Neutrophils in cancer carcinogenesis and metastasis

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Abstract

In recent years, neutrophils have attracted increasing attention because of their cancer-promoting effects. An elevated neutrophil-to-lymphocyte ratio is considered a prognostic indicator for patients with cancer. Neutrophils are no longer regarded as innate immune cells with a single function, let alone bystanders in the pathological process of cancer. Their diversity and plasticity are being increasingly recognized. This review summarizes previous studies assessing the roles and mechanisms of neutrophils in cancer initiation, progression, metastasis and relapse. Although the findings are controversial, the fact that neutrophils play a dual role in promoting and suppressing cancer is undeniable. The plasticity of neutrophils allows them to adapt to different cancer microenvironments and exert different effects on cancer. Given the findings from our own research, we propose a reasonable hypothesis that neutrophils may be reprogrammed into a cancer-promoting state in the cancer microenvironment. This new perspective indicates that neutrophil reprogramming in the course of cancer treatment is a problem worthy of attention. Preventing or reversing the reprogramming of neutrophils may be a potential strategy for adjuvant cancer therapy.

Keywords: Neutrophil, Cancer, Microenvironment, Cell plasticity, Cell reprogramming

Background

Neutrophils have been recognized as the most abundant innate immune cells in both bone marrow and peripheral blood [1]. They are rapidly recruited into sterile or infected inflammation sites and show high plasticity and a strong effector response. Perhaps to avoid unnecessary tissue damage, neutrophils possess a short lifespan [2]. Therefore, the abundance of neutrophils relies on constant replenishment via granulopoiesis in the bone marrow. Their origin is hematopoietic stem cells, which give rise to lymphoid-primed multipotent progenitors (LMPPs). Neutrophils are derived from the early committed neutrophil progenitor (proNeu1), a subtype of granulocyte–monocyte myeloid progenitor (GMP) that develops from LMPPs [3, 4]. Classically, as determined based on nuclear morphology, neutrophils mature through the following sequence: GMPs, myeloblasts, promyelocytes, myelocytes, metamyelocytes, banded neutrophils and segmented neutrophils [1]. According to recent studies, the neutrophil developmental pathway mapped based on single-cell analyses is proNeu1, intermediate progeny (proNeu2), preneutrophil (preNeu), immature neutrophils and, finally, mature neutrophils [4]. Transcription factors, such as C/EBPα and C/EBPε, exquisitely control neutrophil development [5–7]. During neutrophil maturation, migration and immune response functions gradually overtake proliferation. Both microbial and cancer stresses trigger preNeu expansion and immature neutrophil release from bone marrow [8]. Moreover, extramedullary granulopoiesis commonly occurs in the spleen under pathological states [9].

Neutrophils play various roles in different diseases, including infectious diseases, metabolic diseases, autoimmune diseases and aging-associated diseases. On the one hand, neutrophils exert positive functions in host...
defense, including antibacterial [10], antifungal [11] and antiviral [12] functions. In addition, they eliminate apoptotic cell debris, which is beneficial for tissue regeneration and angiogenesis after tissue damage [13]. On the other hand, neutrophils are involved in pathogenesis through diverse mechanisms. First, neutrophils recruited to the lesion site release proteases and produce a large amount of reactive oxygen species (ROS), resulting in tissue damage, rendering the tissue more susceptible to pathogens and even the development of chronic inflammation [14]. This pathological effect on many infectious diseases and pulmonary diseases, including severe cases of coronavirus disease 2019 (COVID-19), has frequently been observed [15]. In addition, neutrophil elastase (NE) causes insulin resistance during the development of obesity and type 2 diabetes [16]. Second, neutrophils may shift their function to immunosuppression characterized by a lower response to chemokines and inhibition of T cell immunity. In sepsis, this functional change is life-threatening [17]. Third, neutrophil extracellular traps (NETs) extruded by activated neutrophils have been reported to participate in the occurrence and development of a wide range of diseases. NETs are large extracellular complexes composed of cytosolic and granule proteins and chromatin [18]. In individuals with atherosclerosis, NETs result in the destabilization of atherosclerotic plaques through the lysis of smooth muscle cells [19]. NETs are also the major inducers of thrombosis [20]. In autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and ANCA-associated vasculitis, NETs are recognized as antigens that contribute to the production of anti-self-antibodies [21]. In general, neutrophils are a double-edged sword in diseases with both defensive and harmful functions.

Neutrophils, the most dominant immune cells [22], also play complex and important roles in cancer. Many studies have reported elevated peripheral blood counts of neutrophils in patients with different cancers. The neutrophil-to-lymphocyte ratio (NLR) has been shown to be an independent prognostic indicators for patients with cancer [23]. This review will describe the multifaceted roles of neutrophils in cancer initiation, growth and metastasis, thereby revealing the heterogeneity and high plasticity of neutrophils in cancer. Based on these findings and those from our own studies, we attempted to analyze the possible mechanism of neutrophil heterogeneity from the perspective of cell reprogramming.

**Neutrophils in carcinogenesis**

**Cancer initiation**

Inflammation plays an essential role in cancer initiation by damaging tissues, and neutrophils are a crucial component of this process. Thus, neutrophils provide a link between inflammation and cancer. Cancer that develops in various mouse models of KRAS-driven ovarian cancer exhibits upregulated levels of neutrophil-related chemokines and an expansion of neutrophils. These phenotypes may result from direct upregulation of neutrophil-related cytokines such as GM-CSF and CXCL8 [24, 25]. In a zebrafish model of HRASG12V-driven melanoma, wounding-induced inflammation with elevated levels of prostaglandin E2 increase the formation of cancer in a neutrophil-dependent manner [26]. Depletion of the entire neutrophil population using anti-Ly6G antibodies impairs carcinogenesis in both chemically induced and spontaneous cancer models. Neutrophils overexpressing CXCR2 are attracted to cancer-prone tissues via the cytokine IL-8 and chemokine ligands CXCL1, CXCL2 and CXCL5. The application of chemical carcinogens in CXCR2-deficient mice, which show impaired neutrophil trafficking, prevents papilloma or adenoma formation [27, 28]. CXCR2-mediated neutrophil trafficking from bone marrow into peripheral blood is antagonized by CXCR4 expression due to the retention of neutrophils by CXCL12-expressing bone marrow stromal cells mediated retention. Bone marrow macrophages subsequently eliminate the retained neutrophils in a rhythmic manner.

**Neutrophils induce DNA damage**

The evidence described above has indicated that neutrophils are crucial for carcinogenesis, but the exact mechanisms by which neutrophils foster carcinogenesis require further elucidation. Neutrophils produce and release genotoxic DNA substances that increase DNA instability. In an in vitro coculture model mimicking intestinal inflammation in ulcerative colitis, neutrophils increase errors in the replication of colon epithelial cells. In individuals with chronic colon inflammation, activated neutrophils cause an accumulation of target cells in G2/M phase, consistent with the installation of a DNA damage checkpoint [29]. Neutrophil-derived elastase, neutrophil production of ROS, reactive nitrogen species (RNS) and angiogenic factors such as MMP-9 and the immunosuppressive ability of neutrophils may be associated with this process. ROS released by neutrophils during chronic inflammation, such as hypochlorous acid (HOCl, formed by myeloperoxidase (MPO)), cause DNA damage and are mutagenic in lung cells in vitro. HOCl is a major neutrophil oxidant. MPO-catalyzed formation of HOCl during lung inflammation is an important source of neutrophil-induced genotoxicity. Neutrophils cause DNA damage by releasing ROS and inducing gene mutations in premalignant epithelial cells, thus driving oncogenic transformation in lung cancer. Additionally, at physiological concentrations, HOCl induces mutations in
the hypoxanthine phosphoribosyl transferase (HPRT) gene, inducing three major types of DNA lesions [30]. Haqqani and coworkers analyzed a mouse model of subcutaneous cancer and showed that inducible nitric oxide synthase (iNOS) and nitric oxide synthase (NOS) contents and neutrophil infiltration were significantly correlated with the number of mutations in the Hprt locus [31]. However, a new mechanism that does not rely on ROS was also recently identified. In clinical samples from patients with inflammatory bowel disease and injury models, activated tissue-infiltrating neutrophils release particles carrying proinflammatory microRNAs, including miR-23a and miR-155, which increase DNA double-strand breaks and genomic instability [32]. miR-155 is also responsible for neutrophils-induced DNA damage and DNA repair landscape in acute colon injury, resulting in colorectal cancer initiation even shaping the progression [33].

