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REVIEW

Complex role of neutrophils in the tumor microenvironment: an avenue for novel immunotherapies

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ABSTRACT Neutrophils, which originate from the bone marrow and are characterized by a segmented nucleus and a brief lifespan, have a crucial role in the body's defense against infections and acute inflammation. Recent research has uncovered the complex roles of neutrophils as regulators in tumorigenesis, during which neutrophils exhibit a dualistic nature that promotes or inhibits tumor progression. This adaptability is pivotal within the tumor microenvironment (TME). In this review, we provide a comprehensive characterization of neutrophil plasticity and heterogeneity, aiming to illuminate current research findings and discuss potential therapeutic avenues. By delineating the intricate interplay of neutrophils in the TME, this review further underscores the urgent need to understand the dual functions of neutrophils with particular emphasis on the anti-tumor effects to facilitate the development of effective therapeutic strategies against cancer.

KEYWORDS Neutrophil; plasticity; tumor microenvironment; immunotherapy

Introduction

Neutrophils, which comprise a significant proportion of circulating leukocytes in humans (50%–70%) and mice (10%–25%)[1,2], have pivotal roles in responding to infection and acute inflammation[3]. However, neutrophil involvement in tumor progression is multifaceted. Neutrophils, which are derived from bone marrow granulocyte monocyte progenitors (GMPs), are characterized by segmented nuclei, a short lifespan, and rapid turnover[4,5]. These granulocytes are essential in host defense and profoundly impact tumor dynamics by infiltrating tumors, demonstrating remarkable phenotypic traits[6].

Recent studies have unveiled the dichotomous nature of neutrophil behavior in tumors with the ability to promote or inhibit tumor growth likely reflecting neutrophil plasticity in response to environmental cues[7-10]. Of note, some studies have shown that the cytokine and chemokine profiles within the tumor microenvironment (TME) may dictate neutrophil recruitment and functional orientation[11-13]. While an elevated neutrophil level within solid tumors often correlates with unfavorable clinical outcomes[11], emerging evidence suggests the potential of neutrophils to impede tumor progression through mechanisms, such as direct tumor cell cytotoxicity and modulation of innate and adaptive immune responses[14,15], which were previously underestimated.

This review aimed to unravel the intricate roles of neutrophils, specifically elucidating how distinct neutrophil subsets exert dual effects on the TME. By meticulously exploring neutrophil heterogeneity and diverse neutrophil functions within the TME, this review offers a comprehensive synthesis of the latest research findings. Moreover, the prospect of leveraging these dynamic neutrophil behaviors to forge innovative therapeutic strategies for cancer treatment is discussed.

Development, maturation, and recruitment of neutrophils

Neutrophil development is initiated within the bone marrow from self-renewing hematopoietic stem cells in a process termed "granulopoiesis" (**Figure 1**). These stem cells

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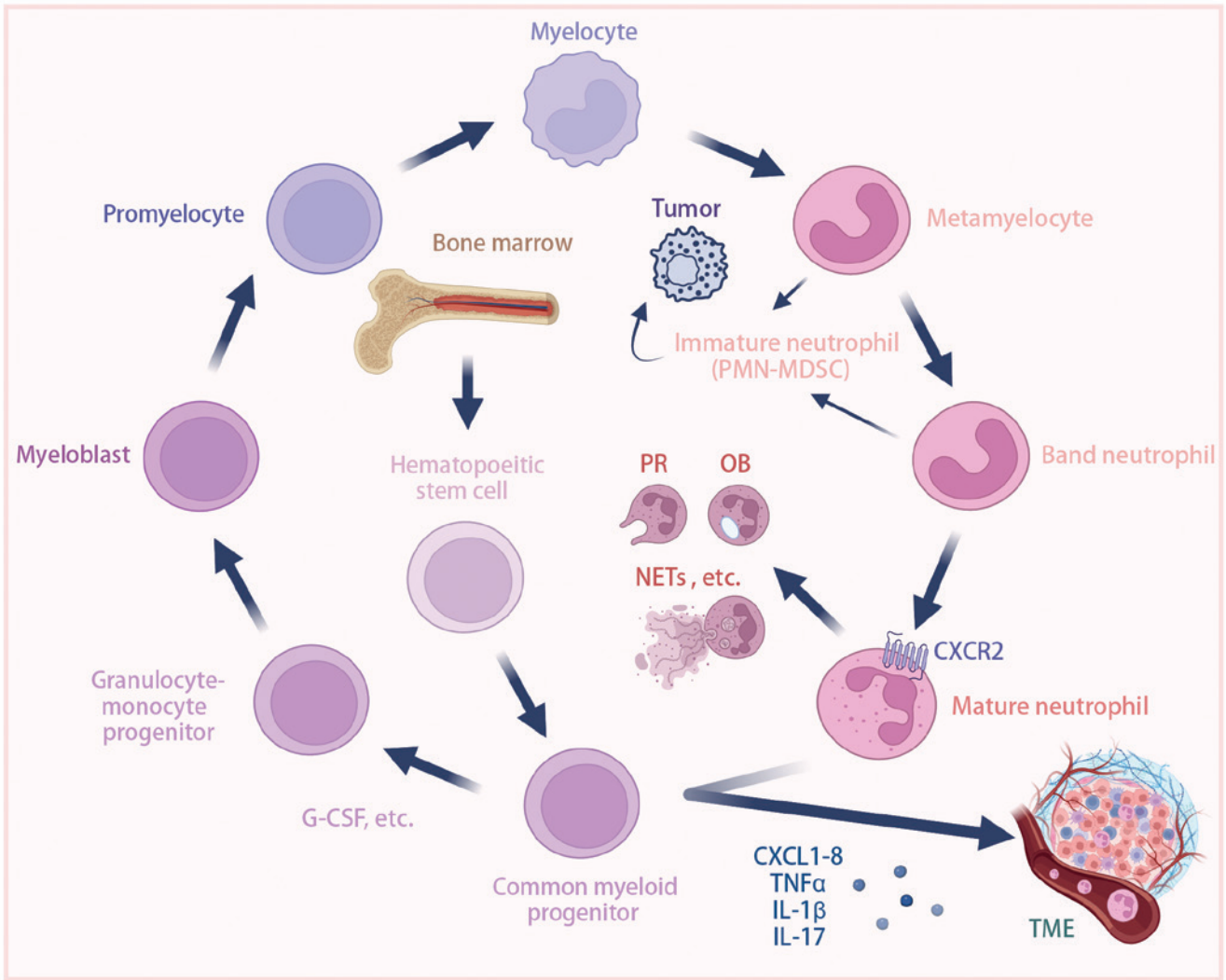

