysocki RW, Amoozgar Z, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. *Sci Transl Med*. 2016;8:361ra138.
7. Singhal S, Bhojnagarwala PS, O'Brien S, et al. Origin and role of a subset of tumor-associated neutrophils with antigen-presenting cell features in early-stage human lung cancer. *Cancer Cell*. 2016;30:120–135.
8. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci U S A*. 2006;103:12493–12498.
9. Zhou S-L, Zhou Z-J, Hu Z-Q, et al. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. *Gastroenterology*. 2016;150:1646–1658. e17.
10. Berry RS, Xiong M-J, Greenbaum A, et al. High levels of tumor-associated neutrophils are associated with improved overall survival in patients with stage II colorectal cancer. *PLoS One*. 2017;12:e0188799.
11. Fainsod-Levi T, Gershkovitz M, Völs S, et al. Hyperglycemia impairs neutrophil mobilization leading to enhanced metastatic seeding. *Cell Rep*. 2017;21:2384–2392.
12. Tabariès S, Ouellet V, Hsu BE, et al. Granulocytic immune infiltrates are essential for the efficient formation of breast cancer liver metastases. *Breast Cancer Res*. 2015;17:45.
13. Wu QD, Wang JH, Condron C, Bouchier-Hayes D, Redmond HP. Human neutrophils facilitate tumor cell transendothelial migration. *Am J Physiol Cell Physiol*. 2001;280:C814–C822.
14. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell*. 2011;20:300–314.
15. Faget J, Groeneveld S, Boivin G, et al. Neutrophils and snail orchestrate the establishment of a pro-tumor microenvironment in lung cancer. *Cell Rep*. 2017;21:3190–3204.
16. Glodde N, Bald T, van den Boorn-Konijnenberg D, et al. Reactive neutrophil responses dependent on the receptor tyrosine kinase c-MET limit cancer immunotherapy. *Immunity*. 2017;47:789–802. e9.
17. Steele CW, Karim SA, Leach JDG, et al. CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. *Cancer Cell*. 2016;29:832–845.
18. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell*. 2009;16:183–194.
19. Andzinski L, Kasnitz N, Stahnke S, et al. Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. *Int J Cancer*. 2016;138:1982–1993.
20. Shaul ME, Levy L, Sun J, et al. Tumor-associated neutrophils display a distinct N1 profile following TGFbeta modulation: a transcriptomics analysis of pro- vs. antitumor TANs. *Oncoimmunology*. 2016;5:e1232221.
21. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol*. 2012;12:253–268.
22. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer*. 2016;16:431–446.
23. Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol*. 2010;22:231–237.
24. Galdiero MR, Varricchi G, Loffredo S, Mantovani A, Marone G. Roles of neutrophils in cancer growth and progression. *J Leukoc Biol*. 2018;103:457–464.
25. Satpathy SR, Jala VR, Bodduluri SR, et al. Crystalline silica-induced leukotriene B4-dependent inflammation promotes lung tumour growth. *Nat Commun*. 2015;6:7064.
26. Jablonska J, Wu C-F, Andzinski L, Leschner S, Weiss S. CXCR2-mediated tumor-associated neutrophil recruitment is regulated by IFN-beta. *Int J Cancer*. 2014;134:1346–1358.

27. Raccosta L, Fontana R, Maggioni D, et al. The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils. *J Exp Med*. 2013;210:1711–1728.

28. Patsialou A, Bravo-Cordero JJ, Wang Y, et al. Intravital multiphoton imaging reveals multicellular streaming as a crucial component of in vivo cell migration in human breast tumors. *Intravital*. 2013;2:e25294.

29. Harney AS, Arwert EN, Entenberg D, et al. Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by TIE2hi macrophage-derived VEGFA. *Cancer Discov*. 2015;5:932–943.

30. Arwert EN, Harney AS, Entenberg D, et al. A unidirectional transition from migratory to perivascular macrophage is required for tumor cell intravasation. *Cell Rep*. 2018;23:1239–1248.

