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Neutrophils in the Tumor Microenvironment

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Abstract

Neutrophils are the first responders to inflammation, infection, and injury. As one of the most abundant leukocytes in the immune system, neutrophils play an essential role in cancer progression, through multiple mechanisms, including promoting angiogenesis, immunosuppression, and cancer metastasis. Recent studies demonstrating elevated neutrophil to lymphocyte ratios suggest neutrophil as a potential therapeutic target and biomarker for disease status in cancer. This chapter will discuss the phenotypic and functional changes in the neutrophil in the tumor microenvironment, the underlying mechanism(s) of neutrophil facilitated cancer metastasis, and clinical potential of neutrophils as a prognostic/diagnostic marker and therapeutic target.

Keywords

Tumor microenvironment; Neutrophil; CXCR2 ligands; IL17; Pro-tumor chemokines; Pro-tumor cytokines; Neutrophil-released proteases; Metastasis; Angiogenesis; NETs; NLR

1.1 Introduction

Neutrophils or polymorphonuclear (PMN) leukocytes originate from the myeloid lineage and are the most abundant white blood cell types. Every day, nearly 10^{11} neutrophils are produced in the bone marrow and represent the most active cell type for the innate immune system [1, 2]. The name neutrophil is derived from the positive staining of both hematoxylin and eosin dyes. Neutrophils are first responders of acute inflammation and capture invading microorganisms through different mechanisms such as phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs) [2]. Until recently, host defense, immune modulation, and tissue injury were considered the only function of neutrophils [3]. However, it has been observed that other than simply killing the microbe, neutrophils function in a more complicated mechanism(s). Thus, neutrophils play a pivotal role in chronic inflammatory diseases such as cancer. Accumulating evidence suggests that neutrophils display phenotypic heterogeneity and functional versatility and are transcriptionally active cells as they respond to multiple signals by producing several inflammatory cytokines and factors that regulate the immune system [4, 5].

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Current literature suggests an important role of neutrophils in the tumor microenvironment [1]. However, the pro- or antitumor nature of neutrophils in different cancer types is still inconclusive [6, 7]. The tumor microenvironment plays a crucial role in cancer metastasis [8] and significantly affects the therapeutic response and the overall outcome of cancer patients. This chapter will discuss the phenotypic and functional changes in the neutrophil in the tumor microenvironment, the underlying mechanism(s) of neutrophil facilitated cancer metastasis, and clinical potential of neutrophils as a prognostic/diagnostic marker and therapeutic target.

1.2 Neutrophil Life Cycle

Neutrophils compose a significant part of granulocytes and play pivotal roles during inflammation, infection, and cancer progression [9, 10]. Additionally, the neutrophils are the most abundant leukocytes in multiple species, including human. In whole blood, the proportion of neutrophils in healthy adults ranges from 30% to 70%; meanwhile, the neutrophil numbers may fluctuate under disease conditions [11]. The neutrophils are commonly short-lived cell types compared to other immune cell types (less than 24 h). Meanwhile, the half-disappeared time in the circulation of neutrophils was around 8 h [12]. However, in vivo labeling in humans with the use of $^2\text{H}_2\text{O}$ under homeostatic conditions demonstrated the neutrophil lifetime could be as long as 5.4 days [13].

1.2.1 Granulopoiesis

Neutrophils are derived from the common myeloid progenitor cells, which are the precursor of the cells in the innate immune system [14]. The common myeloid progenitor cells (Lin^- , Sca-1^- , c-kit^+ , IL-7R^- , $\text{Fc}\gamma\text{R}^{\text{lo}}$ cell population) further differentiated into granulocyte-monocyte progenitor cells (Lin^- , Sca-1^- , c-kit^+ , IL-7R^- , $\text{Fc}\gamma\text{R}^{\text{hi}}$ cell population), and this process requires the expression of C/EBP- α [15]. The granulocyte-monocyte progenitor cells then further differentiate into monocytes or granulocyte precursor cells [16]. The granulocyte precursor cells give rise to neutrophils by the transition from promyelocyte, myelocyte, metamyelocyte, band cells, then to neutrophils [4, 17]. The commitment to neutrophils during this stage requires the expressions of regulators such as C/EBP- ϵ [16, 18]. Mice without C/EBP- ϵ expression developed usually but failed to generate functional neutrophils and eosinophils [18].

The differentiation of neutrophils requires the gradual replacement of proliferation by differentiation in the myeloid progenitor cells [19] and also requires the neutrophil granulopoiesis. The granulopoiesis is divided into three processes: firstly, the formation of primary granules; secondly, the beginning of nuclear segmentation, the appearance of secondary granules, and exiting from the cell cycle; and thirdly, the final segmented nuclei together with tertiary and secretory granules [19]. Additionally, the neutrophil primary granules formed at myeloblast to promyelocyte stage; the secondary granules can be found at the myelocyte to metamyelocyte stages; the tertiary granules are detected at the band cell stage; meanwhile, only mature neutrophils are with secretory vesicles [4].