**Neutrophils promote angiogenesis and immunosuppression**

Coussens et al. documented that MMP-9 supplied by bone marrow-derived neutrophils and other hematopoietic cells contributes to squamous carcinogenesis [34]. MMP-9 produced by neutrophils also contributes to the carcinogenesis of pancreatic islet carcinoma and lung cancer accelerating angiogenesis [35]. NETs promote inflammation in subjects with nonalcoholic steatohepatitis, resulting in the development of hepatocellular carcinoma, which is inhibited by deoxyribonuclease treatment or peptidyl arginine deaminase type IV knockout, decreasing NET formation [36]. Furthermore, NETs positively correlate with the increased number of regulatory T cells (Tregs) in cancer by facilitating naive CD4+ T cell metabolic reprogramming. Therapies targeting the interaction between these two cell types or inhibiting Treg activity may promote cancer immunosurveillance and prevent hepatocellular carcinoma formation [37].

In summary, neutrophils recruited to inflammatory sites promote cancer initiation mainly by increasing DNA damage, angiogenesis and immunosuppression. However, the mechanism underlying neutrophil-dependent carcinogenesis is complicated and cannot be reduced to one specific molecule. Even the same molecule often exerts different effects on diverse stages. Although CXCR2 promotes neutrophil migration into pro-cancer sites, knockdown of CXCR2 in neutrophils increases ROS production and exerts pro-cancer effect [38]. Thus, in future studies, genetically engineered mouse models (GEMMs) will be extremely valuable for research in the field of cancer-related neutrophil biology, as they enable neutrophils and neutrophil-derived factors to be manipulated as cancer arises de novo.

**Neutrophils in cancer progression**

More than two decades ago, neutrophils were presumed to cause cancer xenograft rejection in mice [39, 40]. Just a few years later, the opposite result was reported: depletion of neutrophils reduced the growth of transplanted cancer [41]. Since then, reports of neutrophils promoting cancer progression have vastly outpaced those of neutrophils inhibiting cancer.

**Neutrophils promote cancer growth**

The mechanisms by which neutrophils promote cancer growth are diverse. Neutrophils are characterized by rich granules, which perform different functions (Table 1). Some granule proteins (MMP-9 and ARG-1) released by activated neutrophils are associated with cancer progression. For example, MMP-9 released by neutrophils degrades the extracellular matrix, which in turn releases vascular endothelial growth factor (VEGF) and promotes angiogenesis [42]. Depletion of neutrophils or blockade of CXCR2 signaling to affect neutrophil recruitment inhibits cancer growth and reduces angiogenesis [43]. In contrast, an injection of cancer cells with neutrophils from cancer-bearing mice increases cancer growth and angiogenesis. In addition, the release of ARG-1 from neutrophils depletes arginine in T cells, causing the downregulation of CD3ξ. This process inhibits CD3-mediated T cell activation and proliferation, creating an immunosuppressive environment that also contributes to cancer growth [44]. In addition, the H+ -pumping ATPase on tertiary granules causes cancer acidosis when it is mobilized to the cell surface, which may lead to cancer progression. Furthermore, an acidic pH inhibits the anticancer activity of T cells and natural killer (NK) cells, resulting in immune escape. Neutrophils also promote cancer growth and progression by recruiting macrophages and Tregs [45]. The structure of NETs formed by granule proteins and DNA induces the proliferation of cancer cells through high mobility group protein B1 (HMGB1) and NE [46–48]. In hematological malignancies, levels of NETs are found to positively correlated with lymphoma progression or childhood acute leukemia development [49, 50].

In addition to granular proteins, neutrophils also play a role in promoting cancer growth by releasing growth factors, including epidermal growth factor, hepatocyte growth factor (HGF) and platelet-derived growth factor. Another study has shown that neutrophils eliminate senescence through IL-1 receptor antagonist (IL-1RA) and thus promote the progression of prostate cancer. Based on cancer promotion effect of neutrophil
| Granule                         | Gene name | Protein name | Functions and Functions References |
|-------------------------------|-----------|--------------|-------------------------------------|
| Azurophil (primary) neutrophil granules | AZU1      | Azurocidin   | Antibacterial activity (Gram-bacteria); monocyte and fibroblast-specific chemotaxis; binds heparin; reprograms stel late cells toward a phenotype affecting the cancer microarchitecture; disrupts vascular endothelial cell morphology [51–55] |
|                               | DEFA1-4   | Neutrophil defensins | Antibacterial, fungicidal, and antiviral activities; enhance anticancer immunity; direct cytolysis (high concentration); induce apoptosis; inhibiting angiogenesis; stimulate cancer growth (low concentration); promotes invasiveness [56–59] |
| PRTN3 (MBN) Myeloblastin      | CD63 (MLA1) | CD63 antigen | Serine protease; facilitates transendothelial neutrophil migration; PRTN3-involved IκBα cleavage leads to abnormal activation of NFκB signaling pathway (carcinogenesis); inhibits T cell proliferation; mediates cancer metastasis to bone [60–62] |
|                               | CTSG      | Cathepsin G  | Antimicrobial, serine protease; facilitates neutrophil anti-cancer cytotoxicity; induces cell migration and multicellular aggregation; promotes metastasis; impairs NKp46-mediated responses of NK cells [65–69] |
|                               | ELA2 (ELANE) | Neutrophil elastase | Serine protease; facilitates primary cancer growth and secondary organ metastasis; selectively kills cancer cells and attenuates carcinogenesis; enhances cancer cell invasion; involved in awakening of dormant cancer cells; cleaves PML-RARα and is important for the development of APL in mice [70–74] |
|                               | MPO       | Myeloperoxidase | Microbicidal activity against a wide range of organisms; cancer cell cytotoxicity; awakening of dormant cancer cells by accumulation of oxidized lipids [75, 76] |
|                               | BPI       | Cap57; bactericidal permeability-increasing protein | Antibacterial, anticancer, and LPS-neutralizing activities; cancer cell cytotoxicity [77] |
| Specific (secondary) neutrophil granules | CHI3L1    | Chitinase-3-like protein 1 | Glycoside hydrolase family 18; binds to chitin, heparin, and hyaluronic acid; plays a critical role in cancer cell growth, proliferation, invasion, metastasis, angiogenesis; activation of tumor-associated macrophages, and Th2 polarization of CD4 + T cells [78] |
|                               | NGAL (LCN2) | Lipocalin 2  | Antimicrobial; functions in innate immune defense; induces apoptosis of B lymphocytes; mediates appetite suppression; induces mesenchymal-epithelial transition of cancer cells thereby facilitating colonization and metastatic outgrowth [79–81] |
|                               | LTF (GIG12) | Lactoferrin  | Antimicrobial, anti-viral, antioxidant, anti-cancer, and anti-inflammatory activities; modulation of immune responses; anti-proliferation of cancer cell line; has a radiation resistance effect; LTF-IC can convert TAMs into M1-like cells [82–85] |
in pancreatic ductal adenocarcinoma (PDAC), lorlatinib inhibiting FES kinase, which is activated in neutrophils by PDAC cells, can attenuate cancer growth [104] (Fig. 1A).

**Neutrophils inhibit cancer growth**
Although fewer studies have assessed the inhibitory effects of neutrophils on cancer, very interesting data have been reported. For example, in models transplanted with different cancer cell lines or spontaneous cancer
Fig. 1 (See legend on previous page.)
Neutrophils in cancer metastasis

In recent years, most studies examining the role of neutrophils in cancer have been related to metastasis. Combined intravenous injection of cancer cells and neutrophils from cancer-bearing rodents was found to increase the incidence of lung metastases as early as the late 1980s [112]. Subsequent studies have shown that the increased levels of neutrophils induced by the IL-17/G-CSF axis or the cholesterol metabolite 27-hydroxycholesterol promote cancer metastasis [113, 114], and the concentration of β2-integrin (CD18) in the intracellular granules of neutrophils is positively correlated with liver metastasis of colorectal cancer in mice [115]. Increased NETs also facilitate hepatocellular carcinoma cell metastasis by activating TLR4/9-COX2 signaling. NET-enabled metastatic activity is abrogated by inhibiting this signaling pathway [116]. Neutrophils are actively involved in each step of the metastatic cascade: formation of the premetastatic niche, cancer cell escape from the primary tumor, intravasation into the blood and/or the lymphatic vascular system, survival in the circulation, extravasation into distant organs, awakening of dormant cancer cells and outgrowth of metastases.

Neutrophils promote cancer cell migration and intravasation

In the early stages of metastasis, neutrophils release MMP-9 to promote angiogenesis, playing an important role again by not only facilitating cancer growth but also providing more routes for cancer cells to escape. Neutrophils also direct cancer cells to endothelial cells, prompting them to enter the bloodstream. One mouse model of melanoma showed that cancer cells clustered around blood vessels and increased lung metastasis but had no effect on the growth of the primary tumor. In this model, cell damage increased HMGB1 levels, leading to the recruitment of neutrophils that subsequently promoted the migration of cancer cells toward blood vessels [117–119]. In vitro, neutrophil-derived tumor necrosis factor (TNF) stimulates melanoma cell migration, suggesting that TNF is one of the factors related to neutrophil-induced metastasis.