Figure 1 Overview of neutrophil development, maturation, and recruitment. Neutrophils develop from hematopoietic stem cells in the bone marrow through several stages: hematopoietic stem cells; common myeloid progenitors; granulocyte-monocyte progenitors; myeloblasts; promyelocytes; myelocytes; metamyelocytes; band neutrophils; and mature neutrophils. Cytokines, such as G-CSF, have a crucial role in each stage of this differentiation process. Immature neutrophils (PMN-MDSCs) can contribute to TME dynamics with pro-tumor effects. However, mature neutrophils participate in immune responses by PR, OB, and forming NETs. Neutrophils are recruited by chemokines (CXCL1-8) and inflammatory mediators (TNF-α, IL-1β, and IL-17) in the TME. Neutrophils express receptors, such as CXCR2, to facilitate migration and have pro- or anti-tumor effects depending on the context. CXCL1-8, C-X-C motif chemokine ligand 1-8; CXCR2, C-X-C motif chemokine receptor 2; G-CSF, granulocyte colony-stimulating factor; IL-1β, interleukin-1beta; IL-17, interleukin-17; NETs, neutrophil extracellular traps; OB, oxidative burst; PR, pathogen recognition; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells; TME, tumor microenvironment; TNF-α, tumor necrosis factor-alpha. Created using BioRender.com.

differentiate into multipotent hematopoietic progenitors that subsequently give rise to common myeloid and lymphoid progenitors. In response to increased cytokine levels, such as granulocyte colony-stimulating factor (G-CSF), common myeloid progenitors differentiate into granulocyte or monocyte progenitors[16]. This orchestrated differentiation leads to the progression of myeloblasts through various stages, including promyelocytes, myelocytes, metamyelocytes, and band neutrophils, culminating in the formation of mature neutrophils[17].

Mature neutrophils contribute to inflammation resolution through diverse mechanisms, including pathogen recognition and phagocytosis, degranulation, oxidative burst, and the generation of neutrophil extracellular traps (NETs[18]; **Figure 1**).

Neutrophils are subsequently cleared from the tissue *via* macrophage phagocytosis[19]. Beyond the classic role in acute infection resolution, emerging evidence underscores the pivotal involvement of neutrophils in tumor regulation[6]. Neutrophils populate the TME in various malignancies[15]. However, the precise neutrophil function remains enigmatic, often contingent upon tumor type, developmental stage, and the interplay with other cellular constituents[20].

Moreover, neutrophils undergo a complex journey from the bone marrow to tumor sites that is coordinated by a finely tuned gene expression program (**Figure 1**). As neutrophils mature, expression of chemokine receptors, such as C-X-C motif chemokine receptor 2 (CXCR2)[21,22], is modulated. Changes in these surface receptors direct neutrophil migration toward their chemokine ligands [C-X-C motif chemokine ligand 1 (CXCL1), CXCL2, CXCL3, CXCL5, CXCL6, and CXCL8[23,24]], which are highly expressed in the tumor environment[6,25,26]. Additionally, inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-17, and IL-1β, have crucial roles in orchestrating neutrophil recruitment into the TME[6,27,28].

Finally, tumor-induced stress can trigger "emergency granulopoiesis," which alters neutrophil maturation and release. This results in the circulation of immature neutrophils, which are sometimes identified as "polymorphonuclear myeloid-derived suppressor cells" (PMN-MDSCs)[29]. PMN-MDSCs may exert pro-tumor effects within the tumor milieu[26] (**Figure 1**). However, the underlying mechanisms have not been elucidated and discerning the impact of these immature neutrophil populations *in vivo* is technically challenging.

Despite significant strides in understanding neutrophil biology in tumorigenesis, the multifaceted roles in tumor immunity have not been fully described, primarily due to the inherent neutrophil plasticity and heterogeneity. Neutrophils encompass diverse subsets, each endowed with unique functions within the TME. Deciphering the regulatory mechanisms governing these processes is paramount for bridging the developmental biology of neutrophils with their intricate roles in tumor immunology.

Neutrophil plasticity and heterogeneity in the TME

Historically viewed as short-lived effector cells with limited plasticity, neutrophils have emerged as remarkably heterogeneous and dynamic entities in the TME, challenging traditional perceptions[30-32]. Extensive evidence underscores the significant plasticity and heterogeneity of neutrophils, reflecting a spectrum of phenotypes and functions akin to the M1/M2 paradigm observed in macrophages. Neutrophils are now classified into anti-tumor (N1) and pro-tumor (N2) subsets, highlighting their diverse roles in tumor progression[6,33,34].