1.2.2 Neutrophil Dynamics: From Bone Marrow to the Circulation

As the first responder in inflammation or infection, neutrophils react quickly and mobilize out of the bone marrow reserve by crossing the sinusoidal endothelium and in an abluminal to the luminal direction [20, 21]. The mobilization of neutrophils from bone marrow to circulation is delicately regulated by factors such as granulocyte colony-stimulating factor (G-CSF), CXCR2, and CXCR2 ligands, together with CXCR4 and CXCR4 ligands [22]. The mobilization of neutrophils requires the upregulation of G-CSF and CXCR2 signaling, together with the downregulation of CXCR4 signaling [20]. The liver and spleen are the primary organs for the neutrophil clearance in the circulation [23]. However, recent studies showed that bone marrow also functions as the sites of neutrophil construction. According to the radiolabel of the senescent neutrophils in mice model, the senescent neutrophils were 32% in bone marrow, 29% in the liver, and 31% in the spleen [24]. The homing of neutrophils to bone marrow requires the upregulation of CXCR4 in neutrophils [25], and the neutrophils backing to the bone marrow will be under apoptosis and digested by bone marrow macrophages [23, 26]. During inflammation, the neutrophils can also be taken up by the macrophages at the sites of inflammation [25]. Under disease conditions such as inflammation or cancer, the half-life of neutrophils are very different, which varies from shorter life spans to longer life spans [26, 27], and also accompanied with dysregulated neutrophil numbers, morphologies, and differentiation states in the circulation system [10, 15, 28]. In cancer cases, the existence of subpopulations of neutrophils made the situation even more complicated [4].

The neutrophils mobilized to the sites where they are required; once the neutrophils arrived, they phagocyte and release chemokines, cytokines, and proteases, and then they are cleaned up by other immune cells, including the macrophages. The dynamic of neutrophils sounds like a straightforward story. However, the population of neutrophils in the human body is not simple. The neutrophils may behave differently according to various stimuli [4]. More research is required to reveal the heterogeneity of neutrophils in a complex disease like cancer.

1.3 Neutrophil Population in Health and Disease

As the most abundant leukocyte in the innate immune system, neutrophils can compose 70% of the leukocyte population [4]. Mature neutrophils are stored in large numbers in the bone marrow. The pool of mature neutrophils is termed as the bone marrow reserve. Typically, individual mice usually have a total number of 120 million neutrophils; meanwhile, in humans, the neutrophil numbers in bone marrow can reach up to 5×10^{10} to 10×10^{10} neutrophils/day, with a total blood granulocyte pool of 65×10^7 cells/kg [26]. In humans, the overall numbers of neutrophil fluctuation depend not only on the blood volume of each individual but also on the ethnic groups, age, health stage, and smoking status. For instance, African American participants possessed significantly lower neutrophil counts in the blood (mean differences, 0.83×10^9 cells/L; $P < 0.001$) relative to white participants, whereas relative to the white participants, Mexican-American participants had higher neutrophil numbers (mean differences, 0.11×10^9 cells/L; $P = 0.026$). Smoking status is positively linked with neutrophil numbers in all three ethnic groups [29].

As discussed previously, the homeostasis of neutrophil numbers requires the sophisticated counterbalance of both positive and negative feedback signaling. The activation of positive neutrophil mobilization pathways spontaneously stimulates the regulation of negative neutrophil mobilization pathways to strike the delicate balance of neutrophil numbers in the human body, for example, feedback inhibition of SOCS3 to STAT3-mediated G-CSF-induced neutrophil granulopoiesis [30]. In disease conditions such as infection, inflammation, congenital disease, and cancer, the homeostasis of neutrophils is disturbed temporarily, or even for a long term, which leads to the variation of neutrophil numbers.

Neutrophils are the crucial regulators during microbe infection. The neutrophils can clear up the microbe by mechanisms including phagocytosis, ROS/RNS production, and NET formation [31]. The number of neutrophils increased dramatically (around 5×10^6 to 10×10^6 increase in neutrophil numbers in 1.5 h) in the peripheral blood once activated by LPS of *Escherichia coli*. Additionally, neutrophils in response to LPS challenges quickly altered their expression profiles such as initialization of the expressions of cytokines such as TNF α and downregulation of surface receptors such as Fc γ RII and TLR4 [32].

Similar to infection, increased neutrophil numbers in the blood are a commonly accepted clinical feature in inflammatory diseases. The acute inflammatory response induced by a thioglycolate injection resulted in the 4.5-fold increases of neutrophil numbers in blood within hours (original numbers of neutrophils in 6–8-week BALB/cJ mouse circulation: $1.5 \times 10^9/L$) [23]. The number of neutrophils also fluctuates during different disease conditions, such as neutropenia seen in patients with solid tumor malignancies filtrated in the bone marrow or patients with lymphoproliferative malignancies such as natural killer cell lymphomas [33]. Radiation therapy used on multiple sites of cancer patients' bone marrow can also result in neutropenia [33]. Additionally, neutropenia observed in cancer patients is mostly due to the administration of chemotherapy drugs [33]. However, higher levels of neutrophils are found in the blood of patients with advanced cancer, and this might be due to the upregulation of G-CSF in multiple cancer types [34]. Moreover, the association study in 5782 tumors and 25 types of cancers showed higher PMN numbers indicated lower survival rates in cancer patients [35], implying the neutrophils are not favorable immune cell type to the majority of the cancer patients.

