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REVIEW

Neutrophils in cancer: from immune defense to tumor promotion

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ABSTRACT Neutrophils are the protagonists of the host immune response, possessing potent antimicrobial and inflammatory capacities. The neutrophil reservoir as well as the development, mobilization, chemotaxis, pro-inflammatory activity, and clearance of neutrophils are strictly regulated to prevent inflammation-induced tissue damage. Inflammation pervades almost every type of cancer. However, there is growing awareness that although the tumor microenvironment has the capacity to recruit neutrophils, the functions are diverse and include roles other than that of sentinels in cancer. This review highlights the heterogeneity of neutrophils in tumors, discusses the dual role of neutrophils as angels and demons in tumorigenesis, invasion, and metastasis, and examines the potential of neutrophils as targets in clinical therapy.

KEYWORDS Neutrophil; cancer; inflammation; tumor microenvironment; metastasis; diversity

Introduction

As the primary sentinels of innate immunity in the body, approximately 10^{11} mature neutrophils are generated daily in the bone marrow (BM) under normal physiologic conditions. Neutrophils are reserved in the BM as well as in peripheral pools in various organs and comprise the largest leukocyte population, which allow neutrophils to participate in construction of the corresponding tissue immune microenvironment. Members of the colony-stimulating factor (CSF) family mobilize myeloid cells in response to infection or other stresses to drive accelerated neutrophil maturation and subsequent BM egress. Circulating blood neutrophils respond to local chemokines and swarm to corresponding sites, where granulocytes recognize and phagocytose microbes. Tissue function is then restored after the inflammation subsides. Neutrophil disorders exacerbate tissue damage and can be life-threatening.

The ability of tumors to escape innate immune surveillance is attributed to the capacity of tumor cells or other cells in the tumor microenvironment (TME) to transform immune cells. Existing research shows a heterogeneous array of neutrophils in tumors. A distinct subset of granulocytes act as executioners and are capable of directly killing tumor cells. Additionally, these cells can also mediate antibody-dependent cell cytotoxicity towards tumor cells and secrete cytokines to recruit cytotoxic T cells. Another neutrophil subset functions as an accomplice, creating an environment that promotes the survival and proliferation of tumor cells. Furthermore, these cells establish a pre-metastatic niche favourable for tumor cell growth in distant organs, thereby promoting metastasis.

The functions of neutrophils in inflammation and tumors have been gradually recognized with the advances in omics technologies and improvement in detection resolution. Recent reviews have focused on the roles of neutrophils in specific diseases, such as cardiometabolic disorders and lung ischemia-reperfusion injury, as well as the mechanisms underlying specific neutrophil activities, including activation

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of the tumor cGAS-STING pathway[1] and neutrophil extracellular traps (NETs) signaling transduction[2]. Additionally, studies have explored neutrophil-based engineering and drug delivery. These investigations have provided precise insights into the specific fates of neutrophils. This review comprehensively examines neutrophil behaviors across a temporal and functional spectrum, encompassing the roles of neutrophils in homeostasis and inflammatory responses in tumor biology. The classification of distinct neutrophil subsets was analysed with a focus on the TME and the dual roles of tumor-associated neutrophils (TANs). Furthermore, the contributions of neutrophils to cancer initiation, progression, and metastasis were systematically explored, while proposing potential therapeutic strategies targeting neutrophils with diverse functions and phenotypes.

Neutrophil homeostasis

Development of neutrophils

Neutrophils are short-lived cells that have a significant function in the innate immune response because neutrophils are required to effectively protect the host against invasion by multifarious pathogenic microorganisms. Neutrophils are predominantly BM residents, where neutrophils undergo proliferation and differentiation and are transiently stored for less than one week. Neutrophils originate from myeloid progenitors through six stages according to cell size, nuclear morphology, and granule content, as follows: myeloblasts; promyelocytes; myelocytes; metamyelocytes; band cells; and mature polymorphonuclear leukocytes (PMNs). The development of myeloblasts into mature neutrophils requires approximately 2 weeks. Mature neutrophils can leave the BM and are characterized by a multilobulated nucleus and a cytoplasm containing primarily glycogen and granules. Approximately 100 billion neutrophils are produced daily under normal conditions with < 2% in the circulation. This dynamic equilibrium is tightly regulated by the interplay of neutrophil production, BM egress, and systemic clearance. As terminally differentiated cell, neutrophils have the shortest survival of any circulating cell with half-lives of 8–16 h under physiologic conditions. Pillay et al.[3] reported an unprecedented 5.4-d lifespan for human circulatory neutrophils but other authors have expressed scepticism about this observation[4]. Specifically, Lahoz-Beneytez et al.[5] reported new stable isotope labelling data showing the half-life of a blood neutrophil is < 1 d. Nevertheless, tissue neutrophils are thought to survive longer than circulatory PMNs.

Clearance of neutrophils

Neutrophil programmed cell death is critical for precise regulation of the neutrophil lifespan. Macrophages clear dead and dying cells in tissues by recognizing "find me, catch me, eat me" signals on apoptotic neutrophils[6]. In addition, ageing circulating neutrophils that do not migrate into tissues return to the BM, where ageing circulating neutrophils are eliminated by bone marrow-resident macrophages. The number of neutrophils in the blood exhibits circadian rhythm changes, which may be associated with the timed release of CXCL12. Interestingly, before neutrophils are eliminated, neutrophils exhibit specific molecular changes, such as a $CD62L^{low}CXCR4^{high}$ phenotype and morphologic alterations, including nuclear hyper-segmentation, features that are collectively defined as "ageing"[7]. Such cells rhythmically oscillate during light-dark cycles under normal conditions, whereas dysregulation of ageing neutrophil clearance results in disease progression.

Reservior of neutrophils

The BM is the largest reservoir of neutrophils *in vivo* with PMNs remaining there for 5–6 d after the last granulocyte precursor division. The CXCR4 axis functions as the major protector of the BM mature neutrophil reserve. Mauer et al.[8] reinfused ^{32}P-labelled autologous neutrophils into the circulation approximately 50 years ago; greater than one-half of the neutrophils disappeared within seconds, indicating the existence of a separate pool of granulocytes. It was then determined that under normal conditions peripheral organs, including the liver, spleen, and especially the lung (the lung has the largest neutrophil pool), act as a granulocyte reserve[9]. Moreover, neutrophils are retained much longer in the lung than in other organs due to the anatomy of the pulmonary microcirculation, i.e., many lung capillaries have a smaller diameter than a neutrophil. Moreover, a study conducted by Andrea demonstrated that there exists a distinct pool of monocytes and neutrophils in the meninges, which are supplied by the adjacent skull and vertebral BM and exhibit distinct transcriptional signatures compared to myeloid cells derived from the blood[10]. Nevertheless, the neutrophil reservoir can be mobilized into the circulation and rapidly

delivered to relevant locations in response to infection or other stressors.

Mobilization of neutrophils

Colony-stimulating factors (CSFs)

Granulocyte-CSF (G-CSF), granulocyte-macrophage-CSF (GM-CSF), and multi-CSF (IL-3) are powerful growth factors that promote proliferation and differentiation of myeloid progenitors *in vitro* and *in vivo*. Each CSF has its own unique effects although the roles of CSFs in the hematopoietic system overlap. The role of G-CSF, as the irreplaceable chemokine for terminal differentiation of myeloid cells to mature neutrophilic granulocytes in the BM, as the irreplaceable chemokine for terminal differentiation of myeloid cells to mature neutrophilic granulocytes, is selective. Specifically, the G-CSF increases the number of neutrophils, whereas other blood lineages remain largely unaffected. The primary role of G-CSF is to support survival of myeloid progenitors in the basic state but G-CSF is unnecessary for neutrophil trafficking. GM-CSF has pleiotropic effects on hematopoietic cells, which substantially delays neutrophil programmed apoptosis to prolong cell survival. In an emerging role of supporting the proliferation of a broad range of hematopoietic cell types, IL-3 promotes earliest progenitor growth by shortening the G0 period. IL-3 also mediates the differentiation of BM cells into monocytes and neutrophils *in vitro*[11]. Overall, these cytokines orchestrate the mobilization of mature neutrophils from the BM to peripheral tissues.