31. Donati K, Sépult C, Rocks N, et al. Neutrophil-derived interleukin 16 in premetastatic lungs promotes breast tumor cell seeding. *Cancer Growth Metastasis*. 2017;10:1179064417738513.

32. Cools-Lartigue J, Spicer J, McDonald B, et al. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. *J Clin Invest*. 2013.

33. Chen MB, Hajal C, Benjamin DC, et al. Inflamed neutrophils sequestered at entrapped tumor cells via chemotactic confinement promote tumor cell extravasation. *Proc Natl Acad Sci U S A*. 2018;115:7022–7027.

34. Jensen HK, Donskov F, Marcussen N, Nordsmark M, Lundbeck F, von der Maase H. Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. *J Clin Oncol*. 2009;27:4709–4717.

35. Ilie M, Hofman V, Ortholan C, et al. Predictive clinical outcome of the intratumoral CD66b-positive neutrophil-to-CD8-positive T-cell ratio in patients with resectable nonsmall cell lung cancer. *Cancer*. 2012;118:1726–1737.

36. Soto-Perez-de-Celis E, Chavarri-Guerra Y, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor-associated neutrophils in breast cancer subtypes. *Asian Pac J Cancer Prev*. 2017;18:2689–2694.

37. Zhao J-J, Pan K, Wang W, et al. The prognostic value of tumor-infiltrating neutrophils in gastric adenocarcinoma after resection. *PLoS One*. 2012;7:e33655.

38. Millrud CR, Kågedal Å, Kumlien Georén S, et al. NET-producing CD16(high) CD62L(dim) neutrophils migrate to tumor sites and predict improved survival in patients with HNSCC. *Int J Cancer*. 2017;140:2557–2567.

39. Jablonska J, Lang S, Sionov RV, Granot Z. The regulation of pre-metastatic niche formation by neutrophils. *Oncotarget*. 2017;8:112132–112144.

40. Yu PF, Huang Y, Han YY, et al. TNFalpha-activated mesenchymal stromal cells promote breast cancer metastasis by recruiting CXCR2(+) neutrophils. *Oncogene*. 2017;36:482–490.

41. Zhou S-L, Dai Z, Zhou Z-J, et al. CXCL5 contributes to tumor metastasis and recurrence of intrahepatic cholangiocarcinoma by recruiting infiltrative intratumoral neutrophils. *Carcinogenesis*. 2014;35:597–605.

42. Gijsbers K, Gouwy M, Struyf S, et al. GCP-2/CXCL6 synergizes with other endothelial cell-derived chemokines in neutrophil mobilization and is associated with angiogenesis in gastrointestinal tumors. *Exp Cell Res*. 2005;303:331–342.

43. Lee L-F, Hellendall RP, Wang Y, et al. IL-8 reduced tumorigenicity of human ovarian cancer in vivo due to neutrophil infiltration. *J Immunol*. 2000;164:2769–2775.

44. Dancey JT, Deubelbeiss KA, Harker LA, Finch CA. Neutrophil kinetics in man. *J Clin Invest*. 1976;58:705–715.

45. Manz MG, Boettcher S. Emergency granulopoiesis. *Nat Rev Immunol*. 2014;14:302–314.

46. Rankin SM. The bone marrow: a site of neutrophil clearance. *J Leukoc Biol*. 2010;88:241–251.

47. Bratton DL, Henson PM. Neutrophil clearance: when the party is over, clean-up begins. *Trends Immunol*. 2011;32:350–357.

48. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood*. 1992;80:2012–2020.

49. Eruslanov EB, Bhojnagarwala PS, Quatromoni JG, et al. Tumor-associated neutrophils stimulate T cell responses in early-stage human lung cancer. *J Clin Invest*. 2014;124:5466–5480.

50. de Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: going forward in reverse. *Nat Rev Immunol*. 2016;16:378–391.