Next, neutrophils guide cancer cells into blood vessels. Cathepsin G, a neutrophil-derived serine protease, induces cell migration, activates insulin-like growth factor 1, increases E-cadherin-mediated intercellular adhesion and cancer cell aggregation, and promotes cancer cell entry into blood vessels [120]. NETs trap circulating cancer cells (CTCs), helping them spread to distant sites and promoting their adhesion to distant sites [121, 122]. The interaction between neutrophils and CTCs promotes cell cycle progression in the blood and expands the metastatic potential of CTCs [123]. According to a recent study, ROS produced by neutrophils increase NETs, especially in obese cancer-bearing mice, which weakens endothelial junctions and promotes the extravasation of cancer cells[124]. In addition, several studies have shown that direct interaction between neutrophils and cancer cells activates neutrophils, increases the migration of cancer cells, promotes the anchoring of cancer cells to endothelial cells, and ultimately helps cancer cells exit blood vessels [123, 125].

Neutrophils facilitate cancer cell extravasation

Finally, metastatic cancer cells in distant tissues typically remain dormant for an extended period, during which infiltrating neutrophils release MMP-9 to promote angiogenesis, triggering the growth of dormant metastases. In addition, continued inflammation induces the formation of NETs, which are needed to wake dormant cancer
cells. A mechanistic analysis has shown that two NEs and MMP-9, which are associated with NETs, cleave laminin. Cleaved laminin induces the proliferation of dormant cancer cells by activating α3β1-integrin signaling [72].

A related interesting phenomenon has been observed. Before disseminated cancer cells arrive, neutrophils accumulate in distant organs, forming the premetastatic niche. Neutrophils have been observed to aggregate in the lungs prior to the occurrence of metastasis in mouse models of MMTV-PyMT mammary cancer, breast cancer with nicotine exposure and melanoma, all of which are closely associated with the occurrence of pulmonary metastasis [79, 126, 127]. Neutrophils also contribute to ovarian cancer metastasis to the omentum by premetastatic sites into the lung before metastatic cancer cells and people; neutrophils migrate from primary breast tumors only observed in patients with cancer and not in healthy people [166]. This diversity results from the effects of com-

Neutrophils inhibit cancer metastasis

In contrast, other researchers have shown that neutrophil depletion facilitates metastasis. CCL2 and G-CSF secreted by the primary tumor activate the cytotoxic functions of these antimetastatic neutrophils mediated by 

Neutrophils in cancer recurrence

According to clinical data, the NLR predicts the prognostic outcome and the absolute neutrophil counts are considered independent prognostic factors for cervical cancer relapse and postoperative recurrence of intrahepatic cholangiocarcinoma [135, 136]. Although the underlying mechanism remains unclear, the interaction between neutrophils and cancer cells may play a role in cancer recurrence. In a zebrafish melanoma model, neutrophils were recruited to the inflammatory site of postoperative trauma and interacted with precancerous cells, providing them with environmental conditions that support their proliferation, and these interactions may be associated with postoperative cancer relapse. In ovarian and lung cancer, stress hormone-induced neutrophil activation reactivates dormant cancer cells and leads to early recurrence. Neutrophil activation is based on the release of S100A8/A9 proteins, myeloperoxidase activation and oxidized lipid accumulation, which finally activate the fibroblast growth factor-related signaling pathway in dormant cancer cells and push them to exit from dormancy [76]. In patients with breast cancer diagnosed with COVID-19, emerging reports show that dormant cancer cells are reawakened by factors released during lung inflammation, including NETs. Severe acute respiratory syndrome coronavirus 2 infection of airway epithelial cells first releases damage-associated molecular patterns followed by inflammatory cytokines and chemokines, which further recruit and activate neutrophils to release NETs [137].

Taken together, these findings show that the premetastatic behavior of neutrophils can be switched in vivo, providing possible opportunities for therapeutic intervention (Table 2). Although cancer recurrence is currently proposed to increase in the presence of neutrophils, our understanding of the role of neutrophils might be altered as this field advances.
| Cancer                                      | Year | Species                  | Mechanism                                                                 | References |
|---------------------------------------------|------|--------------------------|---------------------------------------------------------------------------|------------|
| Cancer-promoting role                        |      |                          |                                                                           |            |
| Lung cancer                                 | 2010 | Mouse                    | Neutrophil elastase accelerates lung cancer growth via degradation of IRS-1 | [138]      |
| Lung carcinoma, melanoma                    | 2016 | Mouse                    | NETosis promotes cancer growth                                            | [139]      |
| Small intestinal cancer                     | 2016 | Mouse                    | Hypercoagulation induced by NETosis promotes carcinogenesis and N2 polarization | [140]      |
| Lung adenocarcinoma                         | 2017 | Human, mouse             | A distinct subset of SiglecF-high neutrophils dependent on cancer-induced osteoblastic cells promote cancer growth | [141]      |
| Melanoma                                    | 2017 | Mouse                    | Neutrophils recruit to TME and acquire immunosuppressive properties       | [142]      |
| Lymphoma, Lung carcinoma, colon carcinoma, pancreatic cancer | 2019 | Mouse                    | Neutrophils acquire immunosuppressive activity mediated by FATP2          | [143]      |
| Lung adenocarcinoma                         | 2020 | Human                    | Multi-omics reveal a potential immunosuppressive role of neutrophil degranulation | [144]      |
| Hepatocellular carcinoma                    | 2011 | Human                    | Neutrophil is correlated with angiogenesis progression                     | [145]      |
| Pancreatic cancer                           | 2016 | Mouse                    | CXCR2 signaling promotes carcinogenesis and metastasis                     | [146]      |
| Lung carcinoma                              | 2013 | Mouse                    | NETs trap circulating cancer cells and promote metastasis                 | [121]      |
| Breast cancer                               | 2015 | Mouse                    | Neutrophil-derived leukotrienes establish the lung pre-metastatic niche   | [126]      |
| Breast cancer                               | 2015 | Mouse                    | Neutrophils polarized by IL-17-producing y0 T cells acquire the ability to suppress cytotoxic T lymphocytes and promotes metastasis | [113]      |
| Lung carcinoma, melanoma                    | 2016 | Mouse                    | Neutrophils recruited by TLR3 promote pre-metastatic niche formation      | [127]      |
| Breast cancer                               | 2016 | Mouse                    | NETs induced by cancer promote metastasis                                 | [147]      |
| Breast cancer                               | 2018 | Mouse                    | NETs produced during inflammation awaken dormant cancer cells              | [72]       |
| Breast cancer                               | 2019 | Mouse                    | WNT-dependent systemic neutrophil inactivation triggered by loss of p53 in cancer cells promotes metastasis | [148]      |
| Breast cancer                               | 2019 | Human, mouse             | Neutrophils escorting CTCs drives cell cycle progression and expands the metastatic potential of CTCs | [123]      |
| Breast cancer, colon cancer                 | 2020 | Human                    | NETs promote metastasis via binding CCDC25 on cancer cells                | [149]      |
| Cancer-suppressing role                     |      |                          |                                                                           |            |
| Breast cancer                               | 2011 | Mouse                    | Neutrophils inhibit lung metastasis by generating H2O2                     | [130]      |
| Lung cancer                                 | 2014 | Human                    | TANs stimulate T cell responses in the early stage of lung cancer         | [150]      |
| Uterine cancer                              | 2015 | Mouse                    | Neutrophils oppose carcinogenesis via clearance of hypoxic cancer cells   | [151]      |
| Lung cancer                                 | 2016 | Human                    | TANs act as APCs in early-stage lung cancer                              | [152]      |
| Colorectal cancer                           | 2017 | Human                    | Neutrophils enhance the responsiveness of CD8+ T cells and improve survival | [153]      |
| Undifferentiated pleomorphic sarcoma (UPS)   | 2019 | Human                    | Neutrophils driving UTCaβ polarization and type 1 immunity mediate resistance against UPS | [154]      |
| Uterine cancer                              | 2020 | Mouse                    | Neutrophils kill cancer cells via their production of ROS and MMP-9 upon relief of hypoxia | [155]      |
| 35 cancer cell lines                        | 2021 | Human                    | Neutrophil elastase selectively kills cancer cells and attenuates carcinogenesis | [70]       |
| Neutrophil-associated complications in cancer|      |                          |                                                                           |            |
| Mammary carcinoma                           | 2015 | Mouse                    | Kidney and heart failure caused by NETosis and inflammation               | [156]      |
| Lung carcinoma                              | 2015 | Mouse                    | HGF/MET-dependent neutrophil recruitment and NO release by neutrophils promotes cancer cell killing | [105]      |
| Small intestinal cancer                     | 2016 | Mouse                    | Coagulation promoted by NETosis                                           | [140]      |
| Myeloproliferative neoplasm                 | 2018 | Mouse                    | Thrombosis promoted by increased NETosis                                  | [157]      |
| Neutrophils with anticancer therapeutic role |      |                          |                                                                           |            |
| Non-Hodgkin lymphoma                        | 2010 | Mouse                    | Neutrophils kill cancer cells by phagocytosis in the treatment of anti-CD47 antibodies synergized with rituximab | [158]      |
microenvironments, conventional therapies and immunotherapy shape neutrophil function.