CXCL/CXCR axis

Neutrophils express several chemokine receptors on the plasma membranes, among which CXCR2 is primarily responsible for neutrophil trafficking. CXCR2 is activated by CXC chemokines, including CXCL1, CXCL2/3, CXCL5, CXCL7, and human CXCL8 (IL-8). Polymorphonuclear (PMN) cells are mobilized to stress sites with high concentrations of chemokines *via* the CXCR2 ligand-CXCR2 axis. In contrast, neutrophils lacking CXCR2 are preferentially retained in the BM. Recently, an unexpected negative feedback effect of G-CSF on the CXCR2 axis that prevents excessive accumulation was identified[12]. While CXCR2 signaling positively regulates the circulating neutrophil count, the CXCL12/CXCR4 axis counteracts this effect by favoring BM retention. CXCR4 gradually decreases when hematopoietic stem cells and progenitor cells (HSPCs) develop during neutrophil maturation into mature neutrophils and exit the BM into the circulation. However, the CXCR4 level is upregulated, which allows for neutrophil return to the BM. CXCL12, a CXCR4 agonist, rapidly increases the blood neutrophil frequency, which is commonly used to correct neutropenia in humans. One proposed mechanism by which G-CSF mobilizes BM neutrophils is blockade of the CXCL12/CXCR4 axis. However, it has been suggested that neutrophilia induced by CXCR4 inhibition is independent of BM mobilization and instead occurs *via* release from the marginated pool in the lungs. In general, multiple mechanisms act synergistically to maintain neutrophil mobilization homeostasis.

Neutrophil inflammatory response

Neutrophil arrival

As sentinel cells, neutrophils express > 30 chemoattractant receptors to identify tissue damage signals and respond quickly. Circulating PMN cells are sensitive to chemically diverse chemoattractants, including chemokines, lipids, complement anaphylatoxins (C3a and C5a) and formyl peptides[13]. Notably, the chemokine released during inflammation and infection always exhibits heterogeneity and overlapping functions in guiding neutrophil migration. PMN cells, when induced by chemokines, roll along vessel walls and enter sites of inflammation and infected tissue through corresponding endothelial cells *via* selectins and adhesion molecules on the neutrophil membrane. Multistep cascade effects lead to rapid recruitment of neutrophil swarms to inflamed tissues. The first few neutrophils to arrive at the site of inflammation act as commandos, acutely amplifying local cell death signals to drive recruitment of additional neutrophils. Subsequent swarming neutrophils rush to the site of inflammation, increasing the radius of the battlefield. Interestingly, several notable studies have revealed that extravascular neutrophils can return from the tissue beds into the vascular lumen, a process termed reverse transendothelial migration (rTEM)[14]. It has been suggested that neutrophils quiet a local inflammatory response through rTEM. However, the paradigm in which rTEM can turn a local inflammatory phenomenon into a systemic response has received greater support from scholars[15].

Neutrophil attack

Neutrophils are the protagonists of the host immune response and possess potent antimicrobial and inflammatory capacities.

Recent studies have unravelled how neutrophils switch from a migratory cell to a killing cell. The neutrophil roles can be categorized into three main processes: phagocytosis; degranulation; and neutrophil extracellular traps (NETs) formation.

PMN cells are dedicated phagocytes that engulf and degrade microorganisms to limit the spread of pathogenic microorganisms in the body. Phagocytic neutrophils can perform a "respiratory burst" that generates reactive oxygen species (ROS) and hypochlorous acid; this process is regarded as the key killing mechanism for most invading pathogens. In addition to well-known antimicrobial properties, recent results have shown that ROS localization acts as a sensor of microbe population size to fine-tune the scale of the inflammatory response. One study also identified an unexpected new role for neutrophil-derived myeloperoxidase (MPO), which restrains lipopolysaccharide (LPS) toxicity instead of aggravating inflammatory damage.

Neutrophil degranulation is divided into oxidative and non-oxidative mechanisms, which are regarded as the defensive arsenal to eliminate foreign agents[16]. As a neutrophil matures, granules of potent antimicrobial peptides and proteolytic enzymes gradually become enriched in the cytoplasm. When activated, these bactericidal bullets are released in the form of secretory vesicles.

The NETs theory was first described in detail in 2004, whereby an extracellular web-like structure is composed of active proteases and DNA from chromatin and mitochondria[17]. Notably, the NETs protein composition varies depending on the stimulus, allowing the NETs to trap and kill different threats, such as bacteria, fungi, viruses, and endoparasites. The interplay between aging and neutrophils has a significant impact, leading to age-related diseases associated with senescence[18]. Recent studies have reported that neutrophil autophagy has a direct correlation with NETs, although the impact on inflammatory development is variable[19].

Neutrophil downregulation

Ensuring the safe termination of the inflammatory response is crucial for maintaining homeostasis. The resolving mechanism is initiated as early as neutrophil attachment to the endothelium and consists of the following steps: 1. downregulation of the PMN pro-inflammatory capacity; 2. intrinsic or extrinsic induction of neutrophil apoptosis; 3. engulfment of apoptotic neutrophils by phagocytes; 4. inhibition of the continued migration of neutrophils to the inflammatory site; 5. secretion of anti-inflammatory factors by macrophages; and 6. initiation of the tissue repair mechanism. In general, post-inflammatory homeostasis cannot be separated from the precise regulation of feedback signals. Specifically, PMN apoptosis accompanied by a decrease in pro-inflammatory capacity and removal by macrophages is vital to the ultimate resolution of inflammation.

Neutrophil departure

Excessive accumulation or hyperactivation of neutrophils or PMN purge dysfunction contributes to inflammation-induced host tissue damage[20]. Interaction with microbes, including bacteria, fungi, parasites, and viruses, can delay neutrophil apoptosis and further aggravate inflammation. In addition, some inhibitory receptor molecules on viable neutrophils, such as CD47, have been identified as "do not eat me" signals to prevent macrophage clearance. Moreover, other immune cells, such as interstitial and alveolar macrophages, act as accomplices in neutrophil hyperactivation. Adhesion molecule dysfunction on neutrophils or the endothelium can further drive PMN accumulation. More interestingly, recent studies have shown that the degree of neutrophil activation is related to metabolic patterns[21]. Beyond a specific threshold, neutrophil phagocytotic, ROS, and NETs functions become superfluous, resulting in necrotic damage. Thus, targeting these abnormal mechanisms can effectively ameliorate persistent inflammatory injury.

Tumor effects on neutrophils

Neutrophil chemotaxis in cancer

Inflammation pervades almost every type of cancer, even in the earliest stages. Although the dual roles of neutrophils in cancer have not been elucidated, there is growing awareness of the capacity for neutrophil recruitment to the TME and various factors are known to be responsible (**Table 1**). Because high levels of the CXC chemokine receptors (CXCR1 and CXCR2) are expressed on neutrophils, these cells can be recruited to tumor sites by chemokines, such as CXCL5, CXCL8 (IL-8), and CXCL6, which are overexpressed in many tumors. Rapid tumor growth usually causes local hypoxia, a vital factor for multiple chemokines in the TME. TGF-β also has a pivotal role in promoting neutrophil recruitment and tumor progression[22]. Furthermore, due to the chemotactic properties of