51. Wang J, Hossain M, Thanabalasuriar A, Gunzer M, Meininger C, Kubes P. Visualizing the function and fate of neutrophils in sterile injury and repair. *Science*. 2017;358:111–116.

52. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst*. 2014;106:dju124.

53. Shinko D, Diakos CI, Clarke SJ, Charles KA. Cancer-related systemic inflammation: the challenges and therapeutic opportunities for personalized medicine. *Clin Pharmacol Ther*. 2017;102:599–610.

54. Sagiv JY, Michaeli J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Rep*. 2015;10:562–573.

55. Brandau S, Trellakis S, Bruderek K, et al. Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. *J Leukoc Biol*. 2011;89:311–317.

56. Coffelt SB, Kersten K, Doornebal CW, et al. IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. *Nature*. 2015;522:345–348.

57. Marini O, Costa S, Bevilacqua D, et al. Mature CD10(+) and immature CD10(-) neutrophils present in G-CSF-treated donors display opposite effects on T cells. *Blood*. 2017;129:1343–1356.

58. Wikberg ML, Ling A, Li X, Öberg Å, Edin S., Palmqvist R. Neutrophil infiltration is a favorable prognostic factor in early stages of colon cancer. *Hum Pathol*. 2017;68:193–202.

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

60. Rakaee M, Busund L-T, Paulsen E-E, et al. Prognostic effect of intratumoral neutrophils across histological subtypes of non-small cell lung cancer. *Oncotarget*. 2016;7:72184–72196.

61. Zhou S-L, Dai Z, Zhou Z-J, et al. Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. *Hepatology*. 2012;56:2242–2254.

62. Li Y-W, Qiu S-J, Fan J, et al. Intratumoral neutrophils: a poor prognostic factor for hepatocellular carcinoma following resection. *J Hepatol*. 2011;54:497–505.

63. Chen C-L, Wang Y, Huang C-Y, et al. IL-17 induces antitumor immunity by promoting beneficial neutrophil recruitment and activation in esophageal squamous cell carcinoma. *Oncoimmunology*. 2017;7:e1373234.