1.3.1. Neutrophil Frequency and Phenotype in Cancer

The life cycle of neutrophils begins with production in the bone marrow, followed by entry into the circulation and migration into the site of infection or inflammation, and finally being cleared by tissue-resident macrophages [25]. During this life cycle, neutrophils can undergo different phenotypic as well as functional changes in the frequency of circulatory neutrophils during tumor progression [4].

One such change is a well-established observation that peripheral neutrophils in the blood are increased in cancer patients [1]. However, this increase in the peripheral neutrophil count is not limited to the cancer condition but is observed under other conditions as described previously. Scientists have tried to use this observation in the form of neutrophil to lymphocyte ratio (NLR) and correlated it with cancer patient outcomes. A metaanalysis study, by Templeton et al. in 2014, compiled observations from 100 such studies with

different types and stages of cancer, which revealed that $NLR > 4$ is associated with lower overall survival rates [36]. A limitation of measuring NLR is that it does not give us any mechanistic insight into the condition.

1.3.2 Low-Density Neutrophils (LDNs)

LDNs are a group of immature cells with banded or segmented nuclei and myelocyte-like cells [4], which represents another subpopulation of neutrophils found in low-density fraction by the Ficoll density gradient [37]. Unlike neutrophils, which are found in the high-density fraction at the bottom of the tube, LDN was associated with many pathological disorders [4] such as asthma or AIDS. However, the LDNs gained attention because of their association with cancer [38, 39]. Specific molecular markers, immunosuppressive characteristics, and functions have not been defined for LDNs, thus leading to different schools of thought about their origin. One possibility is that these immature cells are released from bone marrow during chronic inflammation or cancer or that LDNs are activated neutrophils that have undergone degranulation and, therefore, have a reduced density [4].

1.3.3 Myeloid-Derived Suppressor Cells (MDSCs)

Apart from the increase in the number of neutrophils in cancer patients, there is also an increase in immature myeloid cell populations [38]. These morphologically immature cells with a band or myelocyte-like nuclei [40] are named MDSC because of their immunosuppressive nature and pro-tumor behavior. MDSC has been found to play a critical role during tumor progression [40]. MDSCs are heterogeneous populations which represent cells in different differentiated stages and can be divided into two categories: the granulocytic (G-MDSC) whose morphology and phenotype are similar to neutrophils and represent 80% of the whole MDSC population, and the monocytic (M-MDSC) whose morphology and phenotype are similar to the monocytes and represent around 20% of the whole MDSC population [41]. Other than malignant tumors, MDSC could also appear in infections, autoimmune diseases, diabetes [42], and tuberculosis [43, 44]. The conventional role of MDSCs is usually involved in immunosuppression, and the T cell functions are the main target [15, 44].

In mice, both neutrophils and G-MDSC are defined by $CD11b^+Ly6G^+$, whereas monocytic MDSCs are defined by $CD11b^+Ly6C^+$ [4, 45]. Even though human MDSCs are more complicated with six different markers used to define G-MDSC ($CD11b^+CD14^-CD15^+CD33^+CD66b^+HLA-DR^-$), unfortunately, still there is no clear distinction between neutrophils and G-MDSC [4]; further investigations are warranted to define whether neutrophils and G-MDSC are subpopulations or separate cell types. A possible way to isolate neutrophils from MDSCs is through centrifugation using a standard Ficoll gradient, as neutrophils are high-density cells in comparison with G-MDSCs (enriched in the low-density fraction) [15].

1.3.4 Tumor-Associated Neutrophils (TANs)

The changes in circulatory neutrophils are also reflected in the infiltration of neutrophils inside the tumor [4]. Neutrophils inside the tumor are called TANs. TANs can play dual

roles in cancer progression, and according to the pro- or antitumor properties of these cells, we can classify TAN into N1 and N2 types.

Similar to the classification of tumor-associated macrophages in the tumor microenvironment (M1 as antitumor macrophage and M2 as pro-tumor macrophage), Fridlender et al. proposed the concept of polarization of TANs as N1 with antitumor and N2 with pro-tumor properties. Fridlender presented N1 TANs by blocking transforming growth factor-beta (TGF β) in tumor-bearing mice, which were functionally and morphologically different from N2 TANs [7]. N1 TANs were toxic to cancer cells by using the oxygen radical-dependent mechanism, with increased expression of tumor necrosis factor-alpha (TNF- α), intercellular adhesion molecule 1 (ICAM-1), and FAS. Additionally, N1 were morphologically different from N2 TANs by having hyper-segmented nuclei [7]. On the other hand, N2 TANs with characteristic circular nuclei had pro-tumor characteristics as they suppressed T cell immunity by expressing increased levels of arginase as well as other pro-tumor factors such as CCL2, CCL5, neutrophil elastase (NE), and cathepsin G (CG). Differences in the nuclei of N1 and N2 neutrophils also indicate a possibility that they represent different maturation stages rather than phenotypic subtypes [5].

Various stimuli present in the tumor microenvironment can activate neutrophils to different phenotypes. Thus the primary binary classification of neutrophils is an oversimplification. Neutrophils can have different levels of plasticity with N1 and N2 as extreme phenotypes in complex diseases such as cancer. At present, there are no suitable markers, which define N1 and N2 in humans. Another significant limitation of this system is that the work, which leads to the emergence of the N1 and N2 TANs concept, has only been performed in murine models and is yet to be replicated in humans [46].