Table 1 Factors involved in chemotaxis and neutrophil functions

Factor	Origin/initiate factor	Receptors on neutrophils	Functions
Chemotaxis			
C3a, C5a	Blood serum	Complement receptor	Recruitment; enhanced phagocytosis
N-formyl-methionyl-leucyl-phenylalanine (fMLP)	Polygenetic	β1 integrins	Primary chemoattractant
ATP	Neutrophil at the site of infection	P2Y2 and A3 receptors	Secondary chemoattractants; amplify recruitment signal
Leukotriene B4 (LTB4)	Neutrophil at the site of infection	LTB4 receptor	Secondary chemoattractants; amplifier of neutrophil chemotaxis
P-selectin	Platelets	P-selectin glycoprotein ligand 1 (PSGL-1)	Chemotaxis; enhanced adhesion
CXCL8 (IL8)	Hepatocyte Hep3b and HepG2 cells	CXCR1/2 GPCRs	Chemotaxis; promote pro-inflammatory mediator release
CXCL1	IL-6	CXCR2	IL6-CXCL1-CXC2 axis of chemotaxis
IL-26	Alveolar macrophages	IL-26 receptor complex	Potentiated neutrophil chemotaxis
CXCL14 and CXCL16	$CD11c^+$ macrophages	Not mentioned	Potentiated neutrophil chemotaxis
Vascular endothelial growth factor (VEGF)	Not mentioned	Fms-like tyrosine kinase 1 (FLT-1)	Potentiated neutrophil chemotaxis
Lipocalin-2 (Lcn2)	Psoriatic skin epidermis	Receptors of Lcn2 (24p3R)	Regulated kinase-1/2 and p38-mitogen-activated protein kinase signaling pathways; promote pro-inflammatory mediator release
CXC12	G-CSF	CXCR4	Mobilization and recruitment
CXCL2, human CXCL8	Th17-IL17	CXCR2	IL1β-IL17-CXCL2-CXCR2 axis of chemotaxis
CXCL5	Hypoxia in cancer	CXCR2	Chemotaxis; Enhanced survival; promotes CCL2/CCL17 release
Liver X receptor (LXR) ligands/oxysterols	Released by cancer cells	CXCR2	Chemotaxis; promote neoangiogenesis
HMGB1-CXCL12 complex	Cabozantinib induces	CXCR4	Chemotaxis
PARP-1	Not mentioned	CXCR2	Increased recruitment of neutrophils *via* the CXCL1/CXCR2 pathway and polarizes neutrophils into the pro-angiogenic phenotype
IL-17C	Not mentioned	TLR2/4	Recruitment
HIF2α	Not mentioned	CXCR2	Recruitment
Semaphorin 3E	Not mentioned	Not mentioned	Recruitment *via* the CXCL8/IL8 pathway
Exosomes	Tumor cell	Not mentioned	Recruitment *via* TLR3-mediated lung epithelial cell activation
TNF-α	MSCs	CXCR2	Recruitment
14,15-EET	Not mentioned	Not mentioned	Recruitment by activating the STAT3 and JNK pathways and activating the expression of human IL-8 and murine CXCL15
IL23	Not mentioned	Not mentioned	Recruitment
STK11/LKB1	Tumor cell	Not mentioned	Suppress recruitment

Table 1 Continued

Factor	Origin/initiate factor	Receptors on neutrophils	Functions
Polarization			
TGF-β	Tumor cell	Not mentioned	Polarized into the pro-tumor phenotype
IL-6 and G-CSF	Tumor cell and/or stromal cells	G-CSFR, IL-6R	Polarized into the pro-tumor phenotype
IFN-β	Monocytes/macrophages	Not mentioned	Polarized into the anti-tumor phenotype
IL-6	MSCs	IL-6R	Polarized into the pro-tumor phenotype
TNF-α	MSCs	Not mentioned	Polarized into the pro-tumor phenotype
IL-35	Not mentioned	Not mentioned	Polarized into pro-tumor phenotype *via* an increase in IL-6 and G-CSF production
Retinoic acid	Extraneous	Not mentioned	Polarized into an anti-tumor phenotype
IFN-γ	NK cell	Not mentioned	Polarized into an anti-tumor phenotype
IL-17	IL-17-producing cells	Not mentioned	Polarized into an anti-tumor phenotype
IL-17	γδT17 cell	Not mentioned	Polarized into a pro-tumor phenotype
CCL2	Not mentioned	Not mentioned	Potential tumor cell killing role *in vitro*, increased $CD4^+$ T cell recruitment to the pre-metastatic niche of the lung and enhanced seeding and growth of tumor cells *in vivo*

neutrophils, researchers have utilized neutrophils as drug carriers for targeted tumor therapy.

In addition to the role of TME in recruiting neutrophils, the effects of systemic metabolism and endocrine alteration on neutrophil recruitment have been investigated in several studies. For example, obesity is always associated with chronic inflammation and worse tumor prognosis. Margaroli et al.[23] reported that neutrophils in obese patients exhibit enhanced immunosuppressive activity, thereby promoting renal cancer progression. McDowell et al.[24] found that obesity reprograms neutrophils to increase the production of ROS and NETs, leading to disruption of vascular integrity and promoting breast cancer cell metastasis to the lungs. Systemic changes in hormones also influence neutrophils and Tang et al.[25] discovered that androgens promote the hepatic metastasis of tumor cells by enhancing the recruitment of neutrophils to the liver.

Neutrophil polarization in cancer

Neutrophils are no longer viewed as terminally differentiated cells but rather cells that exhibit plasticity, even after leaving the BM. Numerous studies have explored the factors that drive neutrophil polarization. In 2009 Fridlender et al.[26] concluded that the role of TGF-β in polarization is particularly remarkable. Neutrophils can be divided into two subsets (N1 and N2). N-polarized neutrophils have an anti-tumor role, exhibiting tumor cell toxicity and immune-stimulating activity, with a mature phenotype and short life span. Conversely, N2-polarized neutrophils support tumor progression, displaying low tumor cell toxicity and highly pro-angiogenic, pro-metastatic, and immunosuppressive activities, along with an immature phenotype and long-life span. Neutrophils in the TME can be transformed from N1-to-N2 by TGF-β. Numerous studies have also demonstrated the significant role of TGF-β in inducing polarization of tumor-associated neutrophils (TANs) into a tumor-promoting phenotype, thereby advancing tumor progression[27]. Conversely, inhibiting local TGF-β in the TME can promote neutrophil polarization into an anti-tumor phenotype, inhibiting tumor progression[28]. Mishalian et al.[29] found that neutrophil functions changed by detecting the neutrophil phenotype at different time points during tumor progression; specifically, neutrophils in early stages exhibit the N1 phenotype, whereas neutrophils in later stages acquired the N2 phenotype. These results also suggested that neutrophil depletion would not satisfy the expectations of therapy unless the intervention time point is fully considered. Moreover, Wang et al.[30] identified the hypoxia- and ER stress-induced activation of the *BHLHE40* gene crucially

triggering TAN polarization toward a tumor-promoting phenotype. Siakaeva et al.[31] reported that type I IFN suppresses tumor development by inhibiting G-CSF/NAMPT signaling, thereby reducing the content of tumor-promoting neutrophils in the blood and tumors. Gungabeesoon et al.[32] demonstrated that neutrophils exhibiting elevated IRF1 expression in murine KP and MC38 tumor models correlate with enhanced therapeutic efficacy of tumor immunotherapy. This finding positions IRF1 as a potential upstream regulatory target capable of driving neutrophil differentiation toward an anti-tumor phenotype. The stromal cells in the TME and the level of chemokines in serum can also affect neutrophil polarization. According to Song et al.[33], cancer-associated fibroblasts (CAFs) promote neutrophil N2 polarization by upregulating the CLCF1–CXCL6/TGF-β axis. Tsai et al.[34] showed that CAFs contribute to the formation of vascular mimicry (VM) through the Notch pathway, which facilitates neutrophil N2 polarization. Additionally, serum CCL21 inhibits neutrophil N2 polarization and promotes N1 neutrophil polarization by modulating the NF-κB signaling pathway, thereby enhancing the anti-tumor efficacy in hepatocellular carcinoma (HCC)[35]. The overview of tumor effects on neutrophils is shown in **Figure 1** and **Table 1**.

Pro- and anti-tumor TAN roles

Current studies involving neutrophils in tumors suggest diverse roles for neutrophils. Although most clinical studies indicate that neutrophils are associated with accelerated tumor growth and progression, others reveal the anti-tumor functions of neutrophils, showing that neutrophils are not a homogeneous population (**Table 2**). Recent discoveries regarding subsets of neutrophils in cancer are summarized in **Table 3**.

Pro-tumor role

Promotion of tumor angiogenesis

The ability of TANs to promote neovascularization is not surprising because TANs express high levels of proangiogenic factors, such as MMPs, VEGF-α, and Ang-2. Nozawa et al.[67] found that depletion of neutrophils, which mainly infiltrate dysplasia and tumoir angiogenic islets, significantly suppresss the angiogenic switch by inhibiting the interaction between VEGF and the VEGF receptor. Gao et al.[68] reported that oral squamous cell carcinoma (OSCC) cells upregulate angiogenic factors, such as VEGF-α, MMP-9, MMP-2, and S100A9, in neutrophils *via* the chemerin-activated MEK/ERK signaling pathway, which promotes angiogenesis and tumor growth. Additionally, neutrophils activate the AKT/mTOR axis in HUVECs through NET-mediated signaling, enhancing HUVEC proliferation, migration, and the release of ANGPT2, which initiates neovascularization and promotes gastric cancer growth[69]. Ng et al.[70] identified three neutrophil subsets (T1: dcTRAIL-R1$^-$CD101$^-$; T2: dcTRAIL-R1$^-$CD101$^+$; and T3: dcTRAIL-R1$^+$CD101$^{-/+}$) in pancreatic cancer mouse models based on dcTRAIL-R1 and CD101 expression; the T3 subset promoted tumor growth *via* VEGF-α secretion.