64. Jensen TO, Schmidt H, Møller HJ, et al. Intratumoral neutrophils and plasmacytoid dendritic cells indicate poor prognosis and are associated with pSTAT3 expression in AJCC stage I/II melanoma. *Cancer*. 2012;118:2476–2485.
65. Wang W-Q, Liu L, Xu H-X, et al. Infiltrating immune cells and gene mutations in pancreatic ductal adenocarcinoma. *Br J Surg*. 2016;103:1189–1199.
66. Rao H-L, Chen J-W, Li M, et al. Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. *PLoS One*. 2012; 7:e30806.
67. Donskov F, von der Maase H. Impact of immune parameters on long-term survival in metastatic renal cell carcinoma. *J Clin Oncol*. 2006;24:1997–2005.
68. Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: a systematic review and meta-analysis. *PLoS One*. 2014;9:e98259.
69. Blaisdell A, Crequer A, Columbus D, et al. Neutrophils oppose uterine epithelial carcinogenesis via debridement of hypoxic tumor cells. *Cancer Cell*. 2015;28:785–799.
70. Kuang DM, Zhao Q, Wu Y, et al. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J Hepatol*. 2011;54:948–955.
71. Carus A, Ladekarl M, Hager H, Nedergaard BS, Donskov F. Tumour-associated CD66b+ neutrophil count is an independent prognostic factor for recurrence in localised cervical cancer. *Br J Cancer*. 2013;108:2116–2122.
72. Guerriero JL. Macrophages: the road less traveled, changing anticancer therapy. *Trends Mol Med*. 2018;24:472–489.
73. Casbon A-J, Reynaud D, Park C, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci U S A*. 2015;112:E566–E575.
74. Pillay J, Tak T, Kamp VM, Koenderman L. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. *Cell Mol Life Sci*. 2013;70:3813–3827.
75. Youn J-I, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor bearing mice. *J Immunol*. 2008;181:5791–5802.
76. Youn J-I, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *J Leukoc Biol*. 2012;91: 167–181.
77. Fridlender ZG, Sun J, Mishalian I, et al. Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. *PLoS One*. 2012;7:e31524.
78. Ban Y, Mai J, Li X, et al. Targeting autocrine CCL5-CCR5 axis reprograms immunosuppressive myeloid cells and reinvigorates antitumor immunity. *Cancer Res*. 2017;77:2857–2868.
79. Moses K, Brandau S. Human neutrophils: their role in cancer and relation to myeloid-derived suppressor cells. *Semin Immunol*. 2016;28:187–196.
80. Choi J, Suh B, Ahn Y-O, et al. CD15+/CD16low human granulocytes from terminal cancer patients: granulocytic myeloid-derived suppressor cells that have suppressive function. *Tumour Biol*. 2012;33:121–129.
81. Condamine T, Dominguez GA, Youn J-I, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol*. 2016;1.
82. Lin A, Liang F, Thompson EA, et al. Rhesus macaque myeloid-derived suppressor cells demonstrate T cell inhibitory functions and are transiently increased after vaccination. *J Immunol*. 2018;200:286–294.
83. Suarez-Carmona M, Lesage J, Cataldo D, Gilles C. EMT and inflammation: inseparable actors of cancer progression. *Mol Oncol*. 2017;11:805–823.
84. Vanheule V, Metzemaekers M, Janssens R, Struyf S, Proost P. How post-translational modifications influence the biological activity of chemokines. *Cytokine*. 2018;109:29–51.
85. Subramanian BC, Majumdar R, Parent CA. The role of the LTB4-BLT1 axis in chemotactic gradient sensing and directed leukocyte migration. *Semin Immunol*. 2017;33:16–29.
86. Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M. ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. *Pharmacol Ther*. 2006;112:139–149.
87. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol*. 2017;17:559–572.
88. Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *J Clin Invest*. 2010;120:1151–1164.
89. Satpathy SR, Jala VR, Bodduluri SR, et al. Crystalline silica-induced leukotrieneB(4)-dependent inflammation promotes lung tumor growth. *Nat Commun*. 2015;6:7064–7064.
90. Khandaker MH, Xu L, Rahimpour R, et al. CXCR1 and CXCR2 are rapidly down-modulated by bacterial endotoxin through a unique agonist-independent, tyrosine kinase-dependent mechanism. *J Immunol*. 1998;161:1930–1938.
91. Gaudreault E, Thompson C, Stankova J, Rola-Pleszczynski M. Involvement of BLT1 endocytosis and Yes kinase activation in leukotriene B4-induced neutrophil degranulation. *J Immunol*. 2005;174: 3617–3625.
92. Hartl D, Krauss-Etschmann S, Koller B, et al. Infiltrated neutrophils acquire novel chemokine receptor expression and chemokine responsiveness in chronic inflammatory lung diseases. *J Immunol*. 2008;181:8053–8067.
93. Song J, Wu C, Zhang X, Sorokin LM. In vivo processing of CXCL5 (LIX) by matrix metalloproteinase (MMP)-2 and MMP-9 promotes early neutrophil recruitment in IL-1beta-induced peritonitis. *J Immunol*. 2013;190:401–410.
94. Molon B, Ugel S, Del Pozzo F, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med*. 2011;208:1949–1962.
95. Joseph PRB, Sawant KV, Rajarathnam K. Heparin-bound chemokine CXCL8 monomer and dimer are impaired for CXCR1 and CXCR2 activation: implications for gradients and neutrophil trafficking. *Open Biol*. 2017;7.
96. Sawant KV, Xu R, Cox R, et al. Chemokine CXCL1-mediated neutrophil trafficking in the lung: role of CXCR2 activation. *J Innate Immun*. 2015;7:647–658.
97. Das ST, Rajagopalan L, Guerrero-Plata A, et al. Monomeric and dimeric CXCL8 are both essential for in vivo neutrophil recruitment. *PLoS One*. 2010;5:e11754.
98. Gong L, Cumpian AM, Caetano MS, et al. Promoting effect of neutrophils on lung tumorigenesis is mediated by CXCR2 and neutrophil elastase. *Mol Cancer*. 2013;12:154.
99. Chao T, Furth EE, Vonderheide RH. CXCR2-dependent accumulation of tumor-associated neutrophils regulates T-cell immunity in pancreatic ductal adenocarcinoma. *Cancer Immunol Res*. 2016;4:968–982.