An interesting question is whether TANs can also be associated with survival outcomes. Recently, tumor transcriptomics-based computational study partially answered this question by revealing that TANs are the most adverse prognostic cell population in over 3000 solid tumors, comprised of 14 different cancer types [35]. On the other hand, chemotherapy decreased cancer patients' neutrophils in peripheral blood (neutropenia) numbers, which is a sign of effective chemotherapy treatment. However, to overcome neutropenia, patients are often treated with G-CSF, which has been shown to promote breast cancer metastasis [47]. Thus, it is an interesting question of whether the administration of G-CSF post-chemotherapy is beneficial or detrimental to final clinical outcomes [6].

1.4 Functions of Neutrophils in the Tumor Microenvironment

Generally, TANs represent a pro-tumor factor in different tumor types [1, 10, 48] and are associated with the least favorable overall survival for solid tumor patients in comparison with other leukocytes present in the tumor [35]. In this section, we will discuss different functions associated with neutrophil biology in the light of the tumor microenvironment (Fig. 1.1). The majority of cases discussed in this section reported neutrophils played pro-tumor roles through multiple mechanisms; nevertheless, there are few reports that indicate the antitumor role of neutrophils in cancer [10].

1.4.1 Neutrophil-Released Reactive Oxygen Species (ROS)/Reactive Nitrogen Species (RNS)

As discussed in previous sections, one of the primary functions of neutrophils is to eliminate infection at the inflammatory site during an immune response [3], with phagocytosis being one of the essential killing mechanisms [49]. Neutrophils engulf the pathogen and form a phagosome which later fuses with a lysosome [50]. For killing the pathogen, NADPH oxidase present in neutrophils' granules changes the pH of the fused phagosome and lysosome structure, which is now termed as phagolysosome [51] and results in the production of reactive oxygen species (ROS) through the respiratory burst [52]. However, the released ROS by neutrophils in the tumor microenvironment usually play a protumor role by damaging the DNA bases [53], which results in mutations [53, 54]. In general, the tumor microenvironment has a high level of ROS, which can not only initiate cancer but also lead to epithelial damage and inflammation inside the tumor [1], increasing cellular proliferation, suppressing immune cell [34, 55], chemoresistance [56], and EMT, which leads to an invasive phenotype in multiple cancer types [57]. Hydrogen peroxide, one of the ROS, can regulate different cell signaling pathways, which are important in cellular biology, such as the PI3K/Akt, IKK/NF- κ B, and MAPK/Erk1/2 pathway, by acting as secondary messengers. However, hydrogen peroxide production by neutrophils is also considered as one of the mechanisms of eliminating tumor cells [58]. For instance, neutrophils, after physical contact with cancer cells, can secrete hydrogen peroxide, resulting in tumor cell death by Ca^{2+} influx through the TRPM2 Ca^{2+} channel [59]. Similarly, in TANs, interaction between the Met receptor and its ligand, the hepatocyte growth factor (HGF), triggered the release of nitric oxide to eliminate the tumor cells [60]. Therefore, the level of ROS/RNS production by neutrophils will dictate their pro- or antitumor behavior in the tumor microenvironment [10].

1.4.2 Neutrophil-Secreted Cytokines and Chemokines

Neutrophils respond to different stimuli present in the tumor microenvironment by releasing various cytokines and chemokines [61–63]. These neutrophil-secreted cytokines and chemokines will not only determine the pro- or antitumor response on other tumor-associated stromal cells, but the neutrophil will also educate itself for a pro- or antitumor behavior [7, 10, 48, 64]. For instance, neutrophil-secreted factors, such as oncostatin M (OSM) or TGF- β into the tumor microenvironment, have been shown to polarize the macrophage towards a pro-tumor phenotype (M2 type) [64, 65]. Similarly, nitric oxide secreted by neutrophils has been shown to suppress T cell cytotoxicity [66].

Many recent studies have tipped the balance of neutrophil-secreted chemokines and cytokines towards a pro-tumor behavior. For example, Queen et al. have shown that co-culture of neutrophils with human breast cancer cell lines triggered the release of oncostatin M (OSM) by neutrophils, thereby facilitating angiogenesis through the induction of vascular endothelial growth factor (VEGF) [67]. In another breast cancer study [68], neutrophil-released TGF- β has also been shown to promote tumor cell resistance to gemcitabine by inducing epithelial to mesenchymal changes in tumor cells [69].

