REVIEW ARTICLE

Neutrophils in cancer: dual roles through intercellular interactions

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Neutrophils, the most abundant immune cells in human blood, play crucial and diverse roles in tumor development. In the tumor microenvironment (TME), cancer cells regulate the recruitment and behaviors of neutrophils, transforming some of them into a pro-tumor phenotype. Pro-tumor neutrophils interact with cancer cells in various ways to promote cancer initiation, growth, and metastasis, while anti-tumor neutrophils interact with cancer cells to induce senescence and death. Neutrophils can also interact with other cells in TME, including T cells, macrophages, stromal cells, etc. to exert anti- or pro-tumor functions. In this review, we will analyze the anti- and pro-tumor intercellular interactions mediated by neutrophils, with a focus on generalizing the mechanisms underlying the interaction of neutrophils with tumor cells and T cells. Furthermore, we will provide an overview of cancer treatment strategies targeting neutrophil-mediated cellular interactions.

Oncogene (2024) 43:1163–1177; https://doi.org/10.1038/s41388-024-03004-5

INTRODUCTION

Cancer, a malignant tumor caused by uncontrolled cell division, is a complex disease whose initiation and progression are influenced not only by genetic alterations but also by the external environment, namely the tumor microenvironment (TME) [1, 2]. Recent studies on TME have revealed that cancer is not solely determined by genetic factors but is rather a multifaceted ecosystem. This ecosystem encompasses various non-cancer cells and their diverse interactions within the tumor [3]. TME is a local inflammatory microenvironment that supports tumor initiation, development, and metastasis. It is composed of tumor cells, immune cells (including microglia, lymphocytes, and macrophages, etc.), endothelial cells, stroma cells (including matrix fibroblasts), and non-cellular extracellular matrix constituents (including hyaluronic acid, fibronectin, laminin, and collagen, etc.) [1, 4]. These non-cancer cells were once thought to be bystanders in tumor development but are now known to play a crucial role in cancer pathogenesis [3]. TME can be described as a society that unfolds around cancer cells, where each cell has a complex social network and plays different roles at different stages of cancer development.

Neutrophils form the initial protection against infections [5]. They are the main type of white blood cells in the blood and can be rapidly recruited to the site of infection or injury [6, 7]. The presence of long-lasting neutrophils in tissues is a distinguishing characteristic of chronic inflammation, which subsequently results in harm to the surrounding tissues [5]. In the past decade, there has been an increased focus on studying the significance of neutrophils in cancer; they are now considered major participants in TME and functionally related to all stages of cancer progression [3, 8]. Fridlender et al. put forward a hypothesis in 2009 suggesting that, just like tumor-associated macrophages (TAMs) within the TME that can be divided into anti-tumor (M1 type) and pro-tumor (M2 type) phenotypes, tumor-associated neutrophils (TANs) can also undergo polarization into anti-tumor (N1 type) and pro-tumor (N2 type) phenotypes [9]. This study first proposed the heterogeneity of neutrophils in TME, and related research has continued to progress until it has gradually become a hot spot over the years. However, with the application of single-cell analysis technologies, such as single-cell RNA sequencing (scRNA-seq) and cytometry by time-of-flight (CyTOF), to the analysis of the TME, the heterogeneity of TAMs and TANs has been further revealed [10]. The binary classification of 'M1-M2' and 'N1-N2' is overly simplistic [11, 12]. Ng et al., using the transcriptional profiles obtained from neutrophil scRNA-seq, classified neutrophils in the TME into three clusters: T1, T2, and T3. The T1 cluster and the T2 cluster could potentially transform into the terminally differentiated T3 cluster, presenting the heterogeneity of neutrophils and the convertibility between clusters [13]. Research have shown that anti-tumor neutrophils eliminate tumor cells through both direct cytotoxicity

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Received: 20 November 2023 Revised: 2 March 2024 Accepted: 4 March 2024
Published online: 12 March 2024

and indirect activation of adaptive immune responses [14–20]. Conversely, neutrophils with a pro-tumor phenotype have been found to promote immune suppression, angiogenesis, and epithelial-mesenchymal transition (EMT) [7, 9, 21–24]. In fact, a pan-cancer study conducted on over 3 000 solid tumors across various cancer types revealed that intratumoral neutrophils were identified as the most unfavorable prognostic cell type compared to other groups of white blood cells infiltrating the tumor [7, 25].

As a huge and complex subject with much scattered research, tumor immunity is such a field that timely and comprehensive overviews are always needed. In this review, we first discuss the interactions between neutrophils and other members of TME, particularly cancer cells and T cells, and analyze the mechanisms underlying the anti- and pro-tumor functions of neutrophils. We will then discuss the therapy strategies that target neutrophil-mediated cellular interactions.

FOCUS ON THE ANTI-TUMOR EFFECTS OF NEUTROPHILS IN TME

Neutrophils are developed from hematopoietic stem cells in the bone marrow [6] and enter the circulatory system after being stimulated by cytokines, such as CXC-chemokines and granulocyte colony-stimulating factor (G-CSF) [26]. Though neutrophils have a short lifespan in the blood, they are constantly replenished from the bone marrow and constitute 50%–70% of the leukocytes in the human circulatory system [15, 27]. Neutrophils, as a crucial type of immune cells in the human body, play a critical role in defending against pathogen invasion and function as the first responders of acute inflammation [28, 29]. The pathogen-activated neutrophils kill the pathogens through multiple mechanisms, including direct phagocytosis and release of proteolytic enzymes. The engulfed bacteria through phagocytosis will be digested in a special organelle called phagolysosome, which degrades microorganisms by locally producing large amounts of superoxide, commonly known as “oxidative burst” [30, 31]. Additionally, neutrophils also serve as the bridge between primary and adaptive immunity and lead to the activation of adaptive immune cells. These characteristics of neutrophils lay the foundation for their anti-tumor functions in tumor development. The reactive oxygen species (ROS) and other oxidants secreted by neutrophils, as well as some granule enzymes with cytotoxic effects, can also induce the killing of cancer cells [18, 32]. Meanwhile, the characteristics of downstream immune cells activated by neutrophils also have anti-tumor effects [15]. In a sense, most of the killing effects of neutrophils on tumors are actually an extension of their anti-infection effects.

Anti-tumor effects of neutrophil-tumor interactions

Several neutrophil secretions, including oxidants, cytokines, and enzymes, have anti-tumor effects. The ROS derived from neutrophils activates an H_2O_2-dependent Ca^{2+} channel in the plasma membrane of cancer cells [5, 33]. The influx of calcium ions induces the death of cancer cells [33]. For example, tumor-entrained neutrophils (TENs) are enriched in pre-metastatic lungs. Instead of creating pre-metastatic niches, TENs actually hinder the spread of tumors in the lungs by generating hydrogen peroxide (H_2O_2) [18]. In addition to ROS, an increase in reactive nitrogen species (RNS) secretion by neutrophils also facilitates cancer cell killing [5, 34]. A subpopulation of neutrophils, which express the receptor tyrosine kinase MET, can be stimulated by hepatocyte growth factor (HGF) to increasingly produce nitric oxide (NO), a type of RNS, thereby promoting the killing of cancer cells [34]. Moreover, neutrophils can produce a substance, known as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) [32]. This ligand has the capability to induce programmed cell death, or apoptosis, in cancer cells [32]. Additionally, the effectiveness of this pathway is reinforced by stimulating neutrophils with interferon-gamma (IFNγ) [32]. Enzymes found in neutrophil granules also contribute to the destruction of cancer cells. Neutrophil elastases (NE) derived by neutrophils catalytically release the CD95 death domain. This domain then interacts with histone H1 isoforms, leading to the selective elimination of cancer cells [35]. NE not only hinders the growth of the primary tumor, but also triggers a CD8+ T cell-mediated abscopal effect, which targets distant metastases [35] (Fig. 1).

Anti-tumor effects of neutrophil-T-cell interactions

Some neutrophils can also activate T cells for anti-tumor responses. It has been demonstrated that TANs stimulate the anti-tumor effects of T cells during the initial phases of lung cancer in humans [36]. Radsak et al. found that polymorphonuclear cells (PMNs) can be stimulated to trigger the activation of T cells [19]. Moreover Cui et al. discovered a specific group of neutrophils that could enhance antigen-nonspecific and tumor-specific T cell responses through offering costimulatory signal molecules (OX40L, 4-1BBL, CD86, and CD54) [35]. Moreover, neutrophil-dependent IL-12 can induce UTCαβ, a group of CD4−CD8− unconventional αβ T cells, to polarize toward a type 1 direction that secretes tumor-toxic IFNγ [20]. There are also indirect mechanisms of cooperative anti-tumor pathways, such as the uptake of neutrophil-derived NE by breast tumor cells, which enhanced antigen presentation and activated CD8+ T cells, reducing tumor occurrence at distant sites [35, 37, 38] (Fig. 1).

Developing the anti-tumor therapeutic value of neutrophils

Neutrophils are important components of TME. Full utilization of their anti-tumor effects will improve both targeting and efficiency. Currently, there are two main strategies being explored in this area of research: one is to stimulate the anti-tumor activity of neutrophils, and the other is to utilize the ability of neutrophils to approach tumor cells for drug delivery purposes. Both approaches represent novel strategies for targeting cancer cells.