Interaction with tumor cells: migration and invasion

In addition to a role in promoting tumor angiogenesis, TANs directly modulate the characteristics of tumor cells *via* several mechanisms. Neutrophils induce tumor migratory capabilities by secreting various cytokines. For example, Li et al.[71] reported that TANs promote migration and invasion of gastric cancer cells by inducing epithelial-mesenchymal transition (EMT) *via* IL-17α. Neutrophil NETs can also activate the IL-1β/EGFR/ERK pathway to promote EMT and migration in pancreatic cancer cells. In addition to activating tumor cell EMT, neutrophils can also promote HCC migration by downregulating VE-cadherin expression on endothelial cells through NETs[72]. Moreover, numerous studies have revealed the capacity of TANs to facilitate tumor cell invasion. First, TANs degrade the extracellular matrix (ECM) by secreting several enzymes, such as neutrophil elastase (NE), MMP-8, MMP-9, cathepsin G, and proteinase-3. Second, TANs confer tumor cells with the ability to degrade the ECM *via* granzyme and cytokines, and Queen et al.[73] found that neutrophil-enhanced tumor cell invasion only be obtained by direct contact.

Interaction with tumor cells: proliferation

The proliferation of tumor cells is closely related to granulocytes. Zhu et al.[74] suggested that the formation of NETs in colorectal cancer promotes the expression of integrin α5β1 in tumor cells, thereby enhancing tumor proliferation. Additionally, Wang et al.[75] reported that glioma LINC01116 promotes neutrophil recruitment and tumor proliferation by activating IL-1β expression. Houghton et al.[76] focused on NE in lung cancer, reporting direct promotion of tumor cell proliferation in murine and human lung cancer. Further mechanistic investigation elaborated that NE enter the endosomal compartment and degrade insulin receptor substrate-1 within tumor cells, which increases efficient interaction between

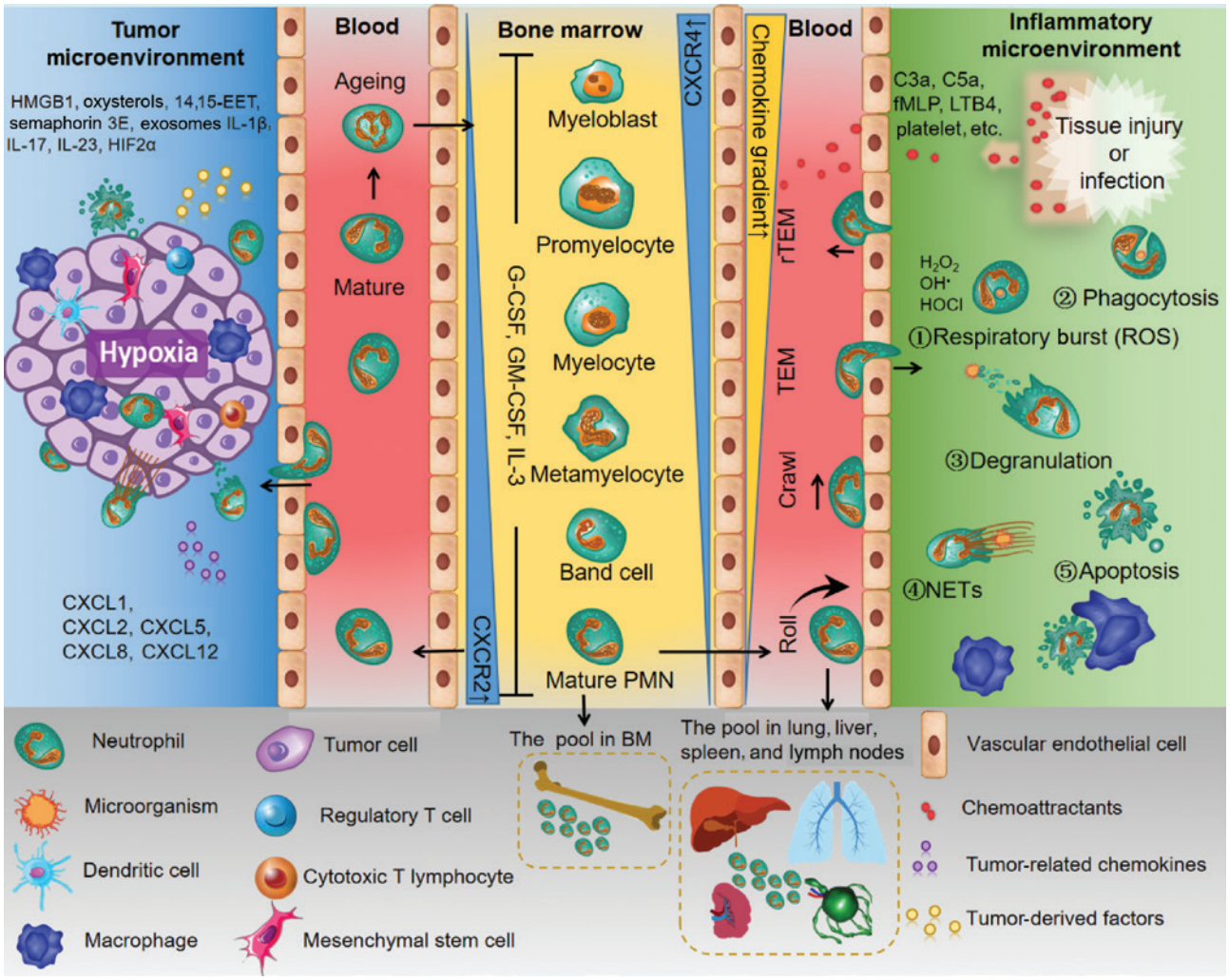


Figure 1 Proposed neutrophil functions in the inflammatory and tumor microenvironment (TME). **(Figure middle)** Neutrophils undergo maturation in the **bone marrow (BM)** under the combined stimulation of granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3). As myeloid progenitors develop, upregulation of CXCR2 and downregulation of CXCR4 occur. The whole maturation process, which is characterized by distinct changes in cell size, nuclear morphology, and granule content, progresses through six sequential stages: myeloblasts; promyelocytes; myelocytes; metamyelocytes; band cells; and mature polymorphonuclear (PMN) leukocytes. Once matured, granulocytes reside in the BM and establish a storage pool in other organs, such as the lungs, liver, spleen, and lymph nodes, standing by and ready to enter the peripheral blood for systemic recycling. In response to infection, inflammation, or tumor-derived signals, mature neutrophils are mobilized from the BM pool or other organ pools into the peripheral blood (PB). In contrast, peripheral mature neutrophils will be gradually aging and return to the BM for the last clearance. **(Figure left)** It is known that the **tumor microenvironment (TME)** is a typically hypoxia position, where activated intracellular hypoxia-inducible factor 2α (HIF2α) induces alteration of the cellular metabolic pathway and the accumulation of some hypoxia-induced factors and chemokines, like high-mobility-group box-1 (HMGB-1), oxysterols, 14,15-epoxyeicosatrienoic acid (14,15-EET), semaphorin 3E, exosomes, and IL-1β, IL-17, and IL-23. In addition, tumor cells and other stromal cells actively recruit neutrophils. Upon crossing the blood vessel, those recruited neutrophils are regulated by surrounding chemokines, some of which are C-X-C motif chemokine ligand 1 (CXCL1), CXCL2, CXCL5, CXCL8, and CXCL12, to recognize, adhere, or kill tumor cells cooperating with other immune effector cells. However, evidence to date supports that tumor-associated neutrophils (TANs) are modified by tumor cells to promote tumor progression rather than to kill tumors. **(Figure right)** With respect to the **inflammatory microenvironment**, injured or infectious tissue releases various chemokines or proteins, like complement component 3a (C3a), complement component 5a (C5a), formyl-methionyl-leucyl-phenylalanine (fMLP), leukotriene B4 (LTB4), and platelets. In response, neutrophils migrate from storage pools to the inflamed tissue by rolling, crawling, and transendothelial migration (TEM) within the PB. This process relies on cytoskeletal rearrangement, which is facilitated by structures. Recruited neutrophils have a crucial role in inflammation