In a GEMM of lung adenocarcinoma, TGFβ polarized neutrophils in a cancer-promoting direction, and TGFβ blockade reversed the neutrophil protumor phenotype to an antitumor phenotype. These two types of neutrophils with opposite functions are named N2 and N1, respectively, which are similar and comparable to tumor-associated macrophages, such as M2 and M1 [167]. In the early stage of non-small cell lung cancer, the anticancer state of neutrophils is also induced by interferon-γ (IFNγ) and GM-CSF. Induced neutrophils indeed develop from immature progenitors through the negative regulation of the transcription factor Ikaros and acquire APCs properties, which as APC-like hybrid cells, promote T cell antitumor responses [152]. Another study has shown that hypoxia is a potent determinant of the TAN phenotype and direct neutrophil-cancer cell interactions. After the removal of hypoxia, the number of neutrophils recruited by the cancer decreased significantly, but the recruited cells were more effective at killing the cancer cells. This activity is mediated by the production of NADPH oxidase-derived ROS and MMP-9. At the same time, the ability of neutrophils to promote cancer cell proliferation, which appears to be mediated by their production of NE, is also reduced [155]. The general trend is that TANs belong to a network of anticancer cells in the early stages of carcinogenesis, but with cancer progression, neutrophil function shifts to immnosuppressive and cancer-promoting states.

Metabolic reprogramming of neutrophils

Neutrophils among TANs with proven immunosuppressive function have been extensively studied and have been named granulocytic myeloid-derived suppressor cells (G-MDSCs) or polymorphonuclear myeloid-derived suppressive cells. G-MDSCs appear as neutrophils at different stages of maturation [168]. G-MDSCs flexibly adapt to the cancer microenvironment. The most important of these adaptations is the metabolic shift, which exerts a substantial effect on cell function.

Table 2 (continued)

| Cancer                     | Year | Species | Mechanism                                                                 | References       |
|----------------------------|------|---------|---------------------------------------------------------------------------|------------------|
| Thymoma, breast cancer     | 2010 | Mouse   | MDSCs are selectively killed by 5-Fluorouracil or doxorubicin selectively resulting in enhanced T cell-dependent anticancer immunity | [159, 160]       |
|                            | 2014 | Mouse   |                                                                           |                  |
| Different cancer cell lines| 2013 | Mouse   | 5-FU and gemcitabine can promote cancer inflammation and resistance to chemotherapy mediated by neutrophils and T cells | [161]            |
| Different cancer cell lines| 2016 | Mouse   | Radiotherapy induces infiltration of neutrophils with cytotoxic activity against cancer cells | [162]            |
| Glioma                     | 2017 | Mouse   | Neutrophil can act as a vector of anticancer drug delivery to cross BBB for suppression of postoperative malignant cancer recurrence | [163]            |
| Different cancer cell lines| 2018 | Mouse   | Neutrophils kill antibody-opsonized cancer cells by trogoptosis           | [107]            |
| Lung carcinoma             | 2018 | Mouse   | TANs are reprogrammed to promote anticancer immunity by blocking LILRB2    | [164]            |
| Different cancer cell lines| 2020 | Mouse   | Neutrophils kill cancer cells via ADCC mediated by IgA and enhanced by CD47–SIRPα checkpoint inhibition | [165]            |
Neutrophil subset identification and markers

Researchers have attempted to identify neutrophil subsets. Specific surface markers proposed to identify neutrophil subsets in cancer include CD101 and CD177 [174, 175], which are associated with cancer regression, and CD117, PDL1, CD170, LOX1, CD84 and JAML [176], which are associated with T cell immunosuppression and cancer progression. In PDAC, the purinergic receptor P2RX-negative neutrophil subset exhibits immunosuppressive role with enhanced PD-L1 expression and mitochondrial metabolism [177]. However, an unequivocal method to detect immunosuppressive neutrophils and other neutrophil subsets using flow cytometry or other strategies remains to be developed. Since the subsets of neutrophils show continuous changes and are highly phenotypically and morphologically similar (even between MDSCs and other cells), a reasonable assumption is that these hypothetical subsets are actually the same type of cells, with larger or smaller changes induced by different local environments. These neutrophils are a single cell type with many different functional phenotypes. The high plasticity of neutrophils enables them to respond quickly to external stimuli, leading to their heterogeneity. Because different stimuli mobilize different cytoplasmic granules, different degrees of exposure of the membrane proteins of each granule to the cell surface can change the cell surface composition of neutrophils, potentially leading to the misidentification of new cell types. Taken together, TANs appear to be more flexible than circulating neutrophils, which enables them to adapt to diverse cancer microenvironments.

Moreover, TANs or normal neutrophils have consistently been shown to be trained to become anticancer neutrophils through various methods to achieve the goal of killing cancer cells. For example, PPM1D/Wip1 is a negative regulator of the cancer suppressor p53 and is overexpressed in several human solid cancers. Ppm1d knockout or chemical inhibition of Wip1 in human or mouse neutrophils exacerbates anticancer phenotypes and increases p53-dependent expression of costimulatory ligands and the proliferation of cocultured cytotoxic T cells [178]. Another study showed that exposure to β-glucan [179], a fungal-derived prototype agonist of trained immunity, trained neutrophils in mice to enhance the anticancer activity of neutrophils. These results, in turn, prove that neutrophils are highly plastic (Fig. 1C).

Interaction between neutrophils and other microenvironmental cells

Cancer is highly heterogeneous and is considered one of its hallmarks. The tumor contains cancer cells and non-cancerous cells such as neutrophils, macrophages, T cells, adipocytes, stromal cells and others constituting the microenvironment. All these cells communicate directly or indirectly. Thus, neutrophils in cancer not only have a relationship with the T cells mentioned above but also affect or are affected by other cells. During advanced colorectal cancer progression, cancer stem cell-derived exosomes containing triphosphate RNAs prime neutrophils for cancer development and depletion of neutrophils with antibodies attenuate the tumorigenicity of these cancer stem cells [180]. In obese patients with pancreatic cancer, crosstalk among pancreatic stellate cells, neutrophils and adipocytes mediated by IL1β promotes PDAC. Genetic or pharmacological targeting of this circuit provides a potential method for pancreatic cancer treatment [181]. Cancer-associated fibroblasts are considered one of the important stromal cells contributing to cancer development. A recent report identified that one of the underlying mechanisms as NET induction. This induction is driven by increased amyloid and β-secretase expression in fibroblasts [182].

Discussion and perspectives

We speculate that the cancer microenvironment may reprogram neutrophils to achieve conversion between anticancer polarity and cancer-promoting one. First, as previously described, neutrophils are heterogeneous in patients with cancer, which may result from the reprogramming of mature neutrophils. Many data indicate that neutrophil precursors support cancer growth and metastatic progression. Second, cancer cells functionally shape the cancer microenvironment by secreting various cytokines, chemokines and other factors, which provides the necessary environmental conditions for the reprogramming of surrounding neutrophils. Neutrophils acquiring new transcriptional activity, which could be characterized as diverse neutrophil subsets, based on single cell RNA sequencing analysis under specific microenvironment support the hypothesis [183]. Our previous review also stated that cancer cells undergo cellular reprogramming either spontaneously or after anticancer treatment [184]. All of these findings suggest the possibility of reprogramming both cancer cells and neutrophils in the cancer microenvironment. Third, our experiments show that mature neutrophils are reprogrammed into multipotent progenitors in the presence of a chemical cocktail [185]. In other words, neutrophils have the potential to undergo cell reprogramming.

More evidence of neutrophil reprogramming is illustrated below. Neutrophils transdifferentiate into other cell types. One study has shown that human postmitotic neutrophils are reprogrammed into macrophages via growth factors. The molecular mechanisms underlying functional changes in neutrophils has been discovered that GM-CSF controls the overexpression of FATP2 in

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neutrophils through the activation of the STAT5 transcription factor, thereby enabling neutrophils to obtain immunosuppressive activity and promote cancer progression in mice [143]. In addition, metabolic reprogramming of neutrophils leads to functional changes, as a metabolic shift of innate immune cells, including neutrophils, is observed in pulmonary diseases, accompanied by an impaired normal immune function of these cells.