Advanced methodologies, such as single-cell RNA sequencing (scRNA-seq), mass cytometry, and spatial transcriptomics, have unveiled the intricate heterogeneity within neutrophil populations[26,35-39]. For example, scRNA-seq delineated 11 distinct neutrophil clusters in primary liver tumors, each characterized by unique gene signatures regulated spatiotemporally. Notably, tumor-associated neutrophils (TANs) were predominant, with specific clusters exhibiting functionalities, such as macrophage recruitment *via* the chemokine (C-C motif) ligand 4 (CCL4)-chemokine (C-C motif) receptor 5 (CCR5) pathway (Neu_11_CCL4) and inhibition of T cell cytotoxicity (Neu_09_IFIT1)[40].

Similarly, mass cytometry analysis of melanoma identified 7 neutrophil subsets, including terminally differentiated subsets exhibiting dynamic changes during tumor progression[41]. Diverse subpopulations within the low-density neutrophil (LDN) and high-density neutrophil (HDN) subsets in lung cancer were identified with implications for disease prognosis[41]. Remarkably, an intermediate cluster ($CD66b^+$/$CD10^{low}$/$CXCR4^+$/PD-L1) exclusive to advanced lung cancer was shown to correlate with poorer outcomes[42].

Our recent study unveiled diverse transcriptional profiles in neutrophils using scRNA-seq in 17 tumor types from 143 patients[15]. Noteworthy findings included enrichment of neutrophils linked to antigen presentation ($HLA\text{-}DR^+CD74^+$) and angiogenesis ($VEGFA^+SPP1^+$) in cancerous tissues, while inflammatory clusters ($IFIT1^+ISG15^+$ and $NFKBIZ^+HIF1A^+$) were prevalent in conditions, such as chronic pancreatitis and COVID-19. Moreover, distinct neutrophil clusters were shown to exhibit associations with specific tumor types and clinical outcomes, underscoring their prognostic significance.

Specifically, $VEGFA^+SPP1^+$ neutrophils, which are prevalent in renal cell carcinoma and gastric adenocarcinoma, display enhanced glycan metabolism and are correlated with poor outcomes. Conversely, $HLA\text{-}DR^+CD74^+$ neutrophils are associated with more favorable prognoses in non-small-cell lung, bladder, and ovarian cancer[15].

These findings underscore the pivotal role of neutrophil plasticity and heterogeneity in tumor progression and offer

critical insight for the development of prognostic models and therapeutic strategies in cancer management.

The distinct role of neutrophils

Pro-tumor mechanisms of neutrophils

Neutrophils may directly promote tumor progression by enhancing genetic instability, promoting tumor cell proliferation, and facilitating angiogenesis or indirectly by suppressing anti-tumor immune responses and facilitating metastatic spread (**Figure 2**).

Extensive research has highlighted that neutrophil-derived enzymes, notably reactive oxygen species (ROS) and neutrophil elastase (NE), contribute to tumor initiation[43-46]. Notably, neutrophil NADPH oxidase 2 (NOX2)-derived ROS has been reported to support tumor colonization through an IL-1β-dependent pathway[47]. Additionally, ROS can induce DNA damage in lung cells, especially when combined with carcinogens, which accelerates tumor formation[48] (**Figure 2**). In contrast to recent results on the role of human NE[49], deletion of NE in prostate and lung cancer murine models resulted in smaller tumors, establishing the crucial role of murine NE in tumor development[43,50] (**Figure 2**). Because murine neutrophils cannot release catalytically active NE[49], these effects may be due to changes in neutrophil biology resulting from the absence of NE. Previous research has demonstrated that $NE^{-/-}$ neutrophils have an altered ability to migrate to inflammatory sites and respond to inflammatory challenges[51,52].

Moreover, *in vivo* studies consistently support the tumor-promoting capacity of neutrophils, particularly through mechanisms, such as induction of angiogenesis, which is imperative for sustained tumor growth[53]. The existence of reprogrammed decoy TNF-related apoptosis-inducing ligand-receptor 1^+ ($dcTRAIL\text{-}R1^+$) neutrophils within hypoxic and glycolytic tumor niches have been shown to exert pro-angiogenic effects that favor tumor expansion[39] (**Figure 2**).

Furthermore, neutrophil-mediated immunosuppression constitutes a significant mechanism facilitating tumor progression[6]. This mechanism includes suppression of NK-mediated tumor cell clearance and promotion of disseminated carcinoma cell extravasation[54]. Our prior work also underscored the critical role of soluble mediators released by neutrophils, notably prostaglandin E2 (PGE_2), in subverting