100. Nywening TM, Belt BA, Cullinan DR, et al. Targeting both tumour-associated CXCR2(+) neutrophils and CCR2(+) macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma. *Gut*. 2017;67:1112–1123.

101. Zarbock A, Stadtmann A. CXCR2: from bench to bedside. *Front Immunol*. 2012;3.

102. Szekanecz Z, Kim J, Koch AE. Chemokines and chemokine receptors in rheumatoid arthritis. *Semin Immunol*. 2003;15:15–21.

103. Veenstra M, Ransohoff RM. Chemokine receptor CXCR2: physiology regulator and neuroinflammation controller?. *J Neuroimmunol*. 2012;246:1–9.

104. Mei J, Liu Y, Dai N, et al. CXCL5 regulates chemokine scavenging and pulmonary host defense to bacterial infection. *Immunity*. 2010;33:106–117.

105. Ritzman AM, Hughes-Hanks JM, Blaho VA, Wax LE, Mitchell WJ, Brown CR. The chemokine receptor CXCR2 ligand KC (CXCL1) mediates neutrophil recruitment and is critical for development of experimental Lyme arthritis and carditis. *Infect Immun*. 2010;78:4593–4600.

106. Nouailles G, Dorhoi A, Koch M, et al. CXCL5-secreting pulmonary epithelial cells drive destructive neutrophilic inflammation in tuberculosis. *J Clin Invest*. 2014;124:1268–1282.

107. Xie K. Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev*. 2001;12:375–391.

108. Wu P, Wu D, Ni C, et al. gammadeltaT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity*. 2014;40:785–800.

109. Asfaha S, Dubeykovskiy AN, Tomita H, et al. Mice that express human interleukin-8 have increased mobilization of immature myeloid cells, which exacerbates inflammation and accelerates colon carcinogenesis. *Gastroenterology*. 2013;144:155–166.

110. Fan X, Patera AC, Pong-Kennedy A, et al. Murine CXCR1 is a functional receptor for GCP-2/CXCL6 and interleukin-8/CXCL8. *J Biol Chem*. 2007;282:11658–11666.

111. Singer M, Sansonetti PJ. IL-8 is a key chemokine regulating neutrophil recruitment in a new mouse model of Shigella-induced colitis. *J Immunol*. 2004;173:4197–4206.

112. Alfaro C, Teijeira A, Oñate C, et al. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). *Clin Cancer Res*. 2016;22:3924–3936.

113. Carpagnano GE, Palladino GP, Lacedonia D, Koutelou A, Orlando S, Foschino-Barbaro MP. Neutrophilic airways inflammation in lung cancer: the role of exhaled LTB-4 and IL-8. *BMC Cancer*. 2011;11:226–226.

114. Miyabe Y, Miyabe C, Luster AD. LTB4 and BLT1 in inflammatory arthritis. *Semin Immunol*. 2017;33:52–57.

115. Lämmermann T, Afonso PV, Angermann BR, et al. Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature*. 2013;498:371–375.

116. Afonso PV, Janka-Junttila M, Lee YJ, et al. LTB4 is a signal-relay molecule during neutrophil chemotaxis. *Dev Cell*. 2012;22:1079–1091.

117. Majumdar R, Tavakoli Tameh A, Parent CA. Exosomes mediate LTB(4) release during neutrophil chemotaxis. *PLoS Biol*. 2016;14:e1002336.