TANs have also been shown to secrete pro-inflammatory cytokines into the tumor microenvironment, such as IL17, CXC, and CC chemokines [10, 70–76]. These pro-inflammatory cytokines, such as IL17, can promote tumor progression by acting directly on pancreatic cancer cells and inducing them with stem cell-like features [77] or indirectly promoting cancer progression by facilitating neutrophil mobilization through upregulation of CXCR2 ligand expression (Fig. 1.2) [76]. Other pro-inflammatory factors, such as CXC chemokines, are well-known for the recruitment of neutrophils to the tumor site [75]. De Oliveria et al. demonstrated higher levels of CXCL8 during an inflammatory response in a zebra fish model, which resulted in higher numbers of neutrophil recruitment [78]. Thus neutrophil-secreted CXCL8 in the head and neck cancer suggests a feedforward loop for neutrophil recruitment in the tumor microenvironment [70]. Apart from CXC chemokines, a number of cancer studies report that neutrophils secrete a significant amount of CC ligands [72, 73], which are chemoattractants for monocyte, regulatory T cells, and other immune cell populations [79]. There are reports suggesting a correlation between the higher levels of CC ligands with lower survival rates for cancer patients [72, 74]. However, it is important to consider that neutrophils are not exclusive in the tumor microenvironment for the secretion of tumor-promoting factors. Other immune cell population present in the tumor microenvironment, such as macrophages [80], lymphocytes [80] (including Th17 cells [81] and $\gamma\delta$ T cells [66]), B cells [82], are also known to secrete tumor-promoting factors. As discussed previously, the proliferation and maturation of neutrophils in bone marrow require cytokines and chemokines such as G-CSF [83]. CXCR2 chemokines, and IL17. Multiple cell types in the tumor microenvironment contribute to the pool of G-CSF, CXCR2 ligands, and IL17. In the tumor microenvironment, the primary source of G-CSF includes cancer cells [84], fibroblasts [85], macrophages, and lymphocytes [86], while the significant contributors of IL17 include Th17 cells [87] and $\gamma\delta$ T cells [88].

1.4.3 Neutrophil-Released Enzymes

The versatile functions of neutrophils are dedicated to the different cytoplasmic granules present inside a mature neutrophil. These cytoplasmic granules are releasable membrane-bound organelle with three major types present in neutrophils: the primary or azurophil, secondary or specific, and tertiary or gelatinase granules [89]. Primary granules are associated with microbicidal functions, whereas secondary and tertiary are associated with extracellular matrix interaction and modification. Various proteases derived from neutrophil granules such as CG, NE, and matrix metalloprotease 9 (MMP-9) play a pro-tumor role through mechanisms [10], including epithelial to mesenchymal transition and extracellular matrix (ECM) remodeling [90], which lead to enhanced metastasis.

NE and CG are serine proteases, which are pre-synthesized in promyelocytes in the bone marrow and then stored in neutrophil primary granules. Both NE and CG are found to be entrapped in negatively charged NETs because of their high isoelectric points [91]. Recent studies suggest that NE can upregulate EGFR/MEK/ERK signaling [92], and phosphatidylinositol 3-kinase (PI3K) signaling [93], and have also been shown to promote cancer cell proliferation and therapy resistance [94, 95]. Also, higher levels of NE in metastatic breast cancer patients are associated with a poor response to tamoxifen therapy [96]. Similarly, inhibition of NE prevents the release of pro-cancer factor TGF- α , thereby

suppressing the growth of gastric carcinoma cells [97], as well as suppressing tumor progression in breast and prostate cancer [95, 98].

Interestingly, cancer cell lacking endogenous NE expression can uptake NE through the neuropilin-1 receptor [99]. CG has been reported to facilitate the E-cadherin-dependent aggregation of MCF-7 mammary carcinoma cells [100], by using insulin-like growth factor-1 signaling [101]. Also, Akizuki et al. showed that higher levels of NE correlated with lower survival rates in breast cancer patients, thereby demonstrating the potential of NE as an independent prognostic marker [102]. Additionally, NE can be utilized as a therapeutic target for colorectal cancer [103], whereas CG can serve as a potential therapeutic target for breast cancer patients [104].

Unlike serine proteases such as CG and NE, MMP-9 is stored in neutrophil tertiary granules [105] and requires zinc as a cofactor for its catalytic activity [106]. An active MMP-9 can remodel the extracellular matrix by the degradation of extracellular proteins [106], facilitating membrane cleavage [107], and activate pro-tumor factors such as TGF- β [108]. TNF, TGF- β , and VEGF [105, 109, 110] are known to regulate the release of MMP-9 by neutrophils. MMP-9 is a pro-angiogenic factor, which promotes resistance to sunitinib (a common chemotherapy drug for multiple cancer types, in renal cell carcinoma patients) [111]. MMP-9 has been explored extensively in breast cancer. MMP-9 has high expression levels in breast cancer tissue in comparison with the healthy tissue [112] and has higher levels present in metastatic breast tumors [113], which suggests an association of MMP-9 with breast cancer development and tumor progression. MMP-9 significantly promotes angiogenesis and metastasis in triple-negative breast cancer [114] and predicts poor survival in hormone-responsive small mammary tumors [115]. All these studies strengthen the potential of MMP-9 as a prognostic biomarker for breast cancer patients.

Neutrophils also release MMP-8 (collagenase-2), which generates chemotactic Pro-Gly-Pro (PGP) tripeptide and is important for neutrophil mobilization [108]; however, unlike other proteases, the role of MMP-8 in tumor progression is controversial. A study in breast cancer has shown an inverse correlation between MMP-8 expression and lymph node metastasis [116]; however, a recent study by Thirkettle et al. demonstrated that MMP-8 can upregulate pro-tumor cytokines, IL-6 and IL-8, thus suggesting pro-cancer behavior [117]. In other cancer types, such as melanoma and the lung carcinoma, the antimetastatic role of MMP-8 has been shown through enhanced adhesion to type I collagen and laminin-1 present in the extracellular matrix [117]; on the contrary, higher levels of MMP-8 in the serum of colorectal cancer patients predict lower patient survival [118].