regulation at the pathologic site. First, neutrophils rapidly initiate a **respiratory burst** that consumes a large amount of oxygen and produces reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radical (.OH), and hypochlorous acid (HOCl), which directly kill the pathogens or diseased cells. Additionally, neutrophils carry out **phagocytosis** during which neutrophils extend pseudopods to surround and engulf target pathogens or cells to form a phagosome. This mechanism also eliminates engulfed materials directly. Moreover, neutrophils undergo **degranulation** to regulate the immune environment. During degranulation, neutrophils will release a variety of intracellular enzymes, like elastase and myeloperoxidase, to the extracellular matrix, which can degrade the cell walls or harmful components involved in regulating inflammation. Furthermore, **neutrophil extracellular traps (NETs)** formation is another latest way to limit the spread of pathogenic microorganisms. NETs release chromatin and antibacterial proteins into the extracellular space to establish a net-like structure that can capture and degrade microorganisms and debris. Finally, after fulfilling PMN leukocyte functions, spent PMN leukocytes with a decrease in proinflammatory capacity undergo **apoptosis** and are removed by macrophages, which is like what occurs in tumor settings. Alternatively, PMN leukocytes will reverse the TEM process back to the PB for the next mobilization. 14,15-EET, 14,15-epoxyeicosatrienoic acid; BM, bone marrow; C3a, complement component 3a; C5a, complement component 5a; CXCL1, C-X-C motif chemokine ligand 1; fMLP, formyl-methionyl-leucyl-phenylalanine; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; H_2O_2, hydrogen peroxide; HIF2α, hypoxia-inducible factor 2α; HMGB-1, high-mobility-group box-1; HOCl, hypochlorous acid; IL-3, interleukin-3; LTB4, leukotriene B4; NETs, neutrophil extracellular traps;. OH, hydroxyl radical; PMNs, polymorphonuclear leukocytes; PB, peripheral blood; ROS, reactive oxygen species; TANs, tumor-associated neutrophils; TEM, transendothelial migration; TME, tumor microenvironment.

PI3K and PDGFR and thereby induces tumor cell proliferation. Nonetheless, some studies have shown that neutrophils do not enhance the growth of tumor cells and even slow proliferation. To date, the exact role of neutrophils in tumor cell proliferation remains in dispute and needs additional exploration.

Creation of an immune-suppressive microenvironment

Several recent studies have reported that TANs help to build an immune-suppressive microenvironment based on the phenomenon of neutrophil recruitment in the TME. Fridlender et al.[26] reported that "N2" TANs inhibit $CD8^+$ T cells by secreting arginase 1 (ARG1), an enzyme that degrades extracellular arginine to inhibit the responsiveness of T cells to CD3/TCR stimulation. Another important mechanism by which TANs suppress T cell proliferation and activation is *via* expression of PD-L1 on the neutrophil surface to activate the PD-L1/PD-1 signaling pathway of T cells[77]. Additional mechanistic research has shown that PD-L1 expression on neutrophils is induced by tumor-derived GM-CSF *via* the JAK and STAT3 signaling pathway[78]. Neutrophil recruitment and PD-L1 expression on TANs are inhibited by tumor suppressors (STK11/LKB1) to restore local immune killing function[79]. Moreover, Taifour et al.[80] found that the tumor-derived cytokine, CHI3L1, promotes neutrophil recruitment and the formation of neutrophil extracellular traps, thereby blocking T-cell infiltration in triple-negative breast cancer. Recently, Wang et al.[81] reported that neutrophil accumulation in the spleen impairs STAT5 signaling by depriving glucose, resulting in the inability of T cells to activate and affecting anti-tumor T-cell immunity in breast cancer. Neutrophils also attract immune-suppressive cells into the TME. Mishalian et al.[82] found that TANs secrete CCL17, an important chemoattractant for regulatory T cells. Zhou et al.[83] indicated that TANs recruit macrophages *via* CCL2 to enhance HCC progression and sorafenib resistance.

Anti-tumor role

Direct tumor cytotoxicity

Neutrophils have the capacity to generate large amounts of antimicrobial mediators, such as protease granules, ROS, MPO, TRAIL, IFN-γ, and H_2O_2. Human neutrophils release neutrophil elastase (ELANE) to selectively kill many types of cancer cells while sparing non-cancerous cells. G-CSF-activated neutrophils produce ROS, triggering oxidative damage to tumor cells in melanoma. Linde[84] also demonstrated that the combined effect of tumor necrosis factor, CD40 agonist, and tumor-binding antibodies activates neutrophils, mediating oxidative killing of tumor cells by inducing the production of C5a and LTB4 and promoting xanthine oxidase (XO) activity. Recently, Yee et al.[85] reported that neutrophils promote ferroptosis in glioblastomas by transferring granules containing myeloperoxidase to tumor cells, inducing iron-dependent accumulation of lipid peroxides within tumor cells. Furthermore, neutrophils exert anti-tumor effects by interacting with microbes. Tintelnot et al.[86] reported that 3-IAA derived from microbiota leads to ROS accumulation in cancer cells under the action of neutrophil myeloperoxidase, inhibiting the proliferation of pancreatic ductal adenocarcinoma (PDAC). Neutrophils also slow down the growth and

Table 2 Prognostic significance of neutrophils in cancer

Tumor type	Country	Enrolled number	Marker	Methods	Prognosis	Reference
HNSCC	Sweden	46	$CD16^{high}$ $CD62L^{dim}$	FCM	Longer OS	Millrud et al. (2017)[36]
OSCC	Brazil	28	CD66b	IHC	No significant association with prognosis	Caldeira et al. (2015)[37]
SCEC	China	36	Morphologic identification	HE	No significant association with OS	Zhang et al. (2014)[38]
ESCC	China	181	MPO	IHC	Longer OS	Chen et al. (2017)[39]
GC	China	105	$CD45^+CD66b^+$/CD15	FCM/IHC	Shorter OS	Wang et al. (2017)[40]
	Italy	273	Morphologic identification	HE	Female: a moderate or extensive amount of TINs with reduced mortality risk Male: no significant association	Caruso et al. (2002)[41]
	China	215	CD66b	IHC	Longer OS, especially in stage III	Zhang et al. (2018)[42]
	China	212	CD15	IHC	Shorter OS	Zhao et al. (2012)[43]
CRC	Sweden	448	CD66b	IHC	Longer OS and CSS	Wikberg et al. (2017)[44]
	Switzerland	1420	MPO	IHC	Significantly improved survival	Droeser et al. (2013)[45]
	China	378	CD177	IHC	Longer 5-year OS and DFS	Zhou et al. (2018)[46]
	Switzerland	677	CD66b	IHC	Longer OS	Governa et al. (2017)[47]
	USA	221	Multi-field manual counting	HE	Longer OS (stage II); no significant association (stage III/IV)	Berry et al. (2017)[48]
	Poland	147	Neutrophil elastase	IHC	No correlation	Väyrynen et al. (2016)[49]
	Italy	271	CD66b	IHC	Longer DFS and DSS	Galdiero et al. (2016)[50]
	UK	130	Morphologic identification	HE	No correlation with CSS	Richards et al. (2012)[51]
HCC	China	259	CXCR2	IHC	Poorer RFS and OS	Li et al. (2015)[52]
	China	238	CD15	IHC	Worse OS and DFS	Kuang et al. (2011)[53]
ECC	Japan	114	CD66b	IHC	Shorter OS	Kitano et al. (2018)[54]
ICC	China	123	CD66b	IHC	Shorter OS	Gu et al. (2017)[55]
PDAC	Japan	212	CD66b	IHC	Shorter survival	Ino et al. (2013)[56]
	China	79	CD15	IHC	Shorter OS	Wang et al. (2016)[57]
NSCLC	Norway	536	CD66b	IHC	Positive prognosticator for DFS in SCC; negative prognostic factor in adenocarcinoma	Rakaee et al. (2016)[58]
TGCT	Japan	102	CD66b	IHC	Shorter OS	Yamada et al. (2016)[59]
UCC	Japan	250	CD66b	IHC	Shorter PFS	Matsumoto et al. (2017)[60]
Localised cervical cancer	Denmark	102	CD66b	IHC	Shorter RFS	Carus et al. (2013)[61]
Localized RCC	Denmark	121	CD66b	IHC	Shorter RFS, CSS and OS	Jensen et al. (2009)[62]