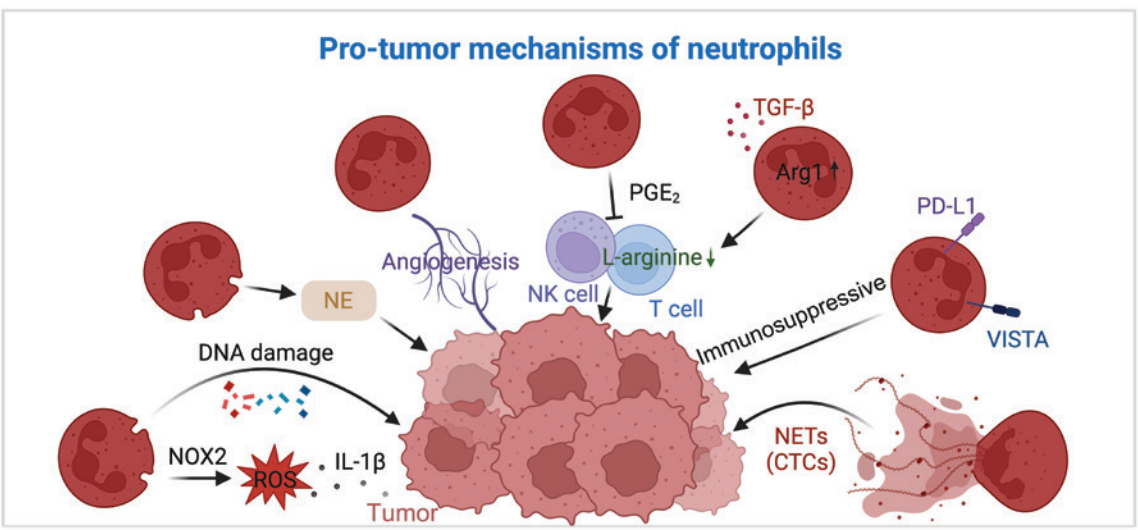

Figure 2 Overview of neutrophil mechanisms in pro-tumor activities. Neutrophils support tumor progression through multiple mechanisms. Neutrophils produce NOX2-derived ROS, which promotes tumor growth *via* an IL-1β-dependent pathway. Additionally, neutrophils induce DNA damage and secrete NE, which enhances tumor development. Neutrophils promote tumor development through angiogenesis. Neutrophils also suppress immune responses by releasing PGE2, which negatively affects NK and T cell activity. Moreover, in response to TGF-β, neutrophils enhance Arg-1 expression, depleting L-arginine in T cells and leading to T cell dysfunction. Neutrophils contribute to immunosuppression by expressing PD-L1 and VISTA. Finally, neutrophils release NETs that trap CTCs and aid in metastasis. Arg-1, arginase-1; CTCs, circulating tumor cells; IL-1β, interleukin-1β; NE, neutrophil elastase; NETs, neutrophil extracellular traps; NOX2, NADPH oxidase 2; PGE_2, prostaglandin E2; ROS, reactive oxygen species; TGF-β, transforming growth factor-beta; VISTA, v-domain immunoglobulin suppressor of T-cell activation. Created using BioRender.com.

the effector functions of T and NK cells[55] (**Figure 2**). Moreover, the substantial production of arginase-1 (Arg-1) by neutrophils in response to transforming growth factor-beta (TGF-β) profoundly impacts T cell metabolism, leading to T cell dysfunction as a result L-arginine depletion[33] (**Figure 2**).

Additional studies have indicated the presence of neutrophils that express PD-L1 or V-domain immunoglobulin suppressor of T-cell activation (VISTA) in human and murine models of hepatocellular carcinoma, melanoma, and gastric cancer[56-59]. Inhibition of VISTA was reported to lead to a pronounced pro-inflammatory response in myeloid cells and reduce the capacity to suppress immune responses in a murine melanoma model[59] (**Figure 2**). Moreover, brain TANs exhibit distinct immunosuppressive and pro-angiogenic capacities compared to their circulating counterparts[60].

Another crucial facet of neutrophil involvement in tumor progression is the release of NETs[61]. Studies have elucidated the role of NETs in promoting the initiation of metastasis, as evidenced by intravital imaging demonstrating the co-localization of tumor cells with endothelial cell-associated neutrophils[62]. NETs facilitate metastasis by entrapping circulating tumor cells (CTCs) and promoting adhesion of CTCs at distant sites, with excessive NET formation correlating with shorter progression-free survival[63,64] (**Figure 2**).

The interaction between neutrophils and CTCs in breast cancer enhances the metastatic potential by driving cell cycle progression within the bloodstream[65]. Finally, the neutrophil-to-lymphocyte ratio (NLR) has emerged as a promising biomarker for tumor patient risk stratification, with alterations in NLR indicating disease recurrence, progression, or response to therapy[66,67]. Moreover, interferon-stimulated neutrophils serve as a predictor of the immunotherapy response[8,68].

Anti-tumor mechanisms of neutrophils

The anti-tumor roles of neutrophils, which are often overlooked, are vital to the immune system arsenal against tumors. In this section we delve into the multifaceted functions and mechanisms of neutrophils in tumor suppression (**Figure 3A–C**).

Neutrophil-mediated direct killing of tumor cells

Neutrophils exhibit potent anti-tumor effects that are partly achieved through direct cytotoxic and cytostatic mechanisms. Notably, ROS, which include crucial components [hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot -}$), and nitric oxide (NO)], have a central role in neutrophil-mediated tumor lysis[69] (**Figure 3A**). Recent studies have unveiled the anti-tumor potential of β-glucan-induced training of granulopoiesis, demonstrating ROS-dependent tumor suppression. Remarkably, the anti-tumor properties of trained neutrophils can be transferred from donor murine bone marrow to recipient naïve mice[70]. ROS-mediated tumor cell killing hinges on tumor cell expression of transient receptor potential cation channel, subfamily M, member 2 (TRPM2), an H_2O_2-dependent calcium channel, which triggers a lethal influx of calcium ions into the cell[71] (**Figure 3A**). Furthermore, neutrophil-derived nitric oxide production, which is potentiated by mesenchymal-epithelial transition (MET) receptor signaling, enhances tumor cell killing[72] (**Figure 3A**). NETs also contribute to tumor cell cytotoxicity. NET components, such as myeloperoxidase, can obliterate melanoma cells, while defensins and histones contribute to tumor cell lysis and destruction of supportive blood vessels and epithelial cells, respectively[73] (**Figure 3A**). Moreover, expelled deoxyribonucleic acid (DNA) strands from NETs entrap tumor cells, impairing their metastatic and proliferative capacities[74] (**Figure 3A**).

Surface receptors, such as Fc receptors, enable neutrophils to engage with antibody-opsonized tumor cells, heightening their cytotoxic and phagocytic capabilities. This interaction, which is particularly potent when tumor cells are opsonized with tumor-targeting monoclonal antibodies, facilitates mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP[75,76]; **Figure 3A**). Neutrophils also demonstrate efficacy in direct tumor cell killing through macrophage-1 antigen (MAC-1)-dependent cellular contacts, which are crucial for cytotoxic activity[77,78] (**Figure 3A**). Additionally, interactions between neutrophils and trastuzumab (IgG)-opsonized cells lead to significant tumor cell destruction *in vitro*. Compared to IgG, IgA antibodies demonstrate enhanced tumor cell-killing capability (**Figure 3A**). Notably, inhibiting the CD47-signal regulatory protein-alpha (SIRP-α) interaction, a crucial target, boosts neutrophil-mediated ADCC[78-80] (**Figure 3A**).