Therapy to skew neutrophils toward an anti-tumor phenotype. Although it is now understood that neutrophils play a role in facilitating the growth and spread of various cancers [6, 15, 17], they also have undeniable potential for anti-tumor activity. Increasing evidence supports the possibility of neutrophils exerting anti-tumor functions in some situations [18, 20, 39, 40]. Activating the anti-tumor potential of neutrophils and using them as anti-tumor effector cells is a novel therapeutic approach for cancer [39]. Linde et al. developed a neutrophil activation therapy, which is a three-component therapy consisting of TNF, anti-CD40 monoclonal antibody, and anti-tumor monoclonal antibody (mAb), that can mobilize and activate neutrophils to exert potent anti-tumor activity [39]. This therapeutic approach has demonstrated favorable efficacy in different established tumor types, such as colorectal cancer, breast cancer and lung cancer [39]. It has also shown the potential to decrease the occurrence of both spontaneous and experimental lung metastasis [39]. In addition, this treatment has the ability to stimulate antigen-presenting cells, generate a memory response in T cells, and develop resistance to cancer rechallenge in successfully treated mice [39]. Mattoscio et al. found that in a human papilloma virus (HPV) model of tumorigenesis, Resolvin D1, an endogenous mediator that promotes the relief of inflammation, reprograms PMNs to an anti-tumor phenotype and stimulates the releasing of MCP-1, which increases the recruitment of antitumoral classical monocytes [41] (Fig. 2).

Neutrophil-based bionic drug delivery. Neutrophils are crucial members in TME and have excellent tumor-targeting ability. A novel research strategy is to modify neutrophils to make some of them act as undercover agents, approaching and lurking in the TME, and releasing anti-tumor drugs at the appropriate time (Fig. 2). Ren et al. proposed a novel drug delivery system using

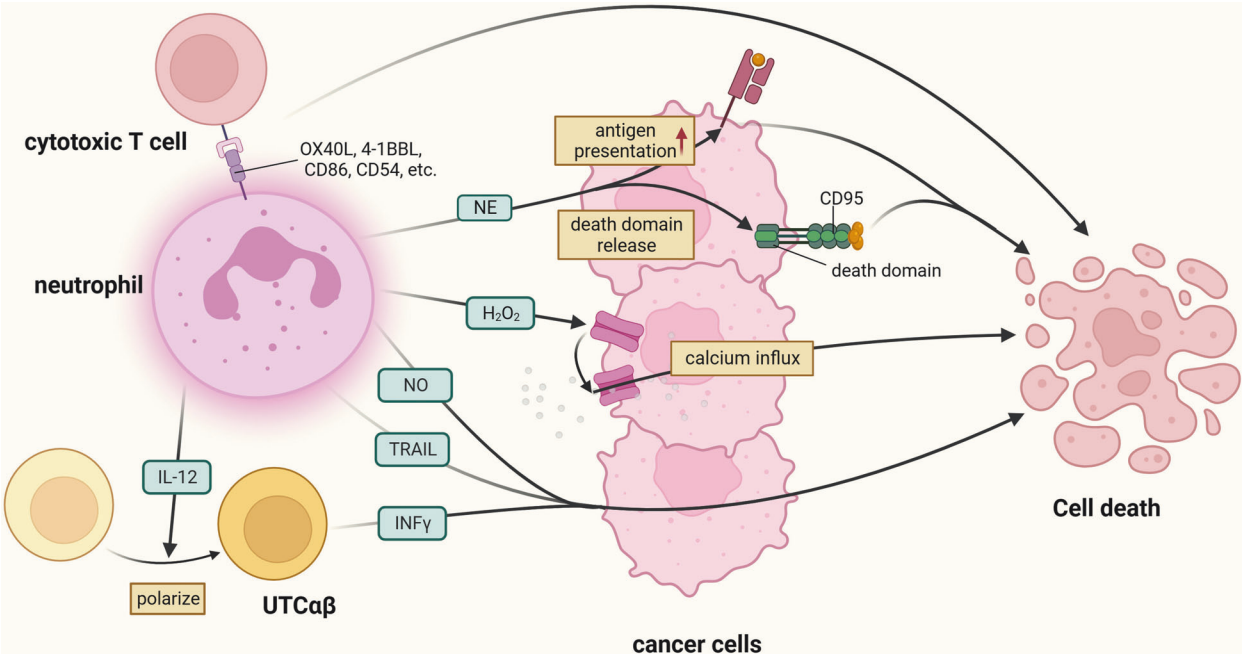

Fig. 1 The anti-tumor effects of neutrophils in TME. In terms of neutrophil-tumor interactions, neutrophils secrete enzymes, oxidants, and cytokines to kill cancer cells. NE has the ability to enhance the presentation of antigens by cancer cells, thereby facilitating the activation of cytotoxic T cells. It can also liberate the CD95 death domain via proteolysis to induce cell death. H_2O_2 activates the Ca^{2+} channels on the cancer cell membrane, and the inward flow of calcium ions induces cell death. Moreover, neutrophil-derived NO and TRAIL can also promote the killing of cancer cells. In terms of neutrophil-T-cell interaction, neutrophils provide co-stimulatory signals through OX40L, 4-1BBL, CD86, and CD54 molecules to enhance T cell responses. Neutrophils can also secrete IL-12 to induce CD4−CD8−UTCαβ to polarize toward a type 1 direction that secretes tumor-toxic IFNγ.

neutrophils as carriers to deliver liposomes containing paclitaxel and hydroxychloroquine, which inhibit autophagy, modulate tumor stemness, and prevent postoperative recurrence and metastasis of triple-negative breast cancer [42]. The effectiveness of this system was validated by both in vitro and in vivo experiments, suggesting its potential clinical application [42]. Chu et al. developed a specially designed biomimetic platform with a neutrophil membrane as a shell, which inherits neutrophil-like tumor-targeting ability. The chemotherapeutic drugs and breast cancer stem cell (CSC) differentiation agents loaded in neutrophils are anchored with nitroimidazole (NI). When the platform, which relies on the natural targeting ability of neutrophils, reaches the metastatic tumor cells, the modified NI group is rapidly reduced to aminoimidazole, and both drugs are explosively released [43]. Similarly, Cui et al. developed a nanodevice that can cause DNA damage in cancer cells, which uses a neutrophil membrane as a natural coating to disguise the nanodevice, enhancing its tumor targeting and immune evasion ability [44]. In addition, there is also research attempting to use neutrophil-derived exosomes for drug delivery. It has been proved in mice that it can effectively inhibit tumor growth and widely prolong the lifespan of mice, which is also a very promising research and development direction [45]. Moreover, the ability of neutrophils to pass through blood brain barrier (BBB) makes neutrophils potential drug carriers for brain tumor [46]. Chang et al. genetically edited human pluripotent stem cells to knock in anti-glioblastoma chimeric antigen receptor (CAR) constructs. The derived CAR-neutrophils are designed to deliver TME-responsive nanodrugs, such as tirapazamine (TPZ), that are released to kill target tumor cells [46].

FOCUS ON THE PRO-TUMOR EFFECTS OF NEUTROPHILS IN TME

If we consider the anti-tumor effects of neutrophils as an extension of their natural attribute, the traits of neutrophils in TME are quite different. Besides their anti-tumor effects, neutrophils also contribute to cancer development through promoting angiogenesis, immunosuppression, and remodeling the extracellular matrix [15], which means that TANs are heterogeneous. Moreover, TANs have a longer lifespan than circulating neutrophils and are associated with poor prognosis in many cancers [14]. From the previous research, we can conclude that the crosstalk between neutrophils and cancer cells is particularly critical in the process of neutrophil transformation, as it is both the key for cancer cells to recruit and transform neutrophils for their own use, and the key for neutrophils to work for cancer cells.

The pro-tumor effects of tumor-neutrophil interaction

Tumor function in orchestrating pro-tumor responses of neutrophils. Recruiting neutrophils and reprogramming them into the pro-tumor phenotype is an important issue for cancer cells to build a liveable TME. The interaction between cancer cells and neutrophils is the main approach for tumor cells to orchestrate the pro-tumor responses of neutrophils, which can be mediated by cytokines, enzymes, micromolecules and membrane proteins, etc. (Fig. 3).