1.4.4 Neutrophil Extracellular Traps (NETs)

NET can be defined as a network of extracellular fibers composed of a DNA scaffold decorated with granule-derived proteins such as NE, CG, MMP-9, and others. For the first time, Brinkmann et al. reported the formation of NET cell death or NETosis, as a new killing mechanism used by neutrophils apart from traditional phagocytosis or degranulation [119]. Initially, neutrophils were reported to form NETs for eliminating the pathogen through rupture of the cytoplasmic membrane, on activation by stimuli such as CXCL8 or lipopolysaccharide (LPS) [120], which also leads to the generation of ROS by NADPH

oxidase [121]. Neutrophils have also been demonstrated to form NETs, without undergoing lytic death, through the release of mitochondrial DNA [121–123].

Similar to other pathological diseases, there are reports suggesting that neutrophils' NET formation in the tumor microenvironment plays an active pro-tumor role during disease progression [10, 124, 125]. There is an increase in the level of NETs in plasma of cancer patients (pancreatic cancer, colorectal cancer, lung cancer, and bladder cancer) in comparison with healthy controls [126, 127]; similarly, Ewing's sarcoma patients with metastasis have higher levels of NETs [128], suggesting that NETs could be considered a potential diagnostic marker target. A recent study has demonstrated that NETs can directly function on tumors cells by enhancing their proliferation through activating NF- κ B signaling pathways [129].

1.5 Role of Neutrophil in Tumor Initiation, Growth, and Metastasis

Neutrophils, an active player in the tumor microenvironment, have been found to play a prominent role in tumor development, growth, and metastasis [130, 131]. Before discussing the different mechanisms through which neutrophils participate in the process of metastasis (Fig. 1.3), we will introduce the metastatic cascade. Metastasis is defined as the migration of cancer cells from the primary tumor site of origin to nearby or distant sites, which lead to the formation of secondary growth of tumor cells. Despite improvements in the treatment of a resectable tumor, metastasis is the driver of mortality. Metastasis is not a random process [132], but a result of the successful completion of multistep biological events, known as the invasion-metastasis cascade [133, 134]. This cascade involves local invasion, entry of cancer cells from a well-defined tumor boundary into the surrounding tumor stroma, followed by a second step intravasation, and the entry of invasive cancer cells into the lumen of lymphatic or blood vessels. After intravasation, the survival ability of tumor cells in the circulation is tested [133]. After surviving this part of their journey, the tumor cells are arrested at a distant organ site. The tumor cells must then extravasate by either involving microcolony growth, which ruptures the wall of the surrounding vessels, or by penetrating the vessel through the endothelial cells and pericytes. Additionally, the tumor cells must survive at the distant site to form micrometastases. After the successful survival of cancer cells in the foreign microenvironment, reinitiation of cancer cell proliferation is necessary for the formation of macrometastasis. Evidence suggests that one or more of the steps of the invasion-metastasis cascade are rarely completed successfully, thereby making the process of metastasis a highly inefficient one.

1.5.1 The Role of Neutrophils in the Early Metastatic Cascade

Neutrophils are well-known to support the early metastatic cascade. However, there is a growing body of literature, which suggests that neutrophils play important roles in all steps of the metastatic cascade [1, 135, 136]. One of the fundamental properties of tumor progression and the beginning of metastatic cascade is the gain of invasive behavior in tumor cells. Neutrophils aid the invasive properties of tumor cells by secreting a wide variety of proteases such as MMP-8, MMP-9, CG, and others. These proteases are well-known to degrade a variety of structural proteins present in the extracellular environment [136–138].

Serine proteases are also known to trigger angiogenesis [139, 140] by releasing factors such as VEGF [141]. Also, an orthotopic breast cancer mouse model suggests that neutrophils induce tumor cells with the production of MMP-12 and MMP-13 [142].

Moreover, neutrophils use myeloperoxidase to produce hypochlorous acid, which can also activate the secreted “inactivate” form of proteases [143]. Recently, TGF- β derived from neutrophils were shown to induce epithelial to mesenchymal transition, a process known to increase the invasiveness of cells in pulmonary adenocarcinoma cells [144]. Until now, we have discussed neutrophil contact-independent mechanisms inducing invasiveness in the tumor cells; however, there are reports which suggest that contact-dependent signaling between TLR4 receptors on neutrophils and hyaluronan on hepatocarcinoma cancer cells promote cellular migration [136, 145]. Similarly, the interaction of neutrophils with gastric cancer cells promotes cellular migration and invasion by inducing epithelial to mesenchymal transition [146].

1.5.2 Role of Neutrophils in Intermediate Metastatic Cascade

In this section, we will discuss how neutrophils support the intermediate steps of the metastatic cascades, such as intravasation, the survival of tumor cells, and extravasation. With the invasive property, tumor cells face a new set of challenges, such as the absence of cell to extracellular matrix interactions, increase in shear forces, and escaping immune cell surveillance to successfully survive the intermediate steps of metastatic cascade [136]. Formation of cell aggregates enhances tumor cell survival [147, 148] and neutrophils aid this process with the help of cathepsin G [149] or cellular markers like CD11a and CD11b [150].