Additionally, neutrophils induce tumor cell death *via* apoptosis and necrosis, as exemplified by the Fas–Fas ligand (FasL) pathway, in which neutrophil-expressed FasL triggers apoptosis upon interaction with the Fas receptor on tumor cells[81]. Neutrophils stimulated by interferon-gamma (IFN-γ) express TRAIL, inducing apoptosis by interacting with death receptors on tumor cells[82] (**Figure 3A**).

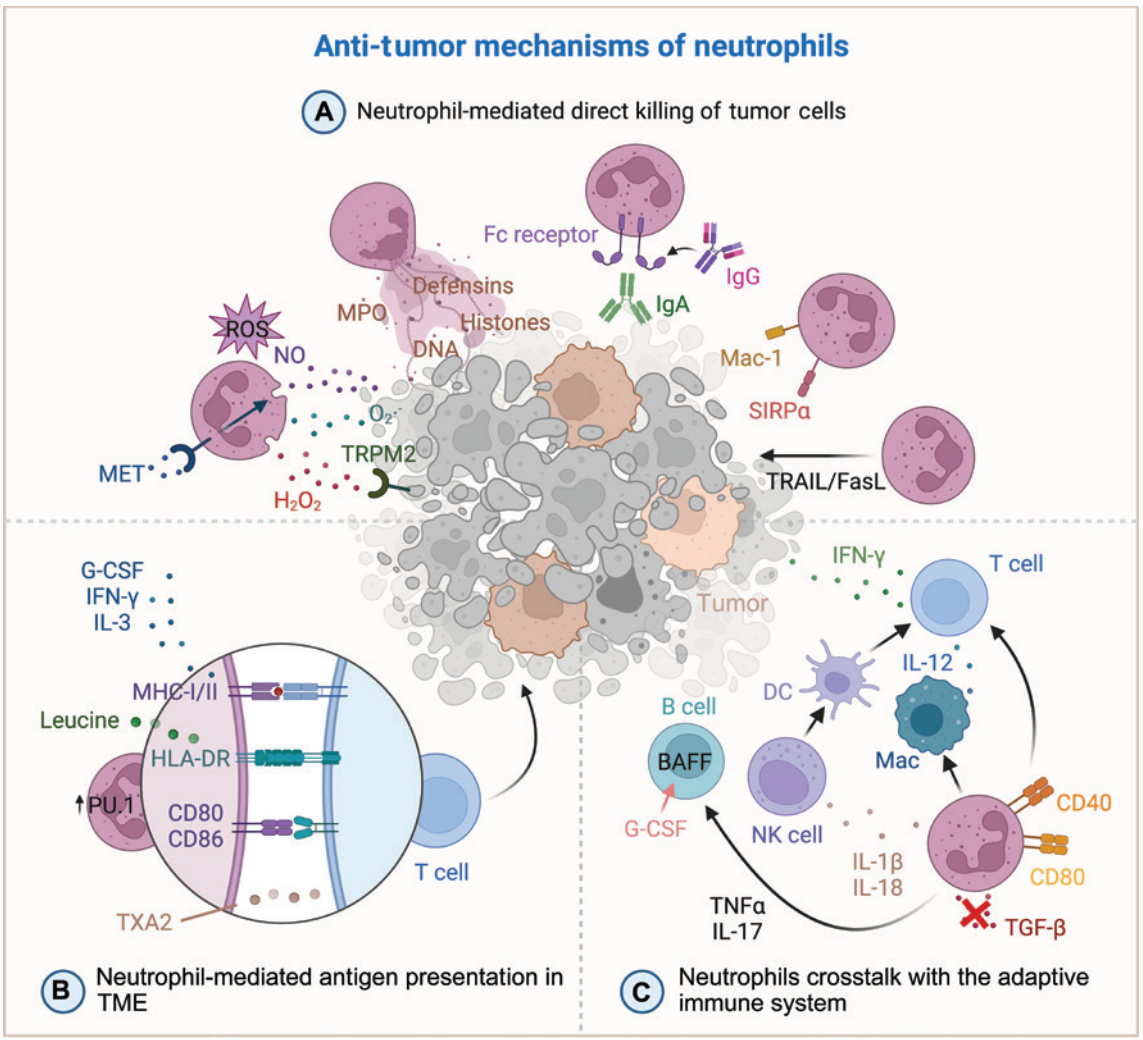

Figure 3 Overview of neutrophil mechanisms in anti-tumor activities. (A) Direct killing of tumor cells mediated by neutrophils. Neutrophils produce ROS, such as NO, $O_2^{\cdot-}$, and H_2O_2, which directly damage tumor cells. The production of NO is enhanced by MET receptor signaling. ROS-mediated tumor cell killing hinges on tumor cell TRPM2 expression, an H_2O_2-dependent calcium channel that triggers a lethal influx of calcium ions into the cell. Additionally, neutrophils release MPO, defensins, histones, and DNA, all of which contribute to the direct killing of tumor cells. Neutrophils also express Fc receptors and Mac-1 adhesion molecules, facilitating ADCC against tumor cells. Notably, inhibiting the CD47-SIRPα interaction is a crucial strategy to boost neutrophil-mediated ADCC. Moreover, through the TRAIL/FasL pathway, neutrophils induce apoptosis and necrosis in tumor cells. (B) Antigen presentation by neutrophils within the TME. Cytokines, such as G-CSF, IFN-γ, and IL-3, along with metabolites (leucine and transcription factor PU.1) influence the antigen-presenting ability of neutrophils. Molecules, such as MHC-I/II, HLA-DR, CD80, and CD86, which are expressed on neutrophils, are involved in antigen presentation to T cells through their corresponding receptors, thereby facilitating the immune response. (C) Crosstalk between neutrophils and the adaptive immune system. Expression of co-stimulatory molecules (CD40 and CD80) on neutrophils enhances the T cell anti-tumor immune response. Blocking TGF-β not only promotes neutrophil recruitment but also facilitates a stronger cytotoxic T cell response. Additionally, neutrophils have a multifaceted role in regulating adaptive immune responses through interactions with various immune cells, such as NK cells, DCs, and B cells. Neutrophils achieve this regulation by secreting cytokines, including IL-1β, IL-17, IL-18, and TNF-α, which significantly influence the broader immune landscape. FasL, fas ligand; Fc, fragment crystallizable; G-CSF, granulocyte colony-stimulating factor; H_2O_2, hydrogen peroxide; IFN-γ, interferon-gamma; IL-1β, interleukin-1beta; IL-3, interleukin-3; IL-12, interleukin-12; IL-17, interleukin-17; IL-18, interleukin-18; Mac-1, macrophage-1 antigen; MET, mesenchymal-epithelial transition; MHC-I/II, major histocompatibility complex-I/II; MPO, granule enzyme myeloperoxidase; NO, nitric oxide; $O_2^{\cdot-}$, superoxide radical; ROS, reactive oxygen species; SIRP-α, signal regulatory protein-alpha; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; TRAIL, tumor necrosis factor-related apoptosis-induced ligand; TRPM2, transient receptor potential cation channel, subfamily M, member 2; TXA2, thromboxane A2. Created using BioRender.com.

Neutrophil-mediated antigen presentation in the TME

Neutrophils have a pivotal role in orchestrating the immune response against tumors by shuttling tumor antigens from the tumor site to the draining lymph nodes. The ability to present antigens to T cells is indispensable for initiating an effective immune response. This capacity is augmented by cytokines, such as GM-CSF, IFN-γ, and IL-3, which enhance phagocytic activity and the expression of major histocompatibility complex (MHC) molecules necessary for efficient antigen presentation[83-85] (**Figure 3B**). However, optimal antigen presentation by neutrophils requires additional signals from activated T cells because innate cues alone, such as signals from Toll-like receptors (TLRs) or damage-associated molecular patterns (DAMPs), are insufficient to induce high levels of MHC molecule expression[86-88].