In conclusion, neutrophils exert both pro-cancer and anticancer effects on the initiation, growth and metastasis of cancer, and these different functions are accompanied by the existence of different neutrophil subpopulations. Because neutrophils normally possess antimicrobial and anticancer functions, functional transformation or abnormal cell differentiation must occur. Here, we propose a hypothesis that the cancer microenvironment or clinical treatment may induce the reprogramming of neutrophils. In clinical practice, an elevated NLR serves as a prognostic indicator and the inhibition or reversal of neutrophil reprogramming can also be employed as a potential therapeutic strategy, e.g., conversion of neutrophils into antigen-presenting cells by FcγR engagement can exhibit immunotherapeutic effect on cancer [186].

**Conclusions**

Neutrophils would be a promising cell target population for anticancer therapy, although their roles in cancer are dual and remain to be further investigated. Direct target neutrophils or indirect target microenvironment factors reprogramming neutrophil plasticity might be potential therapeutic strategies.

**Abbreviations**

ADCC: Antibody-dependent cellular cytotoxicity; COVID-19: Coronavirus disease 2019; CTCs: Circulating cancer cells; FATP2: Fatty acid transport protein 2; GEMMs: Genetically engineered mouse models; G-MDSCs: Granulocyte–monocyte myeloid-derived suppressor cells; GMP: Granulocyte–monocyte myeloid progenitor; HGF: Hepatocyte growth factor; HMGB1: High mobility group protein B1; HOCl: Hypochlorous acid; HPRT: Hypoxanthine phosphoribosyl transferase; IFNγ: Interferon-γ; IL-1RA: IL-1 receptor antagonist; iNOS: Inducible nitric oxide synthase; LMPs: Lymphoid-primed multipotent progenitors; MPO: Myeloperoxidase; NE: Neutrophil elastase; NETs: Neutrophil extracellular traps; NK: Natural killer; NLR: Neutrophil-to-lymphocyte ratio; NOS: Nitric oxide synthase; PDAC: Pancreatic ductal adenocarcinoma; proNeu1: Early committed neutrophil progenitor; proNeu2: Intermediate progeny; preNeu: Preneutrophil; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; TANs: Tumor-associated neutrophils; TGFβ: Transforming growth factor-β; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

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**Authors’ contributions**

S.X. wrote the manuscript and prepared the figures and tables. L.D. helped writing the manuscript. L.C. reviewed and revised the manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. Nat Rev Cancer. 2016;16(7):431–46.
2. Ballestros I, Rubio-Ponce A, Genua M, Lusito E, Kwock I, Fernández-Calvo G, Khoyratty TE, van Grinsven E, González-Hernández S, Nicolás-Ávila J, et al. Co-option of neutrophil fates by tissue environments. Cell. 2020;183(5):1282-1297.e1218.
3. Drissen R, Buza-Vidas N, Wolf P, Thongjuea S, Gambardella A, Giustacchini A, Mancini E, Zirniv A, Lutteropp M, Grover A, et al. Distinct myeloid progenitor-differentiation pathways identified through single-cell RNA sequencing. Nat Immunol. 2016;17(6):666–76.
4. Kwock I, Becht E, Xia Y, Ng M, Teh YC, Tan L, Evrard M, Li JLY, Tran HTN, Tan Y, et al. Combinatorial Single-cell analyses of granulocyte–monocyte progenitor heterogeneity reveals an early uni-potent neutrophil progenitor. Immunity. 2020;53(2):303-318.e305.
5. Avellino R, Delwel R. Expression and regulation of CEBAFes in normal myelopoiesis and in malignant transformation. Blood. 2017;129(15):2083–91.
6. Muraoka M, Akagi T, Ueda A, Wada T, Koeffler HP, Yokota T, Yachie A. C/EBPε ΔRS derived from a neutrophil-specific granule deficiency patient interacts with HDAC1 and its dysfunction is restored by trichostatin A. Biochem Biophys Res Commun. 2019;519(1):293–9.
7. Avellino R, Havermans M, Erpelinck C, Sanders MA, Hoogenbezoem R, van de Werken HJ, Rombouts E, van Lom K, van Strien PA, Gebhard C, et al. An autonomous CEBPA enhancer specific for myeloid-lineage priming and leukemogenic differentiation. Blood. 2016;127(24):2991–3003.
8. Danek P, Kardosova M, Janeckova L, Karkoulia E, Vanickova K, Fabisik M, Lozano-Asencio C, Benoukraf T, Tirado-Magallanes R, Zhou Q, et al. β-Catenin-TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. Blood. 2020;136(22):2574–87.
9. Mumau MD, Vanderbeck AN, Lynch ED, Golec SB, Emerson SG, Punt JA. Identification of a multipotent progenitor population in the spleen that is regulated by NR4A1. J Immunol. 2020;195(3):1078–87.
10. Thanabalasuriar A, Scott BN, Peiseler M, Willson ME, Zeng Z, Warnere P, Keller AE, Surewaard BGL, Dozier EA, Korhonen JT, et al. Neutrophil extracellular traps confine pseudomonas aeruginosa ocular biofilms and restrict brain invasion. Cell Host Microbe. 2019;25(4):526-536.e524.
11. Drummond RA, Lionakis MS. Measuring in vivo neutrophil trafficking responses during fungal infection using mixed bone marrow chimeras. Methods Mol Biol (Clifton, NJ). 2021;2260:179–96.

12. Iversen MB, Reinert LS, Thomsen MK, Bagdonaite I, Nandakumar R, El-Benna J, Hurtado-Nedelec M, Marzaioli V, Marie JC, Gougerot-Pocidalo MA, Döring Y, Soehnlein O, Weber C. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. Circ Res. 2017;120(4):736–43.

13. Castanheira FVS, Kubes P. Neutrophils and NETs in modulating acute and chronic inflammation. Blood. 2019;133(20):2178–85.

14. El-Benna J, Hurtado-Nedelec M, Marzaioli V, Marie JC, Gougerot-Pocidalo MA, Döring Y, Soehnlein O, Weber C. Neutrophil extracellular traps in host defense and inflammation. Immunol Rev. 2016;273(1):180–93.

15. Laforge M, Elbim C, Frère C, Hémard M, Massaad C, Nuss P, Benoillé J, Becker C. Tissue damage from neutrophil-induced oxidative stress in COVID-19. Nat Rev Immunol. 2020;20(9):515–6.

16. Talukdar S, Lih D, Xu J, McNelis J, Lu M, Li P, Yan Q, Zhu Y, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med. 2012;18(9):1407–12.

17. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. Nat Rev Nephrol. 2018;14(2):121–37.

18. Papayanopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018;18(2):134–47.

19. Doring Y, Soehnlein O, Weber C. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. Circ Res. 2017;120(4):736–43.

20. Perdomo J, Leung HHL, Ahmadi Z, Yan F, Chong JH, Passam FH, Chong BH. Neutrophil activation and NETosis are the major drivers of thrombosis in heparin-induced thrombocytopenia. Nat Commun. 2019;10(1):1322.

21. Loo D, Bianco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, Malech HL, Ledbetter JA, Kaplan MJ. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nat Med. 2016;22(2):121–37.

22. Kargi J, Busch SE, Yang GH, Kim KH, Hanke ML, Metz HE, Hubbard JJ, Lee SM, Madtes DK, Michtosh MW, et al. Neutrophils dominate the immune cell composition in non-small cell lung cancer. Nat Commun. 2017;8:14381.

23. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Oçaña A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014;106(6):dju124.

24. Yoshida M, Taguchi A, Kawana K, Adachi K, Kawata A, Ogishima J, Nakamura H, Fujimoto A, Sato M, Inoue T, et al. Modification of the immune cell composition in non-small cell lung cancer. Nat Commun. 2019;10(1):1322.

25. Powell D, Lou M, Barros Becker F, Huttenlocher A. Cxcr1 mediates recruitment of neutrophils and supports proliferation of tumor-initiating astocytes in vivo. Sci Rep. 2018;8(1):11603.

26. Antonio N, Bønnelykke-Behrndtz ML, Ward LC, Christensen CK, Malech HL, Ledbetter JA, Elkon KB, Kaplan MJ. Neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. J Hepatol. 2021. https://doi.org/10.1016/j.jhep.2021.07.035.

27. Timaxian C, Vogel CFA, Orel C, Vetter D, Durochar C, Chinal C, Ngueny P, Akin ML, Mercier-Name F, Dasy M, et al. Pivotal role for Cxcr2 in regulating tumor-associated neutrophil in breast cancer. Cancers. 2021;13(11):2584.

28. Provini ML, Kangat R, Tabbal E. Efficacy of cancer gene therapy in aging: adenocarcinoma cells engineered to release IL-2 are rejected but do not induce tumor specific immune memory in old mice. Gene Ther. 2000;7(7):624–32.