Neutrophils play a role in helping tumor cells escape immune surveillance by contributing to tumor acidosis through the mobilization of H⁺-pump ATPase, which can hamper the antitumor activity of natural killer (NK) cells and T cells [5]. Recent studies suggest that the presence of neutrophils blunt NK cell [151] or leukocyte activation [146], thus promoting intravascular survival. Lastly, neutrophils can both directly and indirectly aid tumor cell crossing the endothelium lining [136]. Numerous studies have shown the co-localization of neutrophils with tumor cells by expression of selectin molecules present on the neutrophil cell surface, thus facilitating adhesion of tumor cells and neutrophils to the endothelium [135, 152, 153]. Not only the expression of selectins and integrins but also NETs promote metastasis through endothelium and tumor cell adhesion [125, 154–156]. All these studies suggest neutrophil as an important mediator between tumor cells and endothelium lining. However, it remains undetermined whether neutrophils act as a direct bridge between tumor cells and endothelium or neutrophils secrete endothelium activating factors which increase adherence of tumor cells to activated endothelium [136].

1.5.3 Role of Neutrophils in the Late Metastatic Cascade

Successful macrometastasis formation in a new environment is the endgame for a tumor cell. Neutrophils play a central role in the formation of premetastatic niches by arriving at the metastatic site before the arrival of tumor cells and favoring tumor cell survival and proliferation [8, 136, 157]. Neutrophils accumulate in premetastatic niches either through CXCR2-dependent [158, 159] or CXCR4-dependent mechanism [160]. Neutrophil-derived

factors such as oncostatin M [67], elastase [161], and S100A8 and S100A9 [157] trigger tumor cell proliferation. Apart from providing tumor growth-promoting factors, neutrophils can also drive the formation of macrometastasis from micrometastasis by inducing angiogenesis [136, 162, 163], which is similar to the need for vascular supply in primary tumor growth. In multiple cancer types, neutrophil can also support the final establishment of metastasis through immunosuppression of T cells [1, 158, 159, 164].

1.6 The Clinical Significance of Neutrophils

The role of neutrophils in tumor biology is now widely recognized, and its potential as a biomarker or therapeutic agent is being explored. Based on the above discussion, neutrophils function in the tumor microenvironment through the release of ROS, the formation of NET, and the secretion of cytokines. Moreover, neutrophils are not considered neutral towards cancer progression anymore [1, 10]; they encompass plastic phenotype with two extreme polarization state and possess functional heterogeneity [4, 6]. This opens up the potential for therapeutic intervention, but only after overcoming the limitations of our current research tools.

1.6.1 Neutrophils as a Potential Biomarker for Cancer Patients

Most of these studies indicate that higher NLR in cancer patients is correlated with poor clinical outcomes in cancer patients [10, 35, 165–172]. The detection of NLR is easy and inexpensive, as the detection of NLR can be performed using blood analyses [172]. NLR has been proposed as an attractive indicator for treatment decision and risk for cancer patients. However, there are several limitations for NLR application into clinics. It is challenging to translate NLR for personalized prognosis and treatment decision for the individual patient, as cutoff NLR varies for high-risk or low-risk classification in different cancer cases; meanwhile, neutrophil numbers vary between different individuals [1]. One approach to deriving maximum information from NLR is to perform analysis on a regular basis over time, and these results may be combined with other neutrophil-activating and neutrophil-polarizing factors such as IL-1 β and IL-17 in serum [102, 103, 173]. Increased NLR value over time may indicate reoccurrence or progression of the disease.

Neutrophils inside the solid tumors emerged as the least favorable cell populations regarding cancer patient survival [35]. Additionally, compared to healthy tissue, there is a significant increase in the number of TAN in the tumor, which indicates the possibility of using TAN for prognostic tools [110]. However, similar to NLR, an association of TAN with different tumor progression is variable. Markers used to identify TAN (cell morphology, myeloperoxidase, and others) are not expressed uniformly in all tumor types. Thus TAN isolation method is complicated. Thus, an advanced technique is required for TAN use in the clinic.

1.6.2 Neutrophils as Therapeutic Targets in Cancer Patients

Neutrophils and neutrophil-released factors could be considered as a potential prognostic marker for cancer patients. There are several possibilities to target neutrophils, such as preventing neutrophil expansion in the bone marrow, inhibiting neutrophil trafficking to the

tumor, preventing polarization of neutrophils towards N2 type, and lastly targeting neutrophil-associated mediators [1].

Clinically, one of the most appropriate ways of targeting neutrophils is by utilizing agents treating autoimmune and inflammatory diseases. CXCR2 inhibitor, AZD5069, reduced absolute neutrophil counts in bronchiectasis patients [174], but AZD5069 as a therapeutic agent for cancer patients is still under investigation. Similarly, clinical trials with reparixin (CXCR1 and CXCR2 inhibitor) [175] are ongoing in cancer patients [1]. Other molecules that stimulate the expansion of neutrophils are IL-23 and IL-17 [176]. There are approved antagonists for IL-23 and IL-17, which are tested in psoriasis [176]. More preclinical studies are warranted to move these drugs in cancer patients [176].