118. Dreyling KW, Hoppe U, Peskar BA, Morgenroth K, Kozuschek W, Peskar BM. Leukotriene synthesis by human gastrointestinal tissues. *Biochim Biophys Acta*. 1986;878:184–193.

119. Öhd JF, Nielsen CK, Campbell J, Landberg G, Löfberg H, Sjölander A. Expression of the leukotriene D4 receptor CysLT1, COX-2, and other cell survival factors in colorectal adenocarcinomas. *Gastroenterology*. 2003;124:57–70.

120. López-Lago MA, Posner S, Thodima VJ, Molina AM, Motzer RJ, Chaganti RSK. Neutrophil chemokines secreted by tumor cells mount a lung antimetastatic response during renal cell carcinoma progression. *Oncogene*. 2013;32:1752–1760.

121. Liu Y, Gu Y, Han Y, et al. Tumor exosomal RNAs promote lung pre-metastatic niche formation by activating alveolar epithelial TLR3 to recruit neutrophils. *Cancer Cell*. 2016;30:243–256.

122. Kowanetz M, Wu X, Lee J, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proc Natl Acad Sci U S A*. 2010;107:21248–21255.

123. Seubert B, Grünwald B, Kobuch J, et al. Tissue inhibitor of metalloproteinases (TIMP)-1 creates a premetastatic niche in the liver through SDF-1/CXCR4-dependent neutrophil recruitment in mice. *Hepatology*. 2015;61:238–248.

124. Hand F, Harmon C, Elliott LA, et al. Depleted polymorphonuclear leukocytes in human metastatic liver reflect an altered immune microenvironment associated with recurrent metastasis. *Cancer Immunol Immunother*. 2018;67:1041–1052.

125. Kiss M, Van Gassen S, Movahedi K, Saeys Y, Laoui D. Myeloid cell heterogeneity in cancer: not a single cell alike. *Cell Immunol*. 2018;330:188–201.

126. Cortez-Retamozo V, Etzrodt M, Newton A, et al. Origins of tumor-associated macrophages and neutrophils. *Proc Natl Acad Sci U S A*. 2012;109:2491–2496.

127. Shand FHW, Ueha S, Otsuji M, et al. Tracking of intertissue migration reveals the origins of tumor-infiltrating monocytes. *Proc Natl Acad Sci U S A*. 2014;111:7771–7776.

128. Murdoch C. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood*. 2004;104:2224–2234.

129. Qian B-Z, Li J, Zhang H, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature*. 2011;475:222–225.

130. Laubli H, Spanaus K-S, Borsig L. Selectin-mediated activation of endothelial cells induces expression of CCL5 and promotes metastasis through recruitment of monocytes. *Blood*. 2009;114:4583–4591.

131. Hanna RN, Cekic C, Sag D, et al. Patrolling monocytes control tumor metastasis to the lung. *Science*. 2015;350:985–990.

132. Kumar V, Donthireddy L, Marvel D, et al. Cancer-associated fibroblasts neutralize the anti-tumor effect of CSF1 receptor blockade by inducing PMN-MDSC infiltration of tumors. *Cancer Cell*. 2017;32:654–668. e5.

133. Pahler JC, Tazzyman S, Erez N, et al. Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia*. 2008;10:329–340.

134. Jung K, Heishi T, Khan OF, et al. Ly6Clo monocytes drive immunosuppression and confer resistance to anti-VEGFR2 cancer therapy. *J Clin Invest*. 2017;127:3039–3051.

135. Nywening TM, Belt BA, Cullinan DR, et al. Targeting both tumour-associated CXCR2(+) neutrophils and CCR2(+) macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma. *Gut*. 2018;67:1112–1123.

136. Eales KL, Hollinshead KER, Tennant DA. Hypoxia and metabolic adaptation of cancer cells. *Oncogenesis*. 2016;5:e190.

137. Laoui D, Van Overmeire E, Di Conza G, et al. Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population. *Cancer Res*. 2014;74:24–30.