The role of neutrophils in antigen presentation becomes increasingly crucial in the context of decreased migration of dendritic cells (DCs) and T cells to the tumor site, which is often due to increased thromboxane A2 (TXA2) secretion by neutrophils[89,90] (**Figure 3B**). While neutrophils traditionally lack the robust signaling capabilities of professional antigen-presenting cells, such as DCs, the capacity of neutrophils to effectively activate T cells can be enhanced. For example, endocytosis of antibody-antigen complexes *via* Fcγ receptors has been shown to elicit $CD8^+$ T cell-dependent anti-tumor immunity *in vivo*[91].

A cluster of N1 neutrophils, which is characterized by CD86 and HLA-DR expression, has been shown to have antigen-presenting capabilities that potentiate the anti-tumor effect of T cells[92]. In our study using scRNA-seq in multiple tumor types, we observed diverse transcriptional profiles of neutrophils and identified specific subsets with enhanced antigen-presenting capabilities ($HLA\text{-}DR^+CD74^+$ neutrophils). These subsets, which are significantly involved in amino acid metabolism (especially leucine) were shown to co-localize with $CD8^+$ and $CD4^+$ T cells in the TME and appeared to directly activate T cells through effective ligand-receptor interactions[15] (**Figure 3B**).

Furthermore, the transcription factor, PU.1 (spi-1 proto-oncogene), has a pivotal role in augmenting antigen presentation by neutrophils[91] (**Figure 3B**). In recent years there has been a growing interest in innovative immunotherapeutic strategies, including non-canonical antigen presentation by neutrophils. For example, a combination of radiotherapy and radiodynamic therapy with nanoscale metal–organic frameworks have shown promise in enhancing immune-mediated tumor regression. This approach boosts the expression of co-stimulatory molecules, such as CD80 and CD86, as well as MHC-II molecules on neutrophils, which facilitates effective cross-presentation of antigens and enhances the immune response against tumors[93] (**Figure 3B**).

Neutrophils crosstalk with the adaptive immune system

Neutrophils have a dynamic role in shaping the adaptive immune response, particularly in the initial stages of lung cancer, during which neutrophils bolster T cell functions. This interaction fosters enhanced T cell proliferation and elevated IFN-γ release, thereby amplifying proinflammatory factors and upregulating co-stimulatory molecules on T cells[94] (**Figure 3C**). Notably, in a murine colorectal cancer model, interleukin-1 receptor-associated kinase-M (IRAK-M)-deficient neutrophils have been shown to modulate the TME by diminishing PD-L1 and CD11b expression while enhancing CD40 and CD80 levels, consequently promoting a robust T cell anti-tumor immune response[95] (**Figure 3C**). Subsequent studies showed that blocking TGF-β promotes neutrophil recruitment and also supports cytotoxic T cell responses that exhibited significant anti-tumor activity in lung cancer[33,96] (**Figure 3C**).

In addition to T cells, neutrophils engage in intricate crosstalk with NK cells through various mechanisms[97,98]. For example, cytokine-stimulated NK cells and neutrophils exchange contact-dependent activation signals mediated by CD18, intercellular adhesion molecule-1 (ICAM-1), and ICAM-3[97]. Additionally, activated neutrophils attract and activate NK cells by releasing IL-1β and IL-18, which triggers a cascade of events culminating in dendritic cell maturation, T cell proliferation, and IFN-γ production[98] (**Figure 3C**). Moreover, neutrophils collaborate with macrophages to enhance IL-12 secretion, which facilitates the polarization of unconventional αβ ($UTC_{\alpha\beta}$) T cells that produce IFN-γ, thereby bolstering anti-tumor immunity[99] (**Figure 3C**).