Cytokines are the most widely studied signals from cancer cells to neutrophils: Cytokines, including chemokines, interleukins, lymphokines, etc., are one of the main mediators for cancer cells to contact and modify neutrophils. The production, release, and behavior of neutrophils are influenced by a series of cytokines. For example, G-CSF induces emergency granulopoiesis [47, 48], promotes the mobilization of neutrophils from bone marrow to peripheral blood [49], prolongs the lifespan of neutrophils by inhibiting apoptosis [15, 50], and stimulates the differentiation of neutrophils to become either anti- or pro-tumor neutrophils [15]. CXC-chemokines also play an essential role in the communication between neutrophils and cancer cells, which affects the migration, recruitment, and polarization of neutrophils [15]. Neutrophils express high levels of C-X-C chemokine receptors 1 and 2 (CXCR1

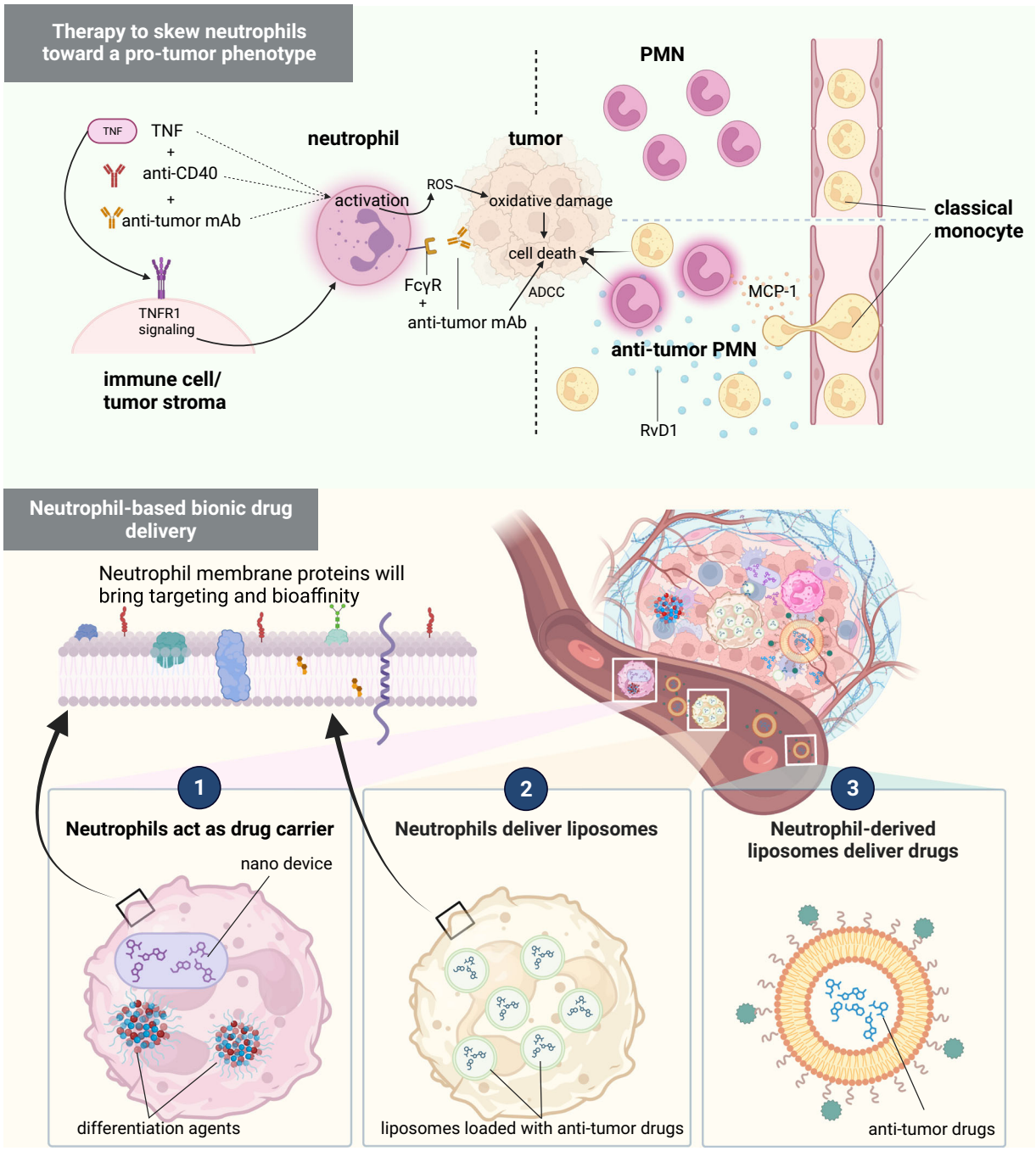

Fig. 2 Developing the anti-tumor therapeutic value of neutrophils. Therapy to skew neutrophils toward a pro-tumor phenotype is a novel therapeutic approach for cancer. Neutrophil activation therapy, a tripartite therapy comprising TNF, anti-CD40 monoclonal antibody, and anti-tumor monoclonal antibody, has the potential to mobilize and activate neutrophils, thereby exerting robust anti-tumor activity (reproduced from Linde et al. [39]). Resolvin D1 (RvD1), an endogenous mediator known for its role in resolving inflammation, can reprogram PMN to adopt an anti-tumor phenotype and stimulate the releasing of MCP-1, to increase the recruitment of antitumoral classical monocytes. Another promising strategy is neutrophil-based bionic drug delivery. Given their high tumor-targeting capability and biocompatibility, neutrophils can be harnessed to deliver anti-tumor drugs and liposomes. Furthermore, liposomes derived from neutrophils present a potential vehicle for drug delivery.

and CXCR2), while their ligands including CXCL1,2,5,6 and IL-8, are widely expressed by tumors, fibroblasts, endothelial cells and tumor-infiltrating leukocytes [15, 51, 52]. The binding of CXCL1 and CXCL2 to CXCR2 mobilizes the release of neutrophils from the bone marrow [53], while CXCL12 binds to neutrophil CXCR4 to mediate the retention of immature neutrophils in the bone marrow [54]. In addition, studies have demonstrated that within the TME, tumor-derived transforming growth factor-β (TGFβ) is able to polarize a population of TANs into a tumor-promoting phenotype, characterized by expressing high levels of CCL17, arginase 1 (ARG1) and CXCL14 and low levels of TNF, CCL6, CXCL10, CXCL13 and intercellular adhesion molecule 1 (ICAM1) [9, 15] (Fig. 3).

Cancer cells secrete large amounts of cytokines to recruit and modify neutrophils for creating a suitable TME. Recent studies have explored the mechanisms by which cancer cells up- or down-

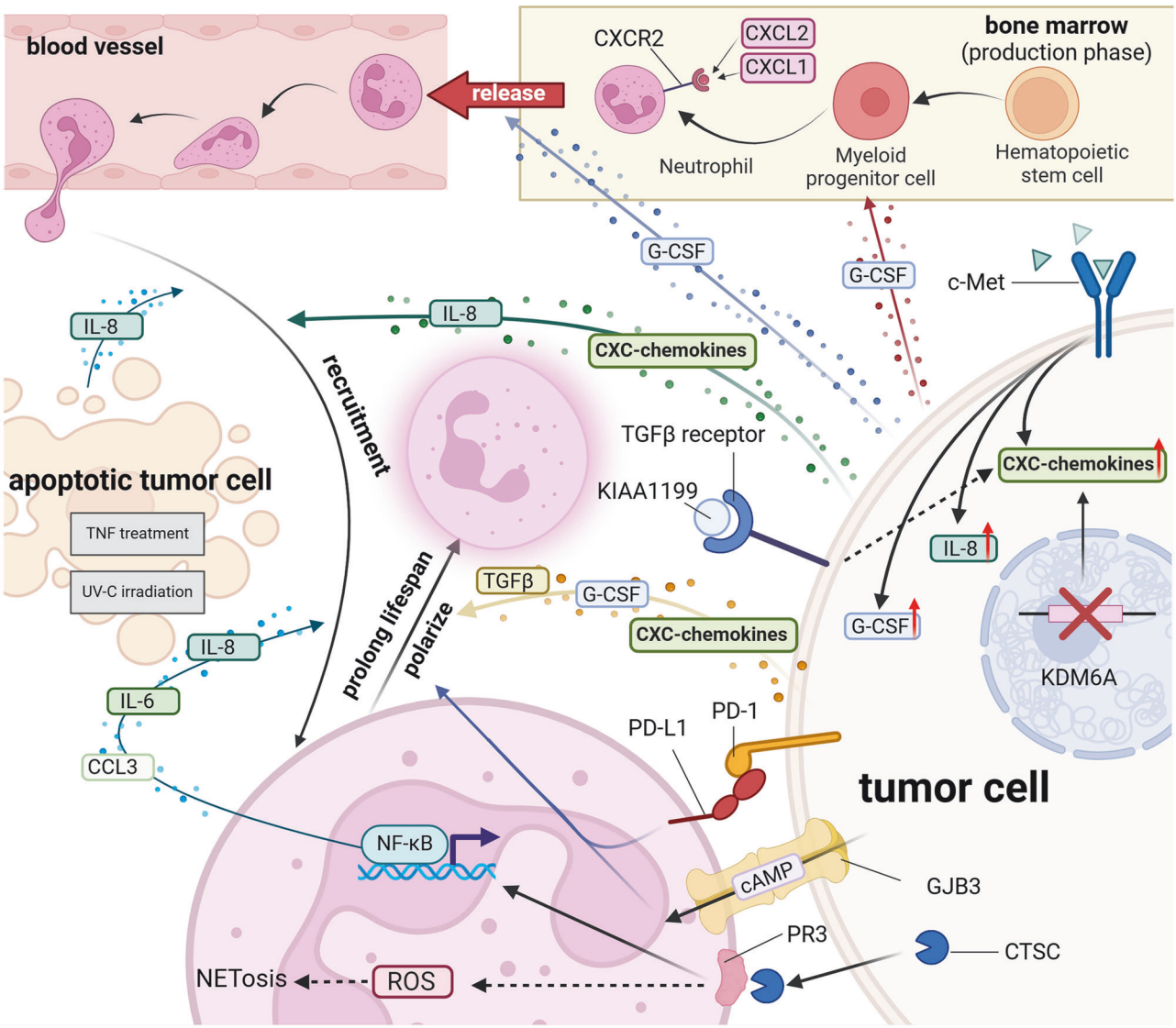

Fig. 3 Tumor function in orchestrating pro-tumor responses of neutrophils. Tumor cells, through changes in endogenous signaling pathways (KDM6A deletion) and external stimuli (KIAA1199 and c-Met pathways), upregulate the expression of a series of cytokines (G-CSF, CXC-chemokines, and IL-8). Tumor-derived G-CSF enhances the generation of neutrophils in the bone marrow. G-CSF, CXCL1, and CXCL2 aid in the release of neutrophils from bone marrow to the blood. Meanwhile, IL-8 and CXC-chemokines secreted by tumor cells (also apoptotic tumor cells) can help recruit neutrophils to TME. Tumor cells also secrete cytokines such as TGFβ to polarize the recruited neutrophils into pro-tumor phenotype. Furthermore, tumor cells can promote the release of chemotactic factors (IL-6, IL-8 and CCL3) from neutrophils, further enhancing the recruitment of neutrophils.

regulate the cytokine expression in a variety of ways. The upstream pathway is often accompanied by the expression conditions of tumor-suppressor or tumor-promoting genes. For example, genome-wide bromouridine sequencing analysis revealed that in pancreatic ductal adenocarcinoma cells (PDACs) with lysine-specific demethylase 6A (KDM6A) knockout, there is an upregulation of many chemotactic cytokines, especially CXCL1 [55]. While brain metastatic cells of breast cancer highly express cellular-mesenchymal epithelial transition factor (c-Met), a tyrosine kinase receptor, which stimulates the expression of various cytokines, including CXCL1, IL8, and G-CSF, by activating downstream signaling pathways [56]. Similarly, KIAA1199, which is highly expressed in colorectal cancer (CRC), can interact with TGFβ receptors to activate the TGFβ/SMAD3 signaling pathway, thereby promoting the secretion of CXCL1 and CXCL3 [57]. Except for expression conditions, the death of tumor cells is also a way to promote cytokine secretion. Schimek et al. found that apoptosis induced by combined UV-C irradiation and TNF treatment, or other chemotherapies, led to a considerable release of chemokines that attract neutrophils (most significantly IL-8) [58] (Fig. 3).

Non-cytokine communication medium from cancer cells to neutrophils: In addition to cytokines, some other plasma proteins, enzymes and micromolecules are also utilized by cancer cells to recruit and modify neutrophils. For example, one such plasma protein is histidine-rich glycoprotein (HRG), a secreted glycoprotein that is mainly produced by hepatocytes [59]. However, hepatocellular carcinoma (HCC) inhibits its secretion, resulting in reduced binding of HRG to neutrophil membrane protein FCγR1, which leads to the activation of PI3K and NF-κB, thereby increasing the secretion of IL-8 [59]. At the same time, there is an upregulation in the production of ROS, stimulating the generation of neutrophil extracellular traps (NETs) [59]. The role of NETs will be discussed in more detail later in the text. Enzymes can

also be utilized. Cancer cells secrete cathepsin C (CTSC), which catalytically activates neutrophil membrane-bound proteinase 3 (PR3), resulting in the upregulation of CCL3 and IL-6 in downstream signaling pathways, promoting the recruitment of neutrophils [60]. Meanwhile, the CTSC-activated CTSC-PR3-IL-1β axis induces the production of ROS in neutrophils, promoting the formation of NETs [60]. High levels of adenosine are a hallmark of TME, and a means by which cancer cells inhibit immunity [61, 62] (Fig. 3). Pannexin 1 (PANX1) is a channel that is capable of releasing intracellular ATP into the extracellular space, leading to an increase in extracellular ATP (exATP) levels under normal physiological circumstances, which is highly expressed in basal-like breast cancer [61, 62]. It's been demonstrated that highly expressed PANX1 is related to TAN infiltration [61, 62].

Cancer cells also communicate with neutrophils in TME directly. Gap junction protein beta 3 (GJB3) is a connexin that is upregulated in cancer cells. Studies have shown that GJB3 forms channels between PDAC and neutrophils in the TME, transferring cAMP from tumor to neutrophils, promoting the viability and polarization of neutrophils [63]. Immune suppression mediated by the PD-L1/PD-1 axis is a crucial mechanism for cancer cells to evade immune surveillance. Yajuk et al. found that PD-1 expressed by cancer cells can bind to PD-L1 on neutrophils, blocking the cytotoxicity of neutrophils [64] (Fig. 3).

Neutrophil-tumor communications facilitate tumor progression. Before cancer formation, neutrophils in the inflammatory microenvironment may inadvertently induce cancer initiation by causing tissue damage. Neutrophils are one of the important factors promoting inflammation developing into cancer [2]. Following the initiation of cancer, cancer cells begin to recruit and reprogram neutrophils, which in turn contribute to cancer development, including primary tumor growth, metastasis, and cancer cell colonization of metastatic sites.

Neutrophil secretions contribute to carcinogenesis: Cancer can develop as a result of persistent inflammation, which is often characterized by the continuous infiltration of neutrophils into tissues [5]. Dysregulated recruitment of neutrophils can cause tissue damage through the secretion of cytotoxic oxidants and proteases, thereby facilitating carcinogenesis by disrupting tissue integrity [65, 66].

In an inflammatory environment, neutrophils release various oxidants such as ROS and RNS (Fig. 4). These neutrophil-derived oxidants can cause genome instability in tissue cells, resulting in damage such as single-strand or double-strand DNA breaks, base or deoxyribose modifications, and DNA crosslinking [67]. DNA bases are susceptible to oxidative modifications by ROS [68, 69]. Among the DNA bases, guanine is especially prone to oxidative damage by ROS due to its low reduction potential [68, 69]. The thoroughly oxidized product of guanine, 7,8-dihydro-8-oxoguanine (8-oxoG), can change its conformation to act like thymine and form a stable pair with adenine [68]. This mismatch will introduce a single base mutation during DNA replication. In addition to the direct effects, ROS can also mediately generate electrophilic products, such as epoxides and malondialdehyde (MDA), through lipid peroxidation of plasma membrane [67, 70]. MDA reacts with several nucleic acid bases (dG, dA, and dC) to form adducts [67, 71]. MDA-DNA adducts appear to be mutagenic, as they induce cell cycle arrest [72], as well as mutations in oncogenes and tumor suppressor genes observed in human tumors [67].

Independent of the damage caused by ROS, a study has found that neutrophils can secrete pro-inflammatory miRNA to damage tissue cells in inflammatory environments [73] (Fig. 4). Pro-inflammatory miRNAs, such as miR-23a and miR-155, derived from neutrophils in the intestinal mucosa facilitate accumulating double-strand breaks by causing the collapse of replication forks and inhibiting homologous recombination (HR) through targeting the HR regulator RAD51 [73].

Neutrophils promote primary tumor progression in multiple aspects: The development of single-cell technologies in the past decade has revealed the vast heterogeneity of neutrophils in TME [6]. It is now understood that neutrophils have high plasticity and can also alter the phenotype of other immune cells. The heterogeneity of neutrophils may enable them to perform multifaceted functions in promoting tumor progression [7].

Neutrophils can promote the survival and proliferation of tumors. As an inflammatory cell, neutrophils can secrete a variety of cytokines to regulate the survival state of cancer cells. For example, neutrophils can release various growth factors, including platelet-derived growth factor (PDGF), HGF and epidermal growth factor (EGF), to promote the growth of cancer cells [65]. At the same time, neutrophils can also secrete IL-1 receptor antagonist (IL-1RA) to eliminate cancer cell senescence, which has been found to promote the progression of prostate cancer [65]. In addition, granule proteins derived from neutrophils also play an important role in promoting cancer development. Lipocalin 2 secreted by neutrophils can enhance the stemness of tumor cells [56], and NE can degrade insulin receptor substrate 1 (IRS1) in cancer cells to activate the proliferation of lung tumor cells, thereby promoting the interaction between PI3K and PDGF receptor [15, 23]. Furthermore, cancer cell proliferation can also be triggered by neutrophil-derived NETs and their associated molecules, including matrix metalloproteinase 9 (MMP9), NE and high mobility group protein B1 (HMGB1) [15, 74, 75]. For example, the activity of MMP9 and NE found within NETs leads to the protein hydrolysis remodeling of laminin 111 in the extracellular matrix, resulting in the creation of a novel epitope, which activated dormant cancer cells through α3β1 integrin [15, 74]. The enzymatic remodeling of HMGB1 protein in NETs triggers Toll-like receptor 9 (TLR9)-dependent activation of cancer cells, enhancing their ability to proliferate, migrate, and invade [15, 75]. Furthermore, tumor cells can recognize NET-DNA through transmembrane protein CCDC25, activating the downstream ILK-β-parvin signaling pathway, thereby enhancing cell proliferation [7, 76] (Fig. 4).

To sustain growth, tumor cells must coordinate establishing a sufficient blood supply for themselves. The indirect interaction between neutrophils and cancer cells includes neutrophil-induced angiogenesis, which provides nutrients for tumor cell survival. The neutrophils in TME can produce pro-angiogenic factors, including BV8, MMP9, and vascular endothelial growth factor A (VEGFA), to promote tumor angiogenesis [15, 77–81]. In the extracellular matrix, neutrophil-derived MMP-9 and NE can also degrade the extracellular matrix to release VEGF [7, 15, 78, 82]. Neutrophil-derived BV8 is a mitogen for endothelial cells [15, 80]. NETs and their associated contents also play a vital role in angiogenesis. Myeloperoxidase (MPO) contained in NETs induces an angiogenesis-promoting reaction in endothelial cells through a TLR4-dependent mechanism, stimulated by H_2O_2 [15, 83]. Soluble cathepsin G in NETs stimulates angiogenesis by cleaving pro-MMP9 and subsequent TGFβ activation, which has been shown to promote tumor vascularity [7, 84] (Fig. 4).

In addition to promoting cancer cell proliferation and angiogenesis, neutrophil-mediated interactions with other immune cells contribute to creating an immunosuppressive niche that supports the growth of the primary tumor. This will be discussed in more detail later in the text.

Neutrophils contribute to tumor metastasis: In recent research, the topic of neutrophils and metastasis has become very popular, especially the relationship between NETs and metastasis. Neutrophils can promote cancer cell metastasis through many pathways. In the following, we will discuss this from three aspects:

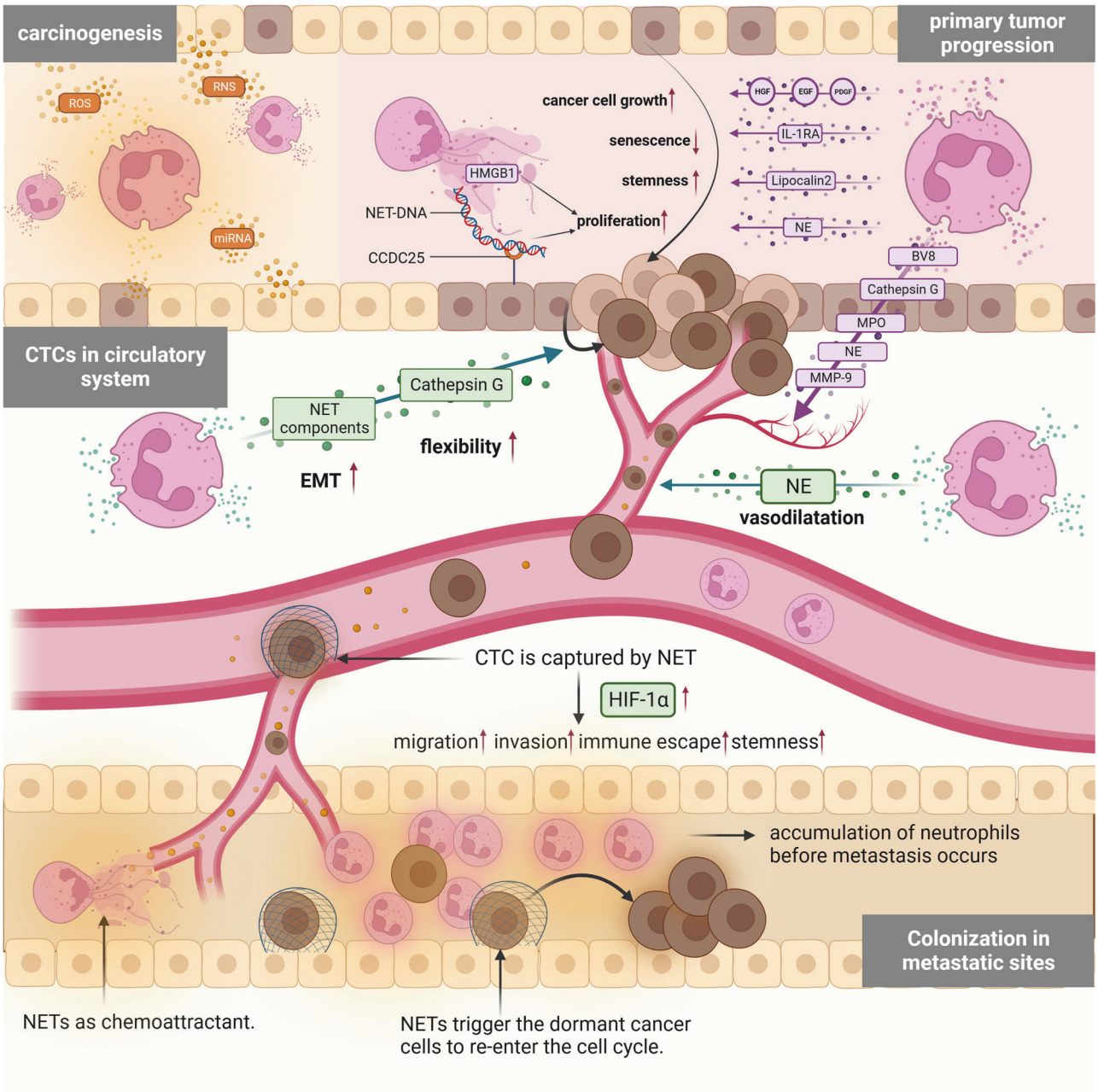

Fig. 4 Neutrophil-tumor communications facilitate tumor progression. During the carcinogenesis stage, neutrophils induce epithelial cell carcinogenesis by secreting oxidants and miRNA. In the primary tumor progression stage, neutrophils enhance tumor cell growth, stemness, and proliferation while inhibiting tumor cell senescence by NETosis and secreting cytokines, enzymes, growth factors, etc. Neutrophils also promote tumor angiogenesis by secreting BV8, Cathepsin G, MPO, NE, and MMP-9, providing nutrients for cancer cells and paving the way for metastasis. Neutrophils enhance the metastatic ability of cancer cells by producing NET components and secreting Cathepsin G, prompting some cancer cells to become circulating tumor cells (CTCs) that enter the circulatory system. In the circulatory system, neutrophils dilate blood vessels by releasing NE to promote cancer cell migration. Meanwhile, the migration ability, immune evasion ability, invasion ability, and stemness of CTCs captured by NET in blood vessels are enhanced, promoting the colonization ability of CTCs. Neutrophils accumulate in the metastatic niche in advance and secrete chemokines to induce CTCs to enter the metastatic niche. Dormant cancer cells in the metastatic niche captured by NET can re-enter the cell cycle and begin to proliferate in the metastatic niche.

infiltration, transit, and the establishment of a metastatic microenvironment.

Cancer cells in primary tumors need to undergo a series of internal and external changes to gain the ability to leave the primary tumor site. Many studies have shown that neutrophils can increase the invasiveness of primary tumors. In addition to the previously mentioned tumor angiogenesis effect, neutrophil-derived cytokines, enzymes, and NETs can also become mediators to promote cancer cell invasion. Cathepsin G and TNF from neutrophils were reported to stimulate cancer cell migration [7, 65]. Cathepsin G can hydrolyze extracellular matrix to give cancer cells the flexibility to move, enabling cancer cells to acquire invasive capabilities [7]; in addition, this enzyme helps to induce multicellular aggregation of human breast cancer MCF-7 cells, which can cause tumor emboli, a symbol of cancer cell infiltration [85]. Furthermore, many studies have pointed out the multi-roles of NETs in promoting invasion. One possible mechanism is that the tumor NET-DNA sensor, such as CCDC25, imparts chemotaxis

to the cancer cells, so that neutrophil-derived NET-DNA can induce epithelial-mesenchymal transformation (EMT) [76]. In non-small cell lung cancer (NSCLC), NETs induce the activation of the NF-κB/NOD-like receptor protein 3 (NLRP3) signaling pathway. This activation is achieved by suppressing the expression of MIR503HG, a long non-coding RNA (lncRNA), which promotes EMT and metastasis [86]. There are also studies showing that in PDAC, NETs promote cancer cell EMT, migration, and invasion through the IL-1β/EGFR/ERK pathway [87]. The occurrence of abdominal infection complications (AIC) following gastrectomy triggers the release of NETs, dependent on TGFβ signaling [88]. TGFβ inhibitors can reduce metastasis in nude mice with AIC-induced NETs in the liver and peritoneal metastasis [88]. In colorectal cancer (CRC) cell lines, purified NETs induced the formation of cancer cell filopodia hence cell movement [89]. This is related to the increased expression of mesenchymal marker mRNA (vimentin, fibronectin) and EMT-promoting transcription factors (ZEB1, Slug), as well as the downregulation of epithelial cell adhesion molecule (EPCAM) and epithelial marker E-cadherin (CDH1) [89] (Fig. 4).

When cancer cells pass through endothelial cells of blood vessels and enter the circulatory system, the NE secreted by neutrophils can cause the expansion of the tumor vascular system, promoting the migration of cancer cells [90]. At the same time, NETs in the circulatory system can capture circulating tumor cells (CTCs). These cancer cells can adhere to the liver sinusoids through NETs, promoting micro-metastasis in the liver [91]. Studies have also shown that after CTCs are captured by NETs, they highly express hypoxia-inducible factor-1α (HIF-1α), thereby enhancing the migration, invasion, immune escape, and stemness of CTCs, promoting tumor metastasis [92] (Fig. 4).

Interestingly, neutrophils that can move freely also prepare new habitats for cancer cells that cannot yet move or have not yet arrived. Neutrophil accumulation in the lungs before metastasis has been observed in mouse models of MMTV-PyMT mammary cancer, nicotine-exposed breast cancer, and melanoma. This accumulation of neutrophils in the lungs has been closely linked to the occurrence of lung metastasis [65, 93–95]. Preventing the recruitment of neutrophils to premetastatic sites or inhibiting the formation of NETs can effectively hinder the occurrence of metastasis [65]. The importance of NETs to metastasis is clear, and its mechanisms can be explained as follows. On the one hand, NETs in pre-metastatic niches can act as chemoattractants for cancer cells [76]; on the other hand, metastatic cancer cells can enter a dormant state in distant tissues for extended periods, and the presence of NETs is crucial for their activation. Within NETs, enzymes such as NE and MMP9 can break down laminin in the ECM. This breakdown of laminin leads to the activation of α3β1 integrin signaling, ultimately stimulating dormant cancer cells to re-enter the cell cycle [74] (Fig. 4).

The interactions between non-cancer cells and neutrophils promote cancer development

The high heterogeneity of TME poses a challenge to the understanding of the cellular interactions and mechanisms involved in tumor progression. Exploring the interactions between neutrophils and non-cancer cells is fundamental to understanding the cellular interactions and mechanisms involved in tumor progression. In the following text, we present a summary of the crosstalk between neutrophils and immune/non-immune cells in TME.

Neutrophil-mediated crosstalk with immune cells. In the TME, immune cells exhibit complex interactions, which can be broadly categorized into two factions: anti-tumor and pro-tumor. Between these factions, there is a complex landscape of conflict and assimilation. Neutrophils, as one of the main components of TME, are increasingly being discovered to mediate pro-tumor effects in conjunction with other immune cells within the TME.

T cells: The effects of neutrophils on T cells include recruiting, remodeling, controlling the direction of differentiation and suppressing tumor cytotoxicity. For example, PMNs, a type of neutrophils, have been observed to impair the activation, proliferation, and viability of CD4+ T cells through direct physical contact [96]. Experimental evidence has shown the presence of PD-L1 on neutrophils can interact with PD-1 present by T cells, leading to the suppression of T cell cytotoxicity; meanwhile, these tumor-infiltrating neutrophils highly express FasL and PD-L2, which can suppress the immune activity of tumor-specific CD8+ T cells [97, 98]. TANs can recruit regulatory T cells (Tregs), promote their infiltration into tumors, and create an immunosuppressive microenvironment [99]. By regulating mitochondrial respiration and metabolic reprogramming in naive CD4+ T cells, NETs can enhance the activity and differentiation of Tregs, ultimately facilitating tumor progression [100, 101]. In addition to these more traditional means, it was found in ascites fluid supernatants (ASC) from ovarian cancer patients that ASC-activated neutrophils can damage T cells by trogocytosis, causing metabolic disruption and paralysis of T cells [102] (Fig. 5).

The effects of T cells on neutrophils include recruitment and induction of neutrophil polarization. γδT17 cells, a subtype of innate-like T lymphocytes, facilitate the enrichment of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) by secreting IL-8, IL-17A, TNF-α and GM-CSF [103–105]. At the same time, studies have also shown that tumor-induced γδ T cells producing IL-17 can cause the polarization of neutrophils, which results in a phenotype of neutrophils that suppresses CD8+ T cells, which facilitates forming metastases in distant organs [106, 107] (Fig. 5).

Other associated immune cells: In addition to the most studied T cells, the social network of neutrophils in the TME also covers immune cells including macrophages, dendritic cells, natural killer (NK) cells, platelets, etc.

An interesting study found that in non-NK-cell-deficient mice, high G-CSF expression (amplifying neutrophils) inhibited tumor metastasis; but in NK-cell-deficient mice, this operation promoted tumor metastasis [108]. The study showed that both NK cells and neutrophils alone can inhibit tumor metastasis, but NK's inhibitory ability is stronger [108]. Neutrophils can inhibit NK's tumor-killing ability by secreting ROS, resulting in a net promotion of tumor metastasis [108]. TANs can also recruit macrophages by secreting CCL2 [99, 109]. Neutrophil-derived cathepsin G can truncate the chemokine CCL15 to increase the migration ability of monocytes by nearly 1000 times, which may be beneficial for the recruitment of macrophages in the tumor microenvironment [7, 110]. Similarly, the ability of cathepsin G to cleave chemerin, a chemotactic protein, can also enhance the chemotaxis of dendritic cells [7, 111]. In addition, during the formation of early metastatic niches, the recruitment of neutrophils depends on the CXCL5 and CXCL7 chemokines secreted by platelets stimulated by tumor cells [112] (Fig. 5).

Neutrophil-mediated crosstalk with non-immune cells. Besides immune cells, tumor cells also mobilize a variety of tissue cells to construct the tumor immune microenvironment. These tumor-stimulated tissue cells exert some functions such as recruiting and reprogramming neutrophils. Mesenchymal stromal cells activated by TNFα can derive CXCR2 ligands (including CXCL1, 2, and 5) to attract CXCR2 neutrophils to promote tumor growth [113]. It has also been reported that lung adenocarcinoma can remotely stimulate bone-resident osteocalcin-expressing osteoblasts in the bone, which provide tumor cells with pro-cancerous neutrophils [114]. Tumor-induced high expression of Ang-1 in hepatocytes facilitates the infiltration of neutrophils [115]. During this process, neutrophils are not only recruited, but they are also likely to undergo phenotypic changes in their characteristics. As a

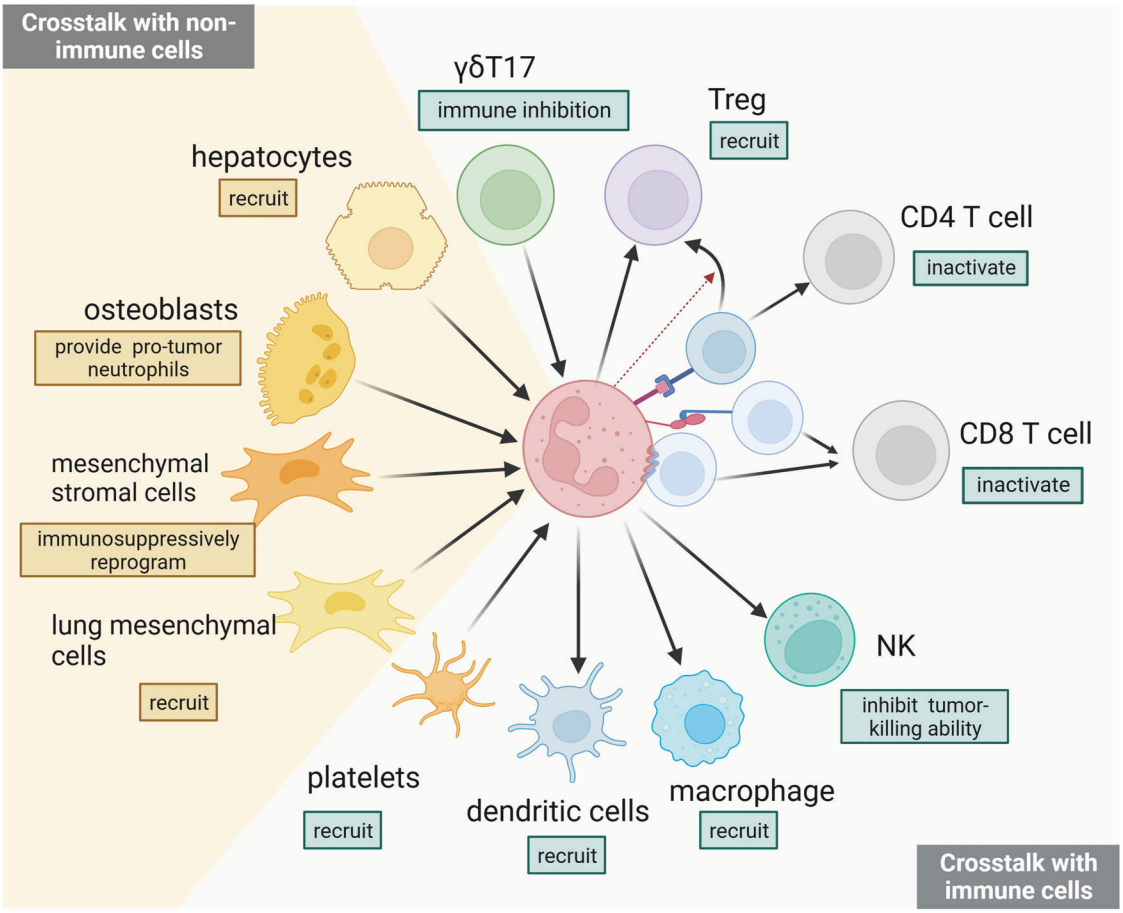

Fig. 5 The interactions between non-cancer cells and neutrophils promote cancer development. In TME, neutrophils interact with both immune and non-immune cells. The effects of neutrophils on other immune cells include recruitment (like macrophages, dendritic cells, and Tregs), the inhibition of tumor cytotoxic immune cells (like CD4+ T cells, CD8+ T cells and NK cells), and the promotion of CD4+ T cell conversion to Tregs. The effects of immune cells on neutrophils include recruitment and immunosuppression. The effects of non-immune cells in the TME on neutrophils include recruitment (hepatocytes and lung mesenchymal cells), immune reprogramming (mesenchymal stromal cells), etc. Moreover, lung adenocarcinoma can remotely activate osteoblasts in the bone to provide pro-cancer neutrophils for tumor cells.

common site of tumor metastasis, the lung contains lung mesenchymal cells that can immunosuppressively reprogram neutrophils to promote breast cancer metastasis [116] (Fig. 5).

The results of such studies are undoubtedly surprising and provide more ideas for the development of anti-cancer therapies. However, as more and more underlying mechanisms have been discovered, it has become increasingly difficult to identify key targeted pathways in the complex social networks in TME.

Therapies based on the inhibition of the pro-tumor neutrophils

Although there exist anti-tumor neutrophils in the TME, intratumoral neutrophils have been identified as the most unfavorable cell type in terms of prognosis among all the different types of white blood cells that infiltrate tumors. This finding is based on a pan-cancer study of over 3000 solid tumors from 14 diverse cancer types [7, 25]. Inhibition of the pro-tumor neutrophils is a new research direction of neutrophil-based cancer therapy. In the following, we will discuss the therapies that target the inhibition of neutrophil recruitment and NET-formation.

Therapy to inhibit neutrophil recruitment. Recruitment of neutrophils is the first step that cancer cells take to utilize neutrophils. Many studies now focus on the inhibition of neutrophil recruitment. In the following, we will discuss two types of representative neutrophil recruitment inhibitors.

TGFβ inhibitor: As previously mentioned, TGFβ in TME will induce TAN polarization towards the pro-tumor phenotype. Furthermore, although it has been proven that TGFβ cannot directly induce neutrophil migration through transwell or under-agarose migration assays, it may potentially facilitate the process of neutrophil recruitment by upregulating chemotactic factors or receptors, and amplifying chemokine signaling [117–120]. TGFβ is a member of a family of multifunctional proteins that regulate cell proliferation, differentiation, and angiogenesis through complex cell signaling pathways [121, 122]. In NSCLC, there is a strong negative association between elevated TGFβ concentrations and prognosis [121, 123]. However, the role of TGFβ in tumors is dual, as studies have also shown that TGFβ has tumor suppressive effects [124–126]. There are several pharmacological approaches to blocking TGFβ signaling, including neutralizing antibodies, antisense oligonucleotides (ASOs), and small molecule inhibitors (SMIs) [127]. Current research on TGFβ-based cancer therapies focuses on inhibiting TGFβ and targeting its downstream signaling pathways. The goal of ASOs is to bind and prevent the translation of TGFβ mRNA, thereby reducing its expression [127]. For instance, experiments conducted on mesothelioma and prostate cancer cell lines have provided evidence that these treatments are effective in decreasing the expression of TGFβ protein and inhibiting the ability of tumor cells to grow independently without anchorage [127–129]. Further in vivo experiments have shown that tumor growth is reduced in animals treated with ASOs [127–129]. Due to

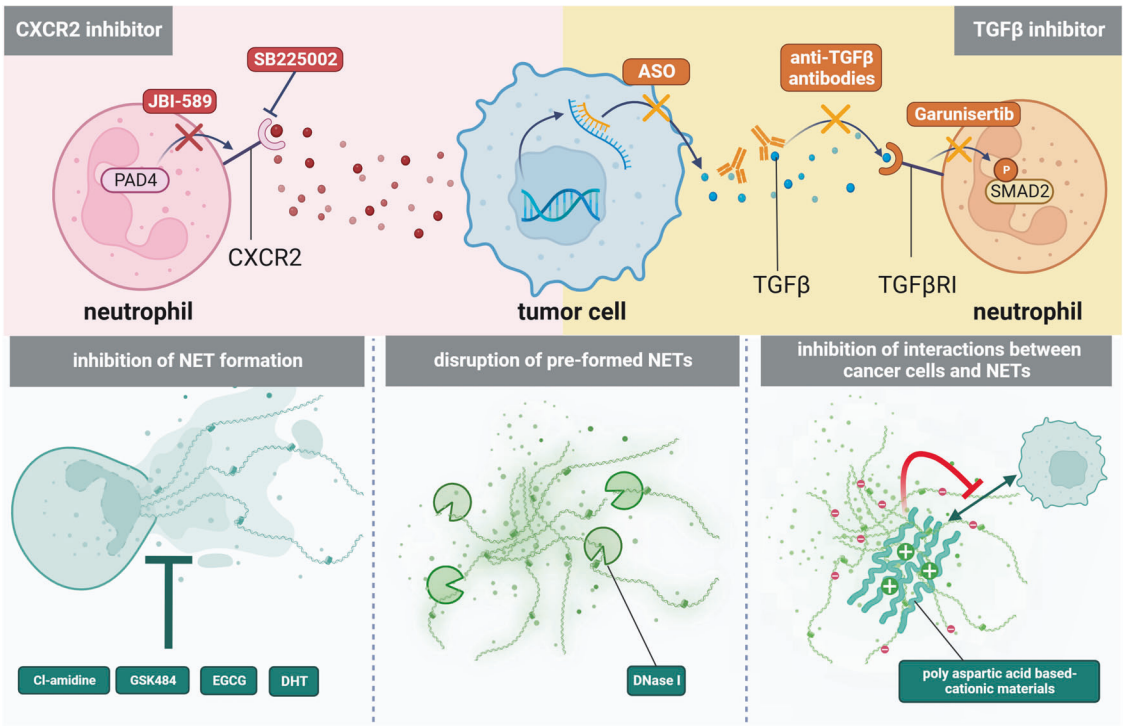

Fig. 6 Therapy to inhibit neutrophil recruitment. TGFβ and CXCR2 play crucial roles in the production and release of neutrophils. Inhibiting the secretion of TGFβ by tumor cells (using ASOs) and inhibiting the interaction between TGFβ, CXCR2, and their target molecules are strategies for anti-tumor drug invention. NETs also play a significant role in cancer progression. Current research primarily aims to inhibit the formation of NETs using drugs such as Cl-amidine, GSK484, EGCG, and DHT. Other strategies involve disruption of the pre-formed NETs by employing DNase I to break down the DNA framework, and inhibition of the interaction between cancer cells and NETs by using poly aspartic acid-based cationic materials, which can neutralize the positive charge of DNA.

its confirmed specificity demonstrated in preclinical models, ASO has progressed into clinical trials [127]. Research on SMIs has found that in co-cultures of neutrophils and SW480 cells (colon adenocarcinoma cells), neutralizing TGFβ with monoclonal antibodies inhibits cancer cell migration and enhances cytotoxicity of neutrophils targeting cancer cells [130, 131]. In vivo experiments using tumor-bearing mice have demonstrated that the administration of anti-TGFβ antibodies leads to a delay in tumor growth compared to controls, and this effect disappears when neutrophils are depleted [130, 131]. Garunisertib (LY2157299 monohydrate), a promising drug, is an oral SMI of the TGFβ receptor I (TGFβRI) kinase that specifically down-regulates the phosphorylation of SMAD2 and eliminates the activation of the typical pathway, which has progressed to phase 3 clinical trials [132] (Fig. 6).

CXCR2 inhibitor: CXCR2 is an effective pro-tumorigenic chemokine receptor that recruits tumor-promoting neutrophils to tissues during tumor induction and tumor-driven inflammation [51], and neutrophils lacking CXCR2 preferentially remain in the bone marrow [53]. In vivo experiments in mice have demonstrated that Ly6G+ neutrophils are the primary contributors to CXCR2 expression in the blood, and CXCR2 deficiency attenuates neutrophil recruitment [51]. CXCR2 expression is elevated in both the stromal and tumor cells of human lung cancer, which is associated with poor patient prognosis [133, 134]. In vivo studies have shown that TANs significantly infiltrate into tumor tissues in mouse models, and suppressing CXCR2 leads to a decrease in the infiltration of neutrophils with inhibitory phenotypes in the TME [133].

The expression of neutrophil CXCR2 is regulated by peptidylarginine deiminase 4 (PAD4) [135]. In lung and colon cancer patients, the expression and activity of neutrophil PAD4, along with the expression of CXCR2, are significantly enhanced compared to healthy donors [135]. Neutrophils from cancer patients have a positive correlation between the expression of CXCR2 and PAD4 [135]. In tumor-bearing mice, the use of JBI-589, a new PAD4 isoform-selective small molecule inhibitor, to pharmacologically inhibit PAD4 resulted in a decrease in CXCR2 expression and prevented neutrophil chemotaxis [135]. The selective inhibitor of CXCR2, known as SB225002, has demonstrated promising therapeutic effects in treating tumors. It achieves this by stimulating the activation of CD8+ T cells, reducing the infiltration of neutrophils, and enhancing the activity of T cells against tumors [133] (Fig. 6). At the same time, the blockade of CXCR2 can improve the therapeutic effect of cisplatin by regulating neutrophil infiltration [133].

NET-targeting therapy. NETs have been implicated as playing a crucial role in the advancement of cancer, and therapies targeting NETs could provide substantial therapeutic benefits. However, the development of drugs specifically targeting NETs remains limited [7]. Current research on anti-cancer drugs targeting NETs focuses on three main approaches: inhibition of NET formation, disruption of pre-formed NETs, and inhibition of interactions between cancer cells and NETs.

PAD4 is a key regulator of NET formation, as it mediates histone citrullination [7]. Despite extensive research into the structure, function, and inhibition of PAD4 over the past several decades, many questions remain regarding its structure and function [136]. Experimental inhibition of PAD4 has shown promise, with small molecule inhibitors such as Cl-amidine or GSK484 effectively blocking NET formation [7, 74, 137, 138]. However, no PAD4-targeting drugs have yet been approved for human use [7]. Zhang et al. found that treatment with Epigallocatechin-3-gallate (EGCG) inhibited NET generation, which inhibits the migration and invasion of SW480 cells [139]. Zhao et al. found that Dihydro

Danshensu (DHT) was effective in counteracting the formation of NETs induced by phorbol 12-myristate 13-acetate [140]. Furthermore, DHT was observed to inhibit the metastasis induced by NETs [140] (Fig. 6).

Destruction of NETs after their formation is also a therapeutic approach. Recombinant DNase can digest the DNA scaffold of NETs and is effective in preclinical models [7]. In a recent study, researchers invented a gene-engineered hybrid liposome. The liposome membrane is embedded with the DNA sensor CCDC25 to target NETs, and the liposome is loaded with DNase I, which can be released upon triggering by the NET-related protein MMP9 [141]. The invention can effectively target and degrade NETs and reshape the microenvironment in situ colorectal cancer mouse models to prevent the formation of primary tumors and pre-metastatic niches [141]. Chen et al. reported the discovery of a nanoplatform that can deliver DNase I to tumor sites and metastatic niches by near-infrared light irradiation and shows that this nanoplatform can enhance cancer immunotherapy and suppress metastasis [142]. However, though DNases target the DNA scaffold, they leave several NET components behind [143], which may be harmful in some cases [7, 144] (Fig. 6).

Another approach to targeting NETs is to block tumor-NET interactions. CCDC25 on the surface of tumor cells can bind to NET-DNA, which acts as a chemokine, promoting the metastasis of cancer cells [76]. Liang et al. introduced cationic materials derived from poly(aspartic acid), which exhibit a robust electrostatic affinity towards DNA. This strong attraction between the cationic polymer and NET-DNA competes with the binding of CCDC25 to NET-DNA, subsequently diminishing the chemotactic potential of NET-DNA in cancer metastasis [145] (Fig. 6). This invention effectively inhibited distant metastasis in a 4T1 orthotopic tumor mouse model. Similar efficacy was also noted in models of metastatic human breast and colon cancer [145].

CONCLUSIONS AND PERSPECTIVES

Neutrophils play an essential role in the TME, and their heterogeneity can be manifested by their pro- and anti-tumor effects mediated by their interactions with various cells. Research on TANs is important for revealing the tumor immune microenvironment. However, due to the fragility, short lifespan, and low RNA content of neutrophils, this research is challenging and there are still many mysteries [6]. Here, we will pose four questions within this field. Firstly, whether neutrophils are reprogrammed before the emergence of cancer cells, or simply because of the tissue damage caused by neutrophils leading to carcinogenesis? Normally, chronic inflammation should lead to faster recognition and killing of mutant cancer cells by neutrophils due to their accumulation, making cancer cells less likely to survive. However, the reality is that chronic inflammation is more prone to carcinogenesis. Secondly, how can we target a specific subgroup of neutrophils? The heterogeneity of neutrophils has been gradually revealed, and it is obvious that when studying tumor-associated neutrophils, the operation of knocking out all circulating neutrophils is too crude. Therefore, we need to explore whether different subgroups of neutrophils have specific surface membrane proteins that can be targeted by antibodies, to achieve precise removal of a certain subtype of neutrophils. Thirdly, if it is possible to change the composition of neutrophils in local organs without disturbing neutrophils in the circulatory system? The TME is a local microenvironment. Modifying the neutrophils in a target site without changing the normal function of circulatory neutrophils may revolutionize research in this field. Finally, how to study neutrophils without separating them from their social network? Neutrophils, as important members of the tumor immune microenvironment, often exert their effects with a variety of cells. Therefore, when studying neutrophils, we cannot just focus on this single point, but need to bring it into the entire immune theme for comprehensive consideration.

Although this review mentions PMN-MDSC, there is still controversy over whether PMN-MDSC belongs to a subset of neutrophils. The term myeloid derived suppressor cell (MDSC) was initially used to describe a population of pathologically activated cells with characters of myeloid origin, immune suppression, and systemic expansion in a cancer-associated context [146]. Therefore, MDSCs are a population of cells defined by function. Based on morphology and cell surface markers, MDSCs are further divided into polymorphonuclear MDSC (PMN-MDSC) and monocytic MDSC (M-MDSC). In mice, the surface marker for M-MDSC is $CD11b^{+}$ $Ly6G^{-}$ $Ly6C^{high}$, while for PMN-MDSC it is $CD11b^{+}$ $Ly6G^{+}$ $Ly6C^{low}$ [146]. However, these markers cannot distinguish between neutrophils and PMN-MDSC, so the isolated $CD11b^{+}$ $Ly6G^{+}$ $Ly6C^{low}$ cell population needs to undergo functional testing (testing whether the cells can suppress T cells) to differentiate them from neutrophils. Currently, the common approach is to consider the cell population that can suppress T cells as PMN-MDSC [146–148]. This means that immunohistochemistry and other staining techniques based on surface antigens will not be able to distinguish between neutrophils and PMN-MDSC. Moreover, as mentioned earlier, the pro-tumor neutrophils can also suppress T cells, overlapping with the phenotype of PMN-MDSC [5]. The similarity of surface antigens and phenotypes does not strictly prove that PMN-MDSC and tumor-promoting neutrophils belong to the same population, but because PMN-MDSC cannot be purified through specific markers, transcriptomic analysis is limited and cannot compare gene expression differences between the two populations [146]. Although there are still studies trying to find more markers (such as LOX-1 and S100A9, etc.) to distinguish MDSC from other populations [149], high-throughput experimental data prove that the MDSC population has extremely high heterogeneity, and the discovery of an additional marker is unlikely to cover the entire MDSC, only including one of its subsets [5]. Given the high heterogeneity of the MDSC population and the overlap with traditional classification methods shown by scRNA-seq data [149], MDSC may not belong to an independent cell population. Some reviews suggest classifying PMN-MDSC as a population of neutrophils with immune suppression function [5, 6].

Several chemoattractants of neutrophils (GRO, CXCL8, GM-CSF) have been identified, which promotes the recruitment of neutrophils to the tumor microenvironment [113, 118, 149, 150]. However, the molecular mechanism by which neutrophils are transformed from an anti-tumor to a pro-tumor phenotype remains unclear. Current tumor therapies related to neutrophils mainly try to activate the anti-tumor phenotype of neutrophils [39], or to inhibit the recruitment or activation of pro-tumor neutrophils by targeting cytokines or their receptors (e.g. CXCR2, CXCR4) [151–154]. However, these therapy categories did not block the transformation of neutrophils by cancer cells or identify the crucial pathways, which limits the development of tumor therapy targeting TANs. Additionally, considering the crucial role of neutrophils in immunity, eliminating them directly is not feasible. Instead, we often have to enhance neutrophil expression in tumor patients to prevent patients from being infected by pathogens [155]. Therefore, new targeted blocking drugs should be developed to inhibit neutrophils from being transformed from an anti-tumor to a pro-tumor phenotype, avoiding interrupting the normal immunity function of normal neutrophils in the whole body. The development of this drug requires an understanding of the underlying molecular mechanisms.

We are like blind men touching an elephant when exploring the tumor immune microenvironment. For this complex and huge topic, we can only explore the mechanisms bit by bit to restore the whole picture of this microenvironment. While this field has

seen numerous scientific advancements, only a few have been able to make a significant impact in the clinic. The fundamental challenge that remains is how to effectively translate these scientific achievements into practical clinical applications.

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ACKNOWLEDGEMENTS

This work was supported by Zhejiang University-University of Edinburgh Institute (ZJE) and Department of Respiratory and Critical Care Medicine, The Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University. We also acknowledge the help of members in JL's lab.

AUTHOR CONTRIBUTIONS

J. Liu designed, supervised and supported the whole project. X. Yu wrote the manuscript. C. Li, Y. Xu, Z. Wang, S. Shao, F. Shao, and H. Wang revised the manuscript. C. Li contributed to some figures. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants to JL from the Natural Science Foundation (NSF) of China (General Grant: 82172899), the NSF of Zhejiang Province (Distinguished Young Scholars: LR22H160002), Dr. Li Dak Sum & Yip Yio Chin Development Fund for Regenerative Medicine, Zhejiang University, and Dynamic Research Enterprise for Multidisciplinary Engineering Sciences (DREMES) at Zhejiang University and the University of Illinois at Urbana-Champaign, funded by Zhejiang University.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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