Table 2 Continued

Tumor type	Country	Enrolled number	Marker	Methods	Prognosis	Reference
MIBC	China	666	CD66b	IHC	Shorter OS	Zhou et al. (2017)[63]
Gallbladder carcinoma	China	98	CD15	IHC	Shorter OS	Zhang et al. (2017)[64]
CSCC	New Zealand	25	CD66b	IHC	Not mentioned	Seddon et al. (2016)[65]
Melanoma	Denmark	186	CD66b	IHC	Poorer RFS, melanoma-specific survival and OS	Jensen et al. (2012)[66]

CRC, colorectal cancer; CSCC, cutaneous squamous cell carcinoma; ECC, extrahepatic cholangiocarcinoma; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ICC, intrahepatic cholangiocarcinoma; MIBC, muscle-invasive bladder cancer; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; SCEC, small cell esophageal carcinoma; TGCT, testicular germ cell tumor; UCC, urothelial cell carcinoma

Table 3 Subset of neutrophils reported in cancer

Subtype	Marker and properties	Functions	Cancer model
N1	Hypersegmented nuclei, TNF-α ↑, ICAM-1 ↑, FAS ↑	Tumor cytotoxic, activate CTL	Mouse, mesothelioma and lung cancer
N2	Circular nuclei, Arginase ↑, CCL2 ↑, CCL5 ↑	Pro-tumor, reduces $CD8^+$ T cell activation	
$SiglecF^{hi}$	$SiglecF^{hi}$	Pro-tumor, myeloid cell recruitment, angiogenesis, T cell suppression	Mouse, KP lung tumor; human, non-small cell lung cancer
High density neutrophils	High density (1.077-1.119), small size, segmented nuclei, migratory, phagocytic, potent oxidative burst, pro-inflammatory	Anti-tumor	Mouse, breast cancer and lung cancer; human, breast cancer and lung cancer
Low density neutrophils	Low density (< 1.077), large size, Banded/ring-shaped to segmented nuclei, reduced migration, reduced phagocytosis, reduced oxidative burst, reduced inflammation	Pro-tumor	
APC-like neutrophil	$CD62L^{lo}$, $CD54^{hi}$, $CXCR2^{lo}$, $CD10^+$, MHC II^+/MHC I^{hi}, $CD14^+$, $Ikaros^{lo}$	Anti-tumor, T cell stimulatory activity	Human, lung cancer
$CD13^{hi}$ neutrophil	$CD13^{hi}$, Arginase-1 ↑	Pro-tumor, suppress monocyte-initiated alloreactive $CD4^+$ or $CD8^+$ T cell proliferation	Human, pancreatic ductal adenocarcinoma
$IL17^+$ neutrophil	$IL17^+$, MMP9 ↑	Pro-tumor, promote angiogenesis	Human, gastric cancer
PD-$L1^+$ neutrophil	PD-$L1^+$, $CD54^+$	Pro-tumor, *in vitro*: suppresses inhibition of T cell proliferation and IFN-γ production; *in vivo*: promotes the progression of human GC	Human, gastric cancer
$CD62L^{dim}$/$CD16^{hi}$	$CD16^{dim}$, $CD16^{hi}$, migratory into tumor site	Anti-tumor, produce NET, predict improved survival	Human, HNSCC
c-MET^+ neutrophils	c-MET^+	Pro-tumor, suppress T cell expansion and effector functions	Mouse, melanoma, breast cancer, CRC, lung cancer
IL-10^+neutrophils	IL-10^+, Arginase-1 ↑	Pro-tumor, suppresses T cell expansion	Human, melanoma
$CD177^+$ neutrophils	CD177	Anti-tumor, suppress epithelial cell tumorigenesis	Human, CRC; mouse, CAC

progression of colon tumors by limiting the number of bacteria and IL-17 expression.

Neutrophil-mediated antibody-dependent cell-mediated cytotoxicity (ADCC)

Neutrophils express FcγR family members and participate in monoclonal antibody (mAb)-mediated immunotherapy to eliminate antibody-targeted cells. However, mAb-induced tumor regression is reversed if neutrophils are depleted. Notably, normal neutrophils or transgenic expression of FcR restores ADCC in FcR-deficient mice. Targeting CD47-SIRPα interaction enhances neutrophil-induced ADCC independent of FcγR or SIRPα genotypes. Overall, this is the most direct evidence that neutrophils protect the body as an innate immune cell.

Enhanced immune system activation

The adaptive immune system, especially the $CD8^+$ cytotoxic T cell component, is now known to be a vital element in anti-tumor processes. Although N2 TANs are reported to inhibit T cell proliferation and activation, N1 TANs attract CTLs into the TME *via* cytokines, including IL-12, TNF-α, and GM-CSF, and chemokines, such as CCL3, CXCL9, and CXCL10. Additionally, during the early stages of cancer, neutrophils migrate to tumor-draining lymph nodes, stimulating T cells as antigen-presenting cells (APCs) to initiate an antitumor immune response. Single-cell transcriptomic analysis of neutrophils from separate 17 cancer types identified a population of neutrophils that function as APCs, activated by leucine[87]. Furthermore, FcγR-mediated endocytosis of antibody-antigen complexes or anti-FcγRIIIB-antigen conjugates transforms neutrophils into nAPCs, triggering $CD8^+$ T cell-dependent anti-tumor immunity in mice. TANs also exhibit anti-tumor effects by promoting the production of IL-12 by macrophages, leading to the polarization of unconventional αβ T cell subsets (UTCαβ) towards a type 1 phenotype[88].

The summary of pro- and anti-tumor functions of TANs is shown in **Figure 2**.

Role of neutrophils in metastasis

Recently, the relationship between neutrophils and metastasis has inspired a new wave of research investigating the participation of neutrophils in various steps of metastasis, as follows: 1. transforming cancer cells and helping cancer cells leave the primary tumor site; 2. supporting cancer cell entry into the circulatory system (blood and/or lymphatics); 3. protecting cancer cells from immune cell attack in the circulatory system; 4. facilitating cancer cell persistence and proliferation in remote tissues; and 5. forming a pre-metastatic niche that promotes the growth of cancer cells. The metastasis-promoting role of neutrophils was first revealed in 1989; specifically, co-injection of cancer cells and neutrophils from tumor-bearing rats into tumor-free rats resulted in increased lung metastases. Although metastasis is closely associated with an elevated number and ratio of neutrophils in the circulation and in the metastatic organ, the role of neutrophils in metastasis is still controversial.

Pro-metastatic role

Neutrophils are well-known to exert the most crucial role in the early cascade of metastasis. Additionally, several studies have indicated that systemic depletion of neutrophils markedly reduces metastasis.

Promotion of tumor cell dissemination

Neutrophils assist tumor cells in escaping from the primary tumor *via* several mechanisms, including enhancing migratory and invasion capacities, as well as angiogenesis. Yang et al.[89] reported that NETs act on breast cancer cells through the NET-DNA receptor, the transmembrane protein, CCDC25, activating the ILK-β-parvin pathway to enhance cell motility, and promoting cancer metastasis. Furthermore, NETs can also promote metastasis in HCC by capturing HCC cells and activating the TLR4/9-COX2 axis to enhance cell invasiveness[90].