Furthermore, neutrophils mediate B-cell chemotaxis by secreting TNF-α, especially in the presence of chemokines (CXCL13 or CXCL12)[100]. Although the direct interaction between neutrophils and follicular B cells remains elusive, evidence suggests that neutrophils accumulate in B-cell zones, where neutrophils secrete B-cell-activating factor (BAFF) through a G-CSF-dependent mechanism and support the accelerated generation of plasma cells[101,102] (**Figure 3C**).

Intriguingly, neutrophils also modulate immunoglobulin production by blocking the BAFF receptor on B cells[103]. Given the multifaceted roles of B cells in anti-tumor immunity and the capacity of B cells to activate other immune cells, such as T and NK cells, elucidating the involvement of neutrophils in this immune crosstalk is of paramount importance[104,105].

Recent studies have further underscored the regulatory role of neutrophils in shaping the tumor-associated microbiota *via* IL-17, thereby fostering B cell activity within the TME and augmenting the overall immune response[106] (**Figure 3C**). These findings highlight the intricate interplay between neutrophils and various components of the adaptive immune system in orchestrating anti-tumor immunity.

Discussion and future perspectives

The increasing interest in the therapeutic potential of neutrophils in tumor treatment has sparked widespread attention[107-109]. However, recent research has predominantly focused on targeting the immunosuppressive and other pro-tumor functions of neutrophils (**Table 1**).

For example, prophylactic therapies, such as G-CSF and its mimetics, have been used to mitigate severe chemotherapy-induced neutropenia, thereby enhancing therapeutic outcomes (NCT00035594)[110]. Moreover, efforts have been made to inhibit neutrophil function due to the pro-tumor phenotype, with a particular focus on targeting the CXCR2 pathway. AZD5069, a CXCR2 inhibitor, is currently being evaluated in a phase I/II study in combination with durvalumab for patients with advanced hepatocellular carcinoma (2020-003346-36)[111]. Furthermore, clinical trials are investigating SX-682, a small-molecule inhibitor of CXCR1 and CXCR2, in combination with pembrolizumab for various conditions, including metastatic melanoma (NCT03161431) and metastatic non-small cell lung cancer (NCT05570825). Reparixin, another promising agent that antagonizes CXCR1 and CXCR2, is being studied in combination with paclitaxel for treatment of metastatic triple-negative breast cancer (NCT02370238)[112].

Additionally, one study in which host CCL5 in bone marrow was targeted using nanoparticle-delivered expression silencing coupled with the CCR5 inhibitor, Maraviroc, led to substantial reductions in immunosuppressive myeloid cells and robust anti-tumor responses[113]. Another promising approach involves combining Sivelestat, an NE inhibitor, with trastuzumab, which may synergistically suppress cancer cell proliferation in HER2-positive breast cancer, offering new therapeutic avenues[114]. In addition, celecoxib, a COX-2 inhibitor, has shown promise in reducing PD-L1$^+$ neutrophils and restoring T cell cytotoxicity, potentially enhancing the effectiveness of lenvatinib[115]. Napabucasin, a STAT3 inhibitor, has demonstrated efficacy in protecting the liver and suppressing alcohol-induced pre-metastatic niche formation by inhibiting neutrophil recruitment and cancer cell plasticity[116]. Pharmacologic inhibition of protein arginine deiminase 4 (PAD4) with JBI-589 in neutrophils has demonstrated efficacy in reducing primary tumor growth and lung metastases, synergistically enhancing the effects of immune checkpoint inhibitors[117]. Of note, our recent study indicated that mice with *KRAS*-mutant intrahepatic cholangiocarcinoma treated with anakinra, an interleukin-1 receptor antagonist, had a significantly enhanced anti-tumor immune response due to altered neutrophil recruitment and phenotypes[24]. Therapies targeting the PD-1/PD-L1 interaction have also shown promise in attenuating pancreatic cancer growth and improving outcomes, especially by modulating neutrophil responses[118]. Neutrophil nanodecoys are being investigated for their potential to inhibit tumor metastasis by blocking interactions between tumor cells and neutrophils[119].

Another emerging research area has harnessed the anti-tumor capabilities of neutrophils in cancer therapy. One promising approach involves MTL-CEBPA, a small activating RNA that is designed to upregulate CCAAT/enhancer binding protein-alpha (C/EBP-α) expression. This upregulation leads to an increase in neutrophil levels. Currently, the anti-tumor effects of MTL-CEBPA are being investigated in a phase 1b trial specifically targeting hepatocellular carcinoma[120]. Of note, by antagonizing various inhibitory receptors on neutrophils, the anti-tumor function may be bolstered, suggesting a concept akin to neutrophil checkpoint blockade[121,122]. Neutrophils, which share characteristics with other myeloid immune cells, engage in intricate interactions that mutually reinforce anti-tumor functions. Therapeutic approaches targeting the "don't eat me" signal mediated by the interaction between SIRP-α expressed on myeloid cells and CD47, which prolongs the lifespan of neutrophils, have shown promise[123]. Owing to the selective expression of Fcα receptors on neutrophils, artificial IgA antibodies have demonstrated the ability to elicit robust antibody-dependent cytotoxicity, which aid in the eradication of tumor cells[124]. Additionally, in our study the modulation of leucine metabolism has emerged as a promising avenue to enhance the anti-tumor properties of neutrophils[15]. Looking ahead, the challenge will be to precisely target