29. Shimizu M, Fortana A, Takeda Y, Yagita H, Yoshimoto T, Matsuzawa A. Induction of antitumor immunity with Fas/APO-1 ligand (CD95L)-transfected neuroblastoma neuro-2a cells. J Immunol (Baltimore, Md:1950). 1999;162(12):7350–7.

30. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci USA. 2006;103(33):12493–8.

31. Christoffersson G, Vågesjö E, Vandooren J, Lidén M, Massena S, Reinert RB, Brissova M, Powers AC, Odenakker G, Phillips PA, VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. Blood. 2012;120(23):4653–62.

32. Purohit A, Saxena S, Varney M, Prajapati DR, Kozel JA, Lazenby A, Singh RK. Host Cxcr2-dependent regulation of pancreatic cancer growth, angiogenesis, and metastasis. Am J Pathol. 2021;191(4):759–71.

33. Romano A, Parmiglio NL, Vettro C, Tibullo D, Giallongo C, La Cava P, Chiarenza A, Motta G, Caruso AL, Villari L, et al. The prognostic value of the myeloid-mediated immunosuppression marker Arginase-1 in classic Hodgkin lymphoma. Oncotarget. 2016;7(41):67333–46.

34. Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, Chen EB, Fan J, Cao Y, Dai Z, Zhou J. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to Sorafenib. Gastroenterology. 2016;150(7):1646-1658.

35. Zha C, Meng X, Li L, Mi S, Qian D, Li Z, Wu P, Hu S, Zhao S, Cai J, et al. Neutrophil extracellular traps mediate the crosstalk between glioma progression and the tumor microenvironment via the HMGB1/RAGE/IL-8 axis. Cancer Biol Med. 2020;17(1):154–68.

36. Yang R, Zhong L, Yang XQ, Jiang KL, Li L, Song H, Liu BZ. Neutrophil elastase enhances the proliferation and decreases apoptosis of leukemia cells via activation of PI3K/Akt signaling. Mol Med Rep. 2016;13(5):4175–82.

37. Lerman I, Ma X, Seger C, Maclaque A, Garcia-Hernandez ML, Rangel-Moreno J, Ackerman J, Nastuki KL, Susiarjo M, Hammes SR. Epigenetic suppression of SFRP1 boosts inflammation-mediated prostate cancer progression. Mol Cancer Res MCR. 2019;17(4):845–59.

38. Nie M, Yang L, Bi X, Wang Y, Sun P, Yang H, Liu P, Li Z, Xie Y, Wang J. Neutrophil extracellular traps induced by IL8 promote diffuse large...
B-cell lymphoma progression via the TLR9 signaling. Clin Cancer Res. 2019;25(6):1867–79.

50. Ostafin M, Ciepiela O, Pruchniak M, Wachowska M, Ulińska E, Mrówka P, Xue Y, Li J, Lu X. A novel immune-related prognostic signature for thyroid cancer. Technol Cancer Res Treat. 2020;19:1533033820935860.

51. Husi H, Fernandes M, Skipworth RJ, Miller J, Cronshaw AD, Fearon KCH, Mayer P, Dinkic C, Jesenofsky R, Klauss M, Schirmacher P, Dapunt U, Zhou M, Kong Y, Wang X, Li W, Chen S, Wang L, Wang C, Zhang Q. A novel approach on neutrophil-derived granule proteins and cytokines. Trends Immunol. 2019;40(7):648–64.

52. Mayer P, Dinkic C, Jesenofsky R, Kluss M, Schirmacher P, Dapunt U, Hackert T, Uhlke F, Hänisch GM, Gaida MM. Changes in the microarchitecture of the pancreatic cancer stroma are linked to neutrophil-dependent reprogramming of stellate cells and reflected by diffusion-weighted magnetic resonance imaging. Theranostics. 2018;8(1):13–30.

53. Jiang X, Qiu Y, Li Y, Li Z, Wang Y, Wang J, Du W, Li Q, Yang Z, Lin J, Li X, Li Y, Guo Y, Zhou X, Li Y, Li Y, Cai X, Li Y, Su Y, Cai Y. Growth factor (IGF) elevation in MCF-7 medium is caused by proteolysis from a computational model. PLoS Comput Biol. 2021;17(2):e1008257.

54. Yang TH, St John LS, Garber HR, Kerros C, Ruisaard KE, Clise-Dwyer K, E, Raskin J, Pauwels P, Baggerman G. Mass spectrometry imaging immunotherapy response in NSCLC patients reveals neutrophil defensins as additional biomarkers for anti-PD-(L)1. Nat Commun. 2020;11(1):5424.

55. Schirmer A, Luo Y, Zhang Z, Li X, Wang Y, Guo J, Guo X, Li Q, Li Y, Zhang H, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. Nat Commun. 2020;11(1):5424.

56. Lee J, Lee D, Lawler S, Kim Y. Role of neutrophil extracellular traps in regulation of lung cancer invasion and metastasis: structural insights from a computational model. PLoS Comput Biol. 2021;17(2):e1008257.

57. Albrengues J, Shields MA, Ng D, Park CG, Ambroco A, Pointederex M, Upadhyay P, Uyeyanmi DL, Pommier A, Kuttner V, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science (New York, NY). 2018;361:6409.

58. Lane AA, Ley TJ. Neutrophil elastase cleaves PML-RARA and is important for the development of acute promyelocytic leukemia in mice. Cell. 2003;113(3):305–18.

59. Hermann I, Hammes SR. Neutrophil elastase in the tumor microenvironment. Steroids. 2018;133:96–101.

60. Yee PP, Wei Y, Kim SY, Lu T, Chih SY, Lawson C, Tang M, Liu Z, Anderson B, Thurburgh K, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. Nat Commun. 2020;11(1):5424.

61. Lee J, Lee D, Lawler S, Kim Y. Role of neutrophil extracellular traps in regulation of lung cancer invasion and metastasis: structural insights from a computational model. PLoS Comput Biol. 2021;17(2):e1008257.

62. Albrengues J, Shields MA, Ng D, Park CG, Ambroco A, Pointederex M, Upadhyay P, Uyeyanmi DL, Pommier A, Kuttner V, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science (New York, NY). 2018;361:6409.

63. Lane AA, Ley TJ. Neutrophil elastase cleaves PML-RARA and is important for the development of acute promyelocytic leukemia in mice. Cell. 2003;113(3):305–18.

64. Hermann I, Hammes SR. Neutrophil elastase in the tumor microenvironment. Steroids. 2018;133:96–101.

65. Yee PP, Wei Y, Kim SY, Lu T, Chih SY, Lawson C, Tang M, Liu Z, Anderson B, Thurburgh K, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. Nat Commun. 2020;11(1):5424.

66. Albrengues J, Shields MA, Ng D, Park CG, Ambroco A, Pointederex M, Upadhyay P, Uyeyanmi DL, Pommier A, Kuttner V, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science (New York, NY). 2018;361:6409.

67. Lane AA, Ley TJ. Neutrophil elastase cleaves PML-RARA and is important for the development of acute promyelocytic leukemia in mice. Cell. 2003;113(3):305–18.

68. Hermann I, Hammes SR. Neutrophil elastase in the tumor microenvironment. Steroids. 2018;133:96–101.

69. Yee PP, Wei Y, Kim SY, Lu T, Chih SY, Lawson C, Tang M, Liu Z, Anderson B, Thurburgh K, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. Nat Commun. 2020;11(1):5424.

70. Albrengues J, Shields MA, Ng D, Park CG, Ambroco A, Pointederex M, Upadhyay P, Uyeyanmi DL, Pommier A, Kuttner V, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science (New York, NY). 2018;361:6409.

71. Lane AA, Ley TJ. Neutrophil elastase cleaves PML-RARA and is important for the development of acute promyelocytic leukemia in mice. Cell. 2003;113(3):305–18.
89. Sokolowska A, Świerzko AS, Gajek G, Golos A, Michalski M, Nowicki M, Szala-Pożdziej A, Wolska-Washer A, Brzezińska O, Wierzbowksa A, et al. Associations of ficolins and mannose-binding lectin with acute myeloid leukemia in adults. Sci Rep. 2020;10(1):10561.

90. Świeżek AS, Michański M, Sokolowska A, Nowicki M, Szala-Pożdziej A, Eppa Ł, Mitrus I, Szmigielska-Kaplon A, Sobczyk-Kruszelnicka M, Michalak K, et al. Associations of ficolins with hematologic malignancies in patients receiving high-dose chemotherapy and autologous hematopoietic stem cell transplantations. Front Immunol. 2019;10:3097.

91. Rasmussen LH, Schultz M, Gaardsting A, Ladefeld S, Garren P, Verden K, Eugen-Olsen J, Helms M, David KP, Kjer J, et al. Inflammatory biomarkers and cancer: CRP and sPAR as markers of incident cancer in patients with serious nonspecific symptoms and signs of cancer. Int J Cancer. 2017;141(1):191–9.