An example of targeting neutrophil-associated factors comes from the application of NE inhibitor to cancer patients. The NE inhibitor, sivelestat sodium hydrate, has been used in patients suffering from thoracic esophagus carcinoma [177]. Another example is the elimination of NET by DNase I digestion, and this method is being tested in several ongoing clinical trials but not in cancer [178]. More clinical studies are needed to evaluate the therapeutic effects of targeting neutrophils in cancer patients. Another concern which still needs to be addressed is the use of G-CSF or GM-CSF to resolve chemotherapy-induced neutropenia, as G-CSF polarizes neutrophils towards pro-tumor behavior. In the future, neutrophil targeting approaches can be combined with anticancer therapies such as chemotherapy and immunotherapy, such as T cell checkpoint inhibitor [1]. Combinational therapy may be more beneficial to cancer patients rather than targeting neutrophil alone.

1.7 Concluding Remarks

Neutrophils are emerging as an important player in the tumor microenvironment, together with a new realization of their role, which extends beyond just microbial elimination during an immune response. The fact that host-related factors are more accessible to target than genetically unstable cancer cells is also bringing new excitement to this field. A plethora of literature now eliminates the myth of neutrophil neutrality and short life span in tumor biology, with evidence accumulated to show that neutrophils are not only important in different stages of tumorigenesis but also in the metastatic cascade. The remarkable ability of neutrophils showing phenotype plasticity, which results in a heterogeneous population, necessitates the urgency to understand the concert between different possible factors, such as metabolite availability or hypoxia in the tumor microenvironment; meanwhile, governing neutrophil maturity and polarization may lead to pro- or antitumor behavior.

With the possibility of such diverse neutrophil phenotypes, simple depletion of neutrophils is not an answer for therapeutic intervention. Thus, we need to fill in the knowledge gap by identifying differentiable markers for various neutrophil populations. Advanced techniques like single-cell sequencing and single-cell fate mapping may provide us with an answer to identify the polarization state of neutrophils in the future. Moreover, neutrophils and the partner in crime interact with cancer cells and may disguise cancer cells from other immune cells by immunosuppression and provide advantages to overcome metastatic cascade. Neutrophils cytoplasmic content and degranulation process plays an important role in

introducing new membrane proteins on the surface of neutrophils and dictates interaction between neutrophils and cancer cells, together with other cell populations in the tumor microenvironment.

Thus, understanding these processes on the molecular level will open the potential therapeutic avenues. Additionally, there exists crosstalk between neutrophils and other immune cells in the tumor microenvironment, similar to the conditions in other inflammatory diseases. Thus, neutrophil inhibitors used in inflammatory diseases may find a role in cancer biology as well. Very importantly, much of our understanding about neutrophil biology in tumor microenvironment comes from mouse models, because of the limitation of short survival period of neutrophils in ex vivo culture. Before extrapolating these mouse model-based findings in clinics, we should be critical about the species-based differences in neutrophils, including tumor evolution and immunity. Our current literature has not merely increased our understanding and excitement about neutrophil biology in the tumor microenvironment but also promoted more research to find a cure for cancer patients in the future. Still, an extensive research effort is needed to completely delineate the neutrophil-facilitated tumor progression and metastasis and translate experiment data into clinical use for cancer patients.

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References

1. Coffelt SB, Wellenstein MD, de Visser KE (2016) Neutrophils in cancer: neutral no more. *Nat Rev Cancer* 16:431–446 [PubMed: 27282249]
2. Selders GS, Fetz AE, Radic MZ, Bowlin GL (2017) An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. *Regen Biomater* 4:55–68 [PubMed: 28149530]
3. Kruger P, Saffarzadeh M, Weber AN, Rieber N, Radsak M, von Bernuth H, Benarafa C, Roos D, Skokowa J, Hartl D (2015) Neutrophils: between host defence, immune modulation, and tissue injury. *PLoS Pathog* 11:e1004651 [PubMed: 25764063]
4. Rosales C (2018) Neutrophil: a cell with many roles in inflammation or several cell types? *Front Physiol* 9:113 [PubMed: 29515456]
5. Mollinedo F (2019) Neutrophil degranulation, plasticity, and cancer metastasis. *Trends Immunol* 40:228–242 [PubMed: 30777721]
6. Grecian R, Whyte MKB, Walmsley SR (2018) The role of neutrophils in cancer. *Br Med Bull* 128:5–14 [PubMed: 30137312]
7. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM (2009) Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. *Cancer Cell* 16:183–194 [PubMed: 19732719]
8. Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, Psaila B, Kaplan RN, Bromberg JF, Kang Y, Bissell MJ, Cox TR, Giaccia AJ, Erler JT, Hiratsuka S, Ghajar CM, Lyden D (2017) Pre-metastatic niches: organ-specific homes for metastases. *Nat Rev Cancer* 17:302–317 [PubMed: 28303905]

9. Janeway CA, Travers P, Walport M, Shlomchik MJ (2001) Immunobiology, 5th edn. Garland Science, New York
10. Wu L, Saxena S, Awaji M, Singh RK (2019) Tumor-associated neutrophils in cancer: going pro. *Cancers (Basel)* 11:E564 [PubMed: 31010242]
11. Palmer C, Diehn M, Alizadeh AA, Brown PO (2006) Cell-type specific gene expression profiles of leukocytes in human peripheral blood. *BMC Genomics* 7:115 [PubMed: 16704732]
12. Dancey JT, Deubelbeiss KA, Harker LA, Finch CA (1976) Neutrophil kinetics in man. *J Clin Invest