Table 1 Clinical trials based on neutrophil targets

Targets	Compounds	Clinical trials	Phases	Aims
G-CSF mimetics	Pegfilgrastim	NCT00035594	III	Neutropenia
	YPEG-rhG-CSF	NCT02005458	II	Neutropenia
CXCR2 inhibitor	AZD5069	NCT03177187	I/II	Metastatic castration-resistant prostate cancer
		2020-003346-36	I/II	Advanced hepatocellular carcinoma
CXCR1/CXCR2 inhibitors	SX-682	NCT03161431	I	Metastatic melanoma
		NCT05570825	II	Metastatic non-small cell lung cancer
		NCT06149481	I/II	Metastatic colorectal cancer
	Reparixin	NCT02001974	I	HER2-negative metastatic breast cancer
		NCT02370238	II	Metastatic triple-negative breast cancer
CCR5 antagonist	Maraviroc	NCT01736813	I	Metastatic colorectal cancer
		NCT03274804	I	Metastatic colorectal cancer
NE inhibitor	Sivelestat	NCT01170845	NA	Esophageal cancer
NSAIDs	Aspirin/ibuprofen	NCT01786200	NA	Colon cancer
	Celecoxib	NCT02429427	III	Breast cancer
		NCT04105335	I	Advanced solid tumors
TGF-β pathway inhibitors	Fresolimumab	NCT02581787	I/II	Non-small cell lung cancer
	Galunisertib	NCT01582269	II	Recurrent glioblastoma
		NCT01682187	I	Recurrent malignant glioma
		NCT02452008	II	Metastatic castration-resistant prostate cancer
		NCT02672475	I	Metastatic triple-negative breast cancer
		NCT02734160	I	Metastatic pancreatic cancer
		NCT03206177	I	Ovarian carcinosarcoma
STAT3 inhibitor	Napabucasin	NCT02358395	I	Advanced hepatocellular carcinoma
		NCT02753127	III	Metastatic colorectal cancer
β-Glucan	Imucell WGP	NCT00682032	NA	Non-small cell lung cancer
C/EBPα	MTL-CEBPA	NCT02716012	I	Advanced hepatocellular carcinoma
CD47-SIRPα inhibitors	Hu5F9-G4	NCT02216409	I	Solid tumors
	CC-90002	NCT02367196	I	Advanced solid and hematologic cancer
	IBI188	NCT03717103	I	Advanced malignancies
CD40 agonist	CP-870, 893	NCT00607048	I	Metastatic solid tumors
		NCT01103635	I	Metastatic melanoma
TRAIL agonists	TRM-1	NCT00092924	II	Non-small cell lung cancer
	AMG 951	NCT00508625	II	Non-small cell lung cancer
	Mapatumumab	NCT01088347	I/II	Advanced cervical cancer

Table 1 Continued

Targets	Compounds	Clinical trials	Phases	Aims
	CS-1008	NCT01220999	I	Metastatic colorectal cancer
	Tigatuzumab	NCT01307891	II	Metastatic triple-negative breast cancer

CCR5, chemokine (C-C motif) receptor 5; C/EBP-α, CCAAT/enhancer binding protein-alpha; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; G-CSF, granulocyte colony-stimulating factor; NE, neutrophil elastase; NSAIDs, non-steroidal anti-inflammatory drugs; SIRP-α, signal regulatory protein-alpha; TGF-β, transforming growth factor-beta; STAT3, signal transducer and activator of transcription 3; TRAIL, tumor necrosis factor-related apoptosis-induced ligand.

neutrophil pro-tumor functions without affecting the anti-tumor and normal functions or directly enhancing anti-tumor capabilities, which are crucial for effective tumor therapy.

Taken together, our research underscores the importance of recognizing the heterogeneity of neutrophils across various tissues and understanding dysfunctional roles within the TME[15,24]. While our understanding of these neutrophil subsets is growing, the origins and complete lifecycle remain unclear. It is uncertain whether these subsets originate from differentiation in the bone marrow, maturation in the circulation, or reprogramming within the TME. Clarifying the origins and complete lifecycle of neutrophil subsets could provide deeper insight into neutrophil heterogeneity, thereby refining the timing of interventions in neutrophil-targeted immunotherapy and potentially improving patient outcomes.

Furthermore, it is imperative to establish a universally accepted definition and methodology for distinguishing each neutrophil cluster, particularly anti-tumor neutrophils. Similarly, deeper insight into their specific roles in tumor suppression are also needed, including interactions with other immune cells. With precise definitions, separation techniques, and purification methods for each cluster, we could propel new neutrophil immunotherapies closer to the promising possibilities seen with chimeric antigen receptor (CAR)-T cell therapy[125]. Engineering neutrophils with CARs could enable a targeted attack on tumor-specific antigens, thereby enhancing their precision and efficacy within the TME. Additionally, CRISPR/Cas9-mediated modifications could enhance neutrophil persistence, improve their ability to localize to tumor sites, and augment resistance to immunosuppressive signals. This endeavor will not only redefine the role of neutrophils in cancer therapy but also pave the way for innovative treatments that can modulate neutrophil behavior in a context-dependent manner. Finally, robust biomarkers are necessary for stratifying patient populations likely to benefit from these therapies. Additionally, thoughtful clinical trial design is crucial for assessing the safety, efficacy, and long-term outcomes of these innovative treatments across diverse patient cohorts.

In conclusion, leveraging the dynamic nature of neutrophils in the TME presents multifaceted opportunities for innovative cancer therapies. By targeting neutrophil plasticity, integrating combination therapies, advancing cell engineering, and identifying new biomarkers we can advance toward more effective and personalized treatments that harness the potential of the immune system in combating cancer.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Mao Zhang, Haokai Qin, Yingcheng Wu, and Qiang Gao.
Collected the data: Mao Zhang and Haokai Qin.
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