Facilitation of tumor cell persistence at remote sites

Huh et al.[91] reported that intravenous co-injection of human melanoma cells with neutrophils into nude mice increases cancer cell retention in the lung compared to injection of melanoma cells alone. Furthermore, a mechanistic investigation revealed that neutrophils help circulating cancer cells anchor to the vascular endothelium *via* interactions between tumor cell ICAM-1 and neutrophil β2-integrin. Szczerba et al.[92] revealed that neutrophils support cell cycle progression in the circulation by directly interacting with circulating tumor cells (CTCs). Additionally, Li et al.[93] discovered that lung mesenchymal cells induce lipid storage in neutrophils. When disseminated tumor cells (DTCs) reach the lungs, lipid-rich neutrophils transfer stored lipids to the DTCs, promoting tumor cell survival and proliferation, and subsequently enhancing breast cancer lung metastasis. Another mechanism that cannot be overlooked is the effect of neutrophil NETs on

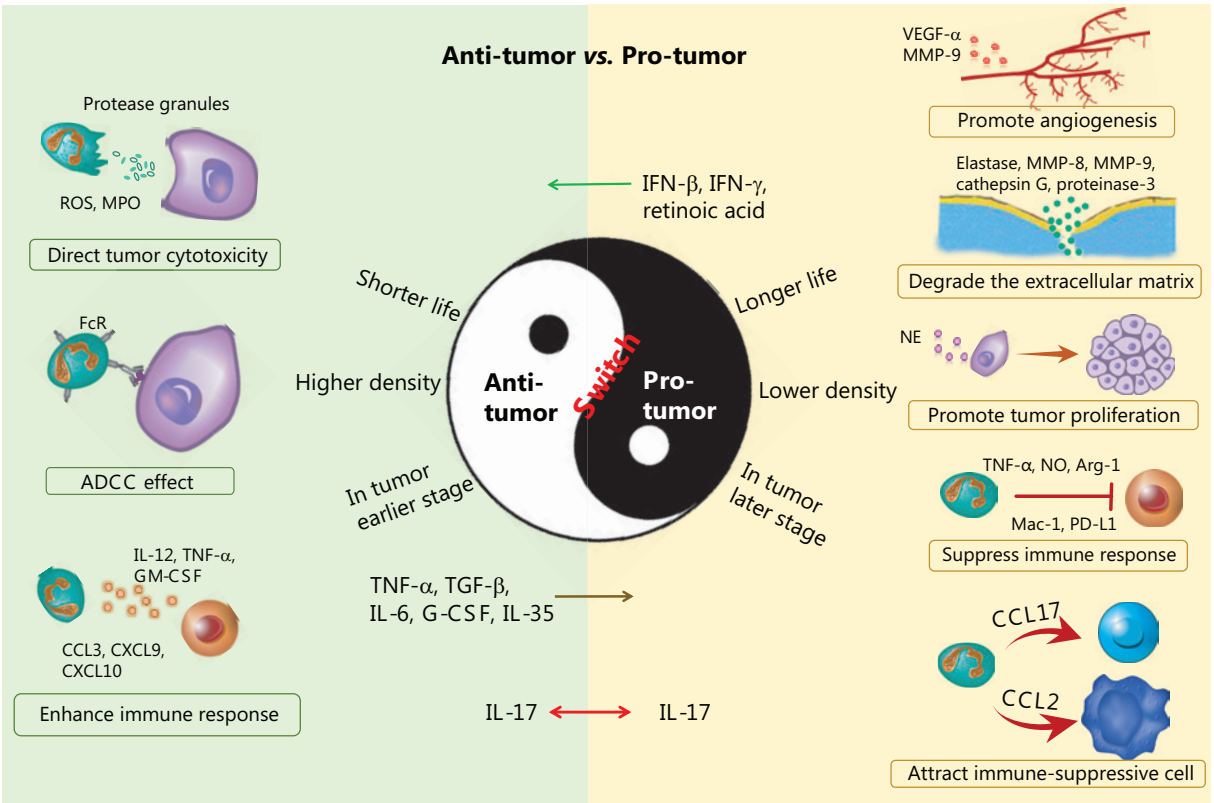


Figure 2 Potential pro- and anti-tumor functions of tumor-associated neutrophils (TANs). TANs may either promote or inhibit tumorigenesis and tumor progression through various immune mechanisms. **(Figure left) Anti-tumor roles of TANs**, which involve **direct tumor cytotoxicity** through secretion of protease granules, like reactive oxygen species (ROS) and myeloperoxidase (MPO). Additionally, TANs mediate the **antibody-dependent cell-mediated cytotoxicity (ADCC) effect**, facilitated by Fc receptors (FcR) on the surfaces, then activate and recruit other immune effector cells, leading to the elimination of antibody-targeted tumor cells by TANs. Apart from ADCC, TANs can further **enhance the immune response**. Dendritic cells, macrophages, and T cells secrete reactive cytokines within the TME, such as IL-12, TNF-α, and GM-CSF, which induce proliferation, maturation, and migration of TANs. TANs sequentially secrete C-C motif chemokine ligand 3 (CCL3), C-X-C motif chemokine ligand 9 (CXCL9), and CXCL10 that attract and recruit T cells, especially Th1 suppressing tumors to the TME. This coordinated action between TANs and other immune cells *via* cytokines and chemokines enhances the anti-tumor immune response. Overall, this dynamic interaction occurs predominantly in the early stage and is marked by a shorter cell lifespan and higher cell density. **(Figure right) Pro-tumor roles of TANs** in different aspects. TANs release abundant vascular endothelial growth factor-α (VEGF-α) and matrix metalloproteinase 9 (MMP-9), **promoting angiogenesis** and an increased blood supply, and a boost of tumor growth. In addition, enzymes, like elastases, MMP-8, MMP-9, cathepsin G, and proteinas-3 secreted by TANs, **degrade the extracellular matrix** and physical barrier facilitating tumor cell invasion into surrounding tissue. Moreover, TANs have an important role in **promoting tumor proliferation** in the late stage *via* secretion of neutrophil elastase (NE). Similarly, TANs have a negative effect on the immune system. Tumor necrosis factor-α (TNF-α), nitric oxide (NO), and arginase 1 (Arg-1) secreted by TANs and macrophage-1 Ag (Mac-1) and PD-L1 expressed on TANs **suppress the immune response** and enable tumors to escape the immune surveillance. CCL17 and CCL2 separately **attract immune suppressive cells**, like Tregs and myeloid-derived suppressor cells (MDSCs), leading to a favorably immune-deficient niche for tumors. In summary, TANs can also impair the anti-tumor immunity with a longer lifespan but lower cell density in the later stage of tumor progression. Notably, neutrophils in the TME can be transformed from pro-tumor phenotypes to anti-tumor phenotypes by IFN-β, IFN-γ, and retinoic acid. Reverse action can be achieved through TNF-α, tumor growth factor-β (TGF-β), IL-6, G-CSF, and IL-35, whereas IL-17 has a dual effect, in which subtle concentration changes influence the dynamic shift of neutrophils. ADCC, antibody-dependent cell-mediated cytotoxicity; Arg-1, arginase 1; CCL3, C-C motif chemokine ligand 3; CXCL9, C-X-C motif chemokine ligand 9; Mac-1, macrophage-1 Ag; MDSCs, myeloid-derived suppressor cells; MMP-9, matrix metalloproteinase 9; MPO, myeloperoxidase; NE, neutrophil elastase; NO, nitric oxide; ROS, reactive oxygen species; TANs, tumor-associated neutrophils; TNF-α, tumor necrosis factor-α; TGF-β, tumor growth factor-β; VEGF-α, vascular endothelial growth factor-α.

tumor cells. For example, NETs exist in distant organs to trap CTCs and help tumor cells adhere, which contributes to tumor metastasis.