92. Sainz B Jr, Alcala S, Garcia E, Sanchez-Ripoll Y, Azevedo MM, Cioff M, Tatari M, Miranda-Lorenzo I, Hidalgo M, Gomez-Lopez G, et al. Microenvironments hCAP-18/LL-37 promotes pancreatic ductal adenocarcinoma by activating its cancer stem cell compartment. Gut. 2015;64(1):1921–35.

93. Chen X, Zou X, Qi G, Tang Y, Guo Y, Si J, Liang L. Roles and mechanisms of human cathelicidin LL-37 in cancer. Cell Physiol Biochem Int J Exp Cell Physiol Pharmacol. 2018;47(3):1060–73.

94. Scheemstra MR, van Harten RM, Veldhuizen EJA, Haasman GP, Coorens M. Cathelicidins modulate TLR-activation and inflammation. Front Immunol. 2020;11:1137.

95. Chen J, Shin YV, Ho J, Liu P, Cheuk IW, Kwong A. Functional implications of cathelicidin antimicrobial protein in breast cancer and tumor-associated macrophage microenvironment. Biomolecules. 2020;10(5):10.

96. Juurikkka K, Butler GS, Salo T, Nyberg P, Åström P. The role of MMP8 in cancer: a systematic review. Int J Mol Sci. 2019;20(18):10.

97. Juurikkka K, Dufour A, Plehak M, Baniomadi P, Campioni Rodrigues P, Solis Oh P, Testa JE, Borgstrom P, Witkiewicz H, Li Y, Schnitzer JE. In vivo assessment of ficolins and mannose-binding lectin with acute myeloid leukemia. Cancer. 2017;141(1):191–9.

98. Brandsma AM, Bondza S, Evers M, Koutstaal R, Nederend M, Jansen et al. Neutrophils restrict tumor-associated microbiota to reduce growth and invasion of colon tumors in mice. Gastroenterology. 2019;156(5):1467–82.

99. Pekala LA, Starr BA, Toledano AJ, Schreiber H. Inhibition of tumor growth by elimination of granulocytes. J Exp Med. 1989;161(1):430–40.

100. Cobert ST, Kersten K, Doomebel CW, Weiden J, Vrijland K, Hau CS, Verstegen NJM, Ciampricotti M, Hawinkels L, Jonkers J, et al. IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature. 2015;522(7556):345–8.

101. Baek AE, Yu YA, He S, Wardell JE, Chang CY, Kwon S, Pillai RV, McDowell HB, Thompson JW, Dubitzky LG, et al. The cholesterol metabolite 27-hydroxysterol facilitates breast cancer metastasis through its actions on immune cells. Nat Commun. 2017;8(1):11864.

102. Benedicto A, Marquez J, Herrero A, Olaso E, Kolaczkowska E, Arteba A. Decreased expression of the B2 integrin on tumor cells is associated with a reduction in liver metastasis of colorectal cancer in mice. BMC Cancer. 2017;17(1):827.

103. Yang LY, Luo Q, Li L, Zhu WW, Sun HT, Wei R, Lin ZF, Wang XY, Wang CQ, Lu M, et al. Increased neutrophil extracellular traps promote metastatic potential of hepatocellular carcinoma via provoking tumors inflammatory response. J Hematol Oncol. 2020;13(1):3.

104. Wang Z, Yang C, Li L, Jin X, Zhang Z, Zheng H, Pan J, Shi L, Jiang Z, Su K, et al. Tumor-derived HMGB1 induces CD62L(dim) neutrophil polarization and promotes lung metastasis in triple-negative breast cancer. Oncogenesis. 2020;9(9):82.

105. Zhang X, Shi H, Yuan J, Xiang J, Qian H, Xu W. Tumor-derived exosomes induce N2 polarization of neutrophils to promote gastric cancer cell migration. Mol Cancer. 2018;17(11):146.

106. Bald T, Quast T, Landsberg J, Rogova M, Glodde N, Lopez-Ramos D, Kohlmeier J, Riesenberg S, van den Boom-Kornienberg D, Hörmig-Hölzel C, et al. Ultraviolet-radiation-induced inflammation promotes angiogenesis and metastasis in melanoma. Nature. 2014;507(7490):109–13.

107. Morimoto-Kamata R, Yui S, Sivin-radiation-induced inflammation promotes angiogenesis in melanoma. Nature. 2014;507(7490):109–13.

108. McDowell SAC, Luo RBE, Alazabzadeh A, Doré S, Bennett NC, Breton V, Karimi E, Resanz M, Yang RR, Lach KD, et al. Neutrophil oxidative stress mediates obesity-associated vascular dysfunction and metabolic transmembrane. Nat Cancer. 2021;2(5):545–62.

109. Najmeh S, Cool-Lartigue J, Rayes RF, Gowing S, Bourque MB, Doré S, Bennett NC, Breton V, Karimi E, Resanz M, Yang RR, Lach KD, et al. Neutrophil oxidative stress mediates obesity-associated vascular dysfunction and metabolic transmembrane. Nat Cancer. 2021;2(5):545–62.

110. Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkountela S, Landin J, Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkountela S, Landin J, et al. Ultraviolet-radiation-induced inflammation promotes angiogenesis and metastasis in melanoma. Nature. 2014;507(7490):109–13.

111. McDowell SAC, Luo RBE, Alazabzadeh A, Doré S, Bennett NC, Breton V, Karimi E, Resanz M, Yang RR, Lach KD, et al. Neutrophil oxidative stress mediates obesity-associated vascular dysfunction and metabolic transmembrane. Nat Cancer. 2021;2(5):545–62.

112. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Cancer Discovery. 2015;5(7):689.

113. Matlung H, Babes L, Zhao XW, van Houdt M, Treffers LW, van Rees DJ, Franke K, Schonnagel K, Verkuijlen P, Janssen H, et al. Neutrophils kill antibody-opsonized cancer cells by tropoegosis. Cell Rep. 2018;23(13):3946-3959.e3946.

114. Hubert P, Heitzmann A, Vel S, Nicolas A, Sastre-Garau X, Oppezzo P, Pfirtsch O, Osinaga E, Argenzona S. Antibody-dependent cell cytotoxicity of synapses form in mice during tumor-specific antibody immunotherapy. Can Res. 2011;71(15):7134–43.

115. van Egmond M, Bakema JE. Neutrophils as effector cells for antibody-based immunotherapy of cancer. Semin Cancer Biol. 2013;23(3):190–9.

116. Brandsma AM, Bondza S, Evers M, Koutstaal R, Nederend M, Jansen JHM, Rosner T, Valenius T, Leenheer JHM, Ten Broeke T. Potent Fc receptor signaling by IgA leads to superior killing of cancer cells by neutrophils compared to IgG. Front Immunol. 2019;10:704.

117. Triner D, Devenport SN, Ramakrishnan SK, Ma X, Frieri RA, Greeneson JK, Inohara N, Guez Y, Colacino JA, Mortensen RM, et al. Neutrophils restrict tumor-associated microbiota to reduce growth and invasion of colon tumors in mice. Gastroenterology. 2019;156(5):1467–82.

118. McDowell SAC, Luo RBE, Alazabzadeh A, Doré S, Bennett NC, Breton V, Karimi E, Resanz M, Yang RR, Lach KD, et al. Neutrophil oxidative stress mediates obesity-associated vascular dysfunction and metabolic transmembrane. Nat Cancer. 2021;2(5):545–62.

119. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Cancer Discovery. 2015;5(7):689.

120. Liu Y, Gu Y, Han Y, Zhang Q, Jiang Z, Zhang X, Huang B, Xu Z, Zheng J, Cao X. Tumor exosomal RNAs promote lung pre-metastatic niche...
formation by activating alveolar epithelial TLR3 to recruit neutrophils. Cancer Cell. 2016;30(2):243–56.

128. Lee W, Ko SY, Mohamed MS, Kenny HA, Lengyel E, Naora H. Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum. J Exp Med. 2019;216(1):76–94.

129. Kaplan RN, Riba RD, Zacharooulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Korns SA, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005;438(7069):820–7.

130. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell. 2011;20(3):300–14.

131. Oberg HH, Wesch D, Kalyan S, Kabelitz D. Regulatory interactions between neutrophils, tumor cells and T cells. Front Immunol. 2019;10:1690.

132. Li P, Lu M, Shi J, Hua L, Gong Z, Li Q, Shultz LD, Ren G. Dual roles of neutrophils in metastatic colonization are governed by the host NK cell status. Nat Commun. 2020;11(1):4387.