138. Casazza A, Laoui D, Wenes M, et al. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. *Cancer Cell*. 2013;24:695–709.

139. Triner D, Xue X, Schwartz AJ, Jung I, Colacino JA, Shah YM. Epithelial hypoxia-inducible factor 2α facilitates the progression of colon tumors through recruiting neutrophils. *Mol Cell Biol*. 2017;37:e00481–e00516.

140. Massena S, Christoffersson G, Vagesjo E, et al. Identification and characterization of VEGF-A-responsive neutrophils expressing CD49d, VEGFR1, and CXCR4 in mice and humans. *Blood*. 2015;126:2016–2026.

141. Lodge KM, Thompson AAR, Chilvers ER, Condliffe AM. Hypoxic regulation of neutrophil function and consequences for Staphylococcus aureus infection. *Microbes Infect*. 2017;19:166–176.

142. Varol C, Sagi I. Phagocyte-extracellular matrix crosstalk empowers tumor development and dissemination. *FEBS J*. 2018;285:734–751.

143. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell*. 2000;103:481–490.

144. Bekes EM, Schweighofer B, Kupriyanova TA, et al. Tumor-recruited neutrophils and neutrophil TIMP-Free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. *AmJ Pathol*. 2011;179:1455–1470.

145. Deryugina EI, Zajac E, Juncker-Jensen A, Kupriyanova TA, Welter L, Quigley JP. Tissue-infiltrating neutrophils constitute the major in vivo source of angiogenesis-inducing MMP-9 in the tumor microenvironment. *Neoplasia*. 2014;16:771–788.

146. Ye X-Z, Xu S-L, Xin Y-H, et al. Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-beta1 signaling pathway. *J Immunol*. 2012;189:444–453.

147. Houghton AM, Grisolano JL, Baumann ML, et al. Macrophage elastase (matrix metalloproteinase-12) suppresses growth of lung metastases. *Cancer Res*. 2006;66:6149–6155.

148. Gutierrez-Fernandez A, Fueyo A, Folgueras AR, et al. Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. *Cancer Res*. 2008;68:2755–2763.

149. Bohrer LR, Chaffee TS, Chuntova P, et al. ADAM17 in tumor associated leukocytes regulates inflammatory mediators and promotes mammary tumor formation. *Genes Cancer*. 2016;7:240–253.

150. Gocheva V, Wang H-W, Gadea BB, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev*. 2010;24:241–255.

151. Afonso PV, McCann CP, Kapnick SM, Parent CA. Discoidin domain receptor 2 regulates neutrophil chemotaxis in 3D collagen matrices. *Blood*. 2013;121:1644–1650.

152. Du R, Lu KV, Petritsch C, et al. HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell*. 2008;13:206–220.

153. Madsen DH, Jürgensen HJ, Siersbæk MS, et al. Tumor-associated macrophages derived from circulating inflammatory monocytes degrade collagen through cellular uptake. *Cell Rep*. 2017;21:3662–3671.

154. Afik R, Zigmond E, Vugman M, et al. Tumor macrophages are pivotal constructors of tumor collagenous matrix. *J Exp Med*. 2016;213:2315–2331.

155. Zhu Y, Herndon JM, Sojka DK, et al. Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity*. 2017;47:323–338. e6.

156. Atai NA, Bansal M, Lo C, et al. Osteopontin is up-regulated and associated with neutrophil and macrophage infiltration in glioblastoma. *Immunology*. 2011;132:39–48.

157. Incio J, Liu H, Suboj P, et al. Obesity-induced inflammation and desmoplasia promote pancreatic cancer progression and resistance to chemotherapy. *Cancer Discov*. 2016;6:852–869.

How to cite this article: SenGupta S, Subramanian BC, Parent CA. Getting TANned: How the tumor microenvironment drives neutrophil recruitment. *J Leukoc Biol*. 2019;105:449–462. https://doi.org/10.1002/JLB.3RI0718-282R