Pre-metastatic niche formation

Metastasis is closely related to pre-metastatic niche formation in distant organs. This niche, a microenvironment formed by stromal cells and immune cells, is driven by the primary tumor and provides a residence for CTCs. Such a niche formation is attracting unparalleled attention and various studies are attempting to illuminate the mechanism by which a distant organ is transformed to create the pre-metastatic niche. Sceneay et al.[94] clarified that hypoxic primary TMEs recruit neutrophils to distant organs, where neutrophils inhibit the activity of natural killer (NK) cells. Similarly, Spiegel et al.[95] focused on the effect of neutrophils on NK cells and found that granulocytes inhibit tumor cell clearance by NK cells and permit CTC persistence. Accumulation of neutrophils in metastatic organs mimics inflammation after injury, which is stimulated by production of leukotriene B4 (LTB4). Recently, Wculek and Malanchi[96] reported that LTB4 from neutrophils in the pre-metastatic niche supports LTB4 receptor $(LTB4R)^+$ metastasis-initiating tumor cells in breast cancer mouse models. In addition, neutrophil accumulation in distant organs appears to be driven by G-CSF; tumor-derived G-CSF guides neutrophils towards a pro-metastatic phenotype. Shojaei et al.[97] focused on the effect of G-CSF on neutrophils and showed that G-CSF induces expression of BV8 in neutrophils, which promotes cancer cell migration, angiogenesis, and metastasis. Moreover, IL-1β secretion from tumor-associated macrophages, through the γδT cell-IL-17-G-CSF axis, leads to neutrophil accumulation and conversion to the immunosuppressive type, which suppresses the anti-tumor role of $CD8^+$ T cells. Furthermore, a subset of neutrophils expressing KIT was reported. Blocking the KIT signaling pathway or inhibiting expression of KITL on tumor cells reduces pulmonary metastasis in a 4T1 breast cancer model. Other studies have shown that VEGFα, TNF, and TGF-β to be key factors in pre-metastatic niche formation. Shaul et al.[98] reported that TANs recruit B cells in a TNF-α-dependent manner and induce differentiation into plasma cells, although the pro- or anti-tumor effects remain unknown. Additionally, TGF-β has been shown to enhance LIF expression by activating the Smad2/3 complex, ultimately leading to the formation of NETs and promoting peritoneal metastasis of GC[99]. Considering the importance of chemokines to neutrophil movement, CXCL1, CXCL2, and CXCL5 have a vital role in recruiting immature myeloid cells and mature neutrophils to create the pre-metastatic niche.

Anti-metastatic role

Although the abovementioned studies support the pro-metastatic capacity of neutrophils, it has been reported that granulocytes inhibit cancer metastasis and that neutrophil depletion increases metastasis. Granot et al.[100] clarified that neutrophil-derived H_2O_2 has a cytotoxic role in tumor cells and is regulated by CCL2. Unexpectedly, despite using the same mouse tumor model, the conclusions of Granot and others differ, highlighting the need to determine the factors responsible for these discrepancies. Considering that the functions of neutrophils in cancer may change during tumor progression, one possible explanation is that the time of depletion intervention was crucially different in different studies, which may influence the effect of neutrophils on metastasis. Another possibility is that the cell lines in different laboratories changed during repeated passaging and any genetic transformation can change cellular characteristics. Moreover, differences in the mouse breeding environment influence neutrophil features, as shown by the influence of microbiome-derived LPS on neutrophil aging.

In addition to H_2O_2, production of Met and Tsp-1 by neutrophils suppresses metastasis. Although Catena et al.[101] reported that neutrophils construct a metastasis-refractory microenvironment *via* Tsp-1, El Rayes et al.[102] found that neutrophils promote metastasis by degrading TSP1 *via* neutrophil-derived protease. These conflicting results can be explained by switching of neutrophil anti-tumor and pro-tumor roles *in vivo*.

Prospects of targeting neutrophils for cancer therapy

The above clinical and research data emphasize the complex role of neutrophils in different cancer types and distinct periods of tumor progression. Considering the importance of the anti-infection function of neutrophils in the innate immune system, indiscriminate elimination of neutrophils is not rational without thorough analysis. Moreover, the short life span and rapid replacement of neutrophils greatly impair the efficacy of therapeutic targeting. Despite the pro-tumor role of neutrophils, the anti-tumor effects of neutrophils should not

be ignored. Therefore, efficient deletion of adverse neutrophils is an arduous task, and the road to success is long.

It now appears that neutrophils are beneficial in the early tumor stage but become more compatible with tumor cells in late stages, and identification of the critical factors that cause this transition is an urgent task. Based on the ambiguous mechanism involved in neutrophil recruitment and polarization, successful therapy may involve blocking recruitment of tumor-promoting neutrophils and suppressing pro-tumor polarization. Considering the role of neutrophils in metastasis, more attention is needed to remodel the pre-metastatic niche and prevent metastasis.

Enhancing the anti-tumor role of neutrophils and decreasing pro-tumor neutrophil accumulation

Several approaches to relieve neutrophil-mediated immunosuppression have been developed. One important method is to restrict recruitment of immunosuppressive neutrophils. Because c-MET$^+$ neutrophils suppress the expansion and functional effects of T cells, the HGF/c-MET pathway appears to participate in neutrophil recruitment. Therefore, Glodde et al.[103] combined a c-MET inhibitor with adoptive T cell transfer and checkpoint immunotherapies to achieve better immunotherapeutic effects. Recently, Ban et al.[104] proposed a therapeutic approach targeting the CCL5-CCR5 axis that combines CCL5-targeting nanoparticles with maraviroc, a U.S. Food & Drug Administration (FDA)-approved CCR5 inhibitor. The results showed that this treatment decreased the number of immunosuppressive myeloid cells and NOS2 expression and enhanced activated CD8$^+$ T cell infiltration.

Another method for switching neutrophils to the anti-tumor phenotype utilized by Chang et al.[105] in a B16 melanoma model involves the synthetic double-stranded RNA analogue poly I:C and inactivated Sendai virus to accumulate FAS$^+$ neutrophils at the TME and enhance CTL activity. Shime et al.[106] adopted a similar strategy in an EL4 lymphoma tumor model and found that this strategy promoted ROS production in neutrophils to directly eliminate tumor cells. Yin et al.[107] constructed an intelligent nano-carrier (designated mP-NPs-DNase/PTX), which combines a paclitaxel (PTX) prodrug nanoparticle core with a poly-l-lysine (PLL) shell conjugated with matrix metalloproteinase 9 (MMP-9)-cleavable Tat peptide and deoxyribonuclease I (DNase I). This nano-carrier degrades the structure of NETs and exerts cytotoxic effects on tumor cells.

Numerous clinical trials are underway using drugs that target TANs and enhance the induction of anti-tumor phenotypes. The predominant strategy involved selectively inhibiting the recruitment, induction, and proliferation of pro-tumor TAN phenotype within the TME to achieve curable outcomes (**Table 4**). Currently, drugs that block TGF-β or relevant receptors to suppress the N2 phenotypes and promote the induction of effective N1 phenotypes are under development. LY3022859, an anti-TGF-β receptor II antibody, and galunisertib, a TGF-β receptor I inhibitor, has been verified to enhance anti-tumoral therapeutic efficacy. Nevertheless, further trials are required to elucidate the underlying mechanisms, particularly with respect to the reliance on neutrophils.

Table 4 Category of inhibitors targeting N1/N2 neutrophils

Class of target	Drug	Cancer type	Phase	Clinicaltrials.gov no.
IFN-β	Bacillus Calmette Guerin	Bladder cancer	III	NCT04806178 (completed)
	Imiquimod	Breast cancer	II	NCT00899574 (completed)
TGF-β	Galunisertib	TNBC	I	NCT02672475 (completed)
		Glioma	I	NCT01682187 (completed)
		Ovarian carcinosarcoma	I	NCT03206177 (completed)
STAT3 inhibitors	Napabucasin	Colorectal cancer	III	NCT02753127 (completed)
CXCR1/CXCR2 inhibitors	Reparixin	Metastatic breast cancer	II	NCT02370238 (completed)
		Metastatic breast cancer	I	NCT02001974 (completed)
	SX-682	Melanoma	I	NCT03161431 (recruiting)
C/EBPα	MTL-CEBPA	Solid tumor	I	NCT04105335 (active, not recruiting)
TRAIL receptor 2 agonist	Tigatuzumab	TNBC and metastatic breast cancer	II	NCT01307891 (completed)

Inhibiting pre-metastatic niche formation

Another issue that needs to be addressed is pre-metastatic niche formation. Kang et al.[108] developed a nanosize neutrophil-mimicking drug delivery system (NM-NP) consisting of poly (lactic-co-glycolic acid) nanoparticles (NPs) that coat the surface of neutrophil membranes. This drug system greatly increases CTC-capture efficiency *in vivo*. By loading with carfilzomib, a second-generation proteasome inhibitor, the NM-NP-based nanoformulation (NM-NP-CFZ), selectively depleted CTCs and elevated the therapeutic effect. Another promising therapy is based on the importance of LTB4 in metastasis[96]. Another alternative therapy to prevent metastasis is blocking LTB4 from neutrophils. Overall, targeting neutrophils for cancer therapy is full of hope but difficult.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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Performed the analysis: Hanwen Hu, Yunjia Bao, Liwei Pang.
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