133. Castano Z, San Juan BP, Spiegel A, Pant A, DeCristo MJ, Lasztesyi TK, Uberbacker JM, Janssen SR, Dongre A, Reinhardt F, et al. IL-1beta inflammatory response driven by primary breast cancer prevents metastasis-initiating cell colonization. Nat Cell Biol. 2018;20(9):1084–97.

134. Vono M, Lin A, Norrby-Teglund A, Koup RA, Liang F, Loré K. Neutrophils acquire the capacity for antigen presentation to memory CD4(+)/T cells in vitro and ex vivo. Blood. 2017;129(4):1991–2001.

135. Carus A, Ladekarl M, Hager H, Nedergaard BS, Donskov F. Tumour-associated neutrophils initiate cell colonization. Nature. 2010;468(7325):243–56.

136. Watanabe A, Harimoto N, Araki K, Kubo N, Igarashi T, Tsukagoshi M, Francescangeli F, De Angelis ML, Zeuner A. COVID-19: a potential driver of neutrophil extracellular trap formation by activating alveolar epithelial TLR3 to recruit neutrophils. Sci Transl Med. 2020;12(541):eaaz8867.

137. Demers M, Wong SL, Martinod K, Gallant M, Cabral JE, Wang Y, Wagner RJ, Silver AJ, Adams D, Castellano CA, Schneider RK, et al. Increased neutrophil extracellular trap formation promotes cancer metastasis via CCDC25. Nature. 2019;572(7760):538–42.

138. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, Huang D, Li J, Li H, Chen F, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature. 2020;583(7814):133–8.

139. Eruslanov EB, Bhojnagarwala PS, Quatromoni JG, Stephen TL, Rangana-than A, Deshpande C, Akimova T, Vachani A, Litzky L, Hancock WW, et al. Tumor-associated neutrophils stimulate T cell responses in early-stage human lung cancer. J Clin Investig. 2014;124(2):5466–80.

140. Blaisdell A, Crequer A, Columbus D, Daikoku T, Mittal D, Dey SK, Erlebacher A. Neutrophils oppose uterine epithelial carcinogenesis via deprivation of hypoxic tumor cells. Cancer Cell. 2015;28(6):785–99.

141. Singhal S, Bhojnagarwala PS, O’Brien S, Moon EK, Garfall AL, Rao TK, Quatromoni JG, Stephen TL, Litzky L, Deshpande C, et al. Origin and role of a subset of tumor-associated neutrophils with antigen-presenting cell features in early-stage human lung cancer. Cancer Cell. 2016;30(1):120–35.

142. Covarria V, Trella E, Mele V, Tomillo L, Amicarella F, Cremosei M, Muraro MG, Xu H, Droese R, Daster SR, et al. The interplay between neutrophils and CD8(+) T cells improves survival in human colorectal cancer. Clin Cancer Res. 2017;23(14):3847–58.

143. Porzetta A, Carriero R, Carnevale S, Barbagallo M, Molgora M, Perucchini C, Magrini E, Gianni F, Kundernfanço P, Polentarutti N, et al. Neutrophils driving unconventional T cells mediate resistance against murine sarcomas and selected human tumors. Cell. 2019;178(2):346-360.e34.

144. Mahieddine K, Blaisdell A, Ma S, Créquier-Grandhomme A, Lowell CA, Erlebacher A. Relief of tumor hypoxia unleashes the tumoricidal potential of neutrophils. J Clin Investig. 2020;130(1):389–403.

145. Cederwall J, Zhang Y, Huang H, Zhang L, Femej D, Dimberg A, Olsson AK. Neutrophil extracellular traps accumulate in peripheral blood vessels and compromise organ function in tumor-bearing animals. Can Res. 2015;75(13):2653–62.

146. Wolach O, Sellar RS, Martinod K, Cherpokova D, McConkey M, Chappell RJ, Silver AJ, Adams D, Castellano CA, Schneider RK, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med. 2018;10:436.

147. Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, Schott AF, Kinugasa-Katayama Y, Lee Y, Won NH, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci Transl Med. 2016;8(361):361ra138.

148. Wellensteiin MO, Coffield SB, Duts DEM, van Miltenburg MH, Slagter M, de Rink I, Henneman L, Kas SM, Prekovic S, Hau CS, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. Nature. 2019;572(7730):538–42.

149. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, Huang D, Li J, Li H, Chen F, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature. 2020;583(7814):133–8.

150. Potentiation with G-CSF. Proc Natl Acad Sci USA. 2016;113(40):11300–5.

151. Bruchard M, Milgrom G, Darengire V, Chalmim F, Chevraux A, Wégran F, Boireau W, Simon B, Ryffel B, Connat JL, et al. Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Can Res. 2010;70(8):3052–61.

152. Cristofanilli M, Andree-Aire D, Gangi P, Giacone W, von Mehren M, et al. Tumor-infiltrating lymphocytes. J Clin Oncol. 2001;19(7):2031–40.

153. Takeshima T, Pop LM, Laine A, Iyengar P, Vitetta ES, Hannan R. Key role of the immunoinhibitory receptor LILRB2 reprograms tumor-associated neutrophils to promote resistance to immune checkpoint inhibitors. Cancer Cell. 2017;31(5):789-802.e789.

154. Veglia F, Turin VA, Blasi M, De Leo A, Kossenkov AV, Doniheddy L, To TKJ, Schug Z, Basu S, Wang F, et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. Nature. 2019;569(7754):73–8.

155. Gillette MA, Satpathy S, Cao S, Dhanasekaran SM, Vasaikar SV, Krug K, Petralia F, Li Y, Liang WW, Reva B, et al. Proteogenomic characterization reveals therapeutic vulnerabilities in lung adenocarcinoma. Cell. 2020;182(1):200-225.e235.

156. Kuang DM, Zhao Q, Wu Y, Peng C, Wang J, Yuan X, Yin XY, Zheng L. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. J Hepatol. 2011;54(5):948–55.

157. Steele CW, Karim SA, Leen JDG, Bailey P, Upstill-Goddard R, Lush I, Foth M, Bryson S, McDaid K, Wilson Z, et al. CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. Cancer Cell. 2016;29(6):832–45.
myeloid cells and promotes antitumor immunity. J Clin Investig. 2018;128(12):5647–62.

165. Treffers LW, Ten Broeke T, Rosner T, Jansen JHM, van Houdt M, Kahle S, Schornagel K, Verkuilen P, Prins JM, Franke K, et al. IgA-mediated killing of tumor cells by neutrophils is enhanced by CD47-SIRPα checkpoint inhibition. Cancer Immunol Res. 2020;8(1):120–30.

166. Shaul ME, Friedlender ZG. Tumor-associated neutrophils in patients with cancer. Nat Rev Clin Oncol. 2019;16(10):601–20.

167. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM. Polarization of tumor-associated neutrophil phenotype by TGF-beta “N1” versus “N2”TAN. Cancer Cell. 2009;16(3):183–94.

168. Mehmeti-Ajradini M, Bergenfelz C, Larsson AM, Carlsson R, Liesbeck K, Ahl J, Janolis H, Wulft M, Bredberg A, Kallberg E, et al. Human G-MDSCs are neutrophils at distinct maturation stages promoting tumor growth in breast cancer. Life Sci Alliance. 2020;3(11):10.

169. Cané S, Bronte V. Detection and functional evaluation of arginase-1 isolated from human PMNs and murine MDSC. Methods Enzymol. 2020;632:193–213.

170. Raber PL, Thevenot P, Sierra R, Wyczewskowska D, Halle D, Ramirez ME, Ochoa AC, Fletcher M, Velasco C, Wilk A, et al. Subpopulations of myeloid-derived suppressor cells impair T cell responses through independent nitric oxide-related pathways. Int J Cancer. 2014;134(12):2853–64.

171. Al-Khamsi AA, Zheng L, Del Valle L, Hossain F, Wyczewskowska D, Zabaleta J, Sanchez MD, Dean MJ, Rodriguez PC, Ochoa AC. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. Oncogenesis. 2017;6(10):e1344804.

172. Rogers T, DeBerardinis RJ. Metabolic plasticity of neutrophils: relevance to pathogen responses and cancer. Trends Cancer. 2021;7(8):700–13.

173. Grieshaber-Bouyer R, Radtke FA, Cunin P, Stifano G, Levescot A, Vijaykumar B, Nelson-Maney N, Blaustein RB, Monach PA, Nigrovic PA. The neutrotine transcriptional signature defines a single continuum of neutrophils across biological compartments. Nat Commun. 2021;12(1):2856.

174. Mysore V, Cullere X, Mears J, Rosetti F, Okubo K, Liew PX, Zhang F, Madera-Sáucedo I, Rosenbauer F, Stone RM, et al. FcγR engagement reprograms neutrophils into antigen cross-presenting cells that elicit acquired anti-tumor immunity. Nat Commun. 2021;12(1):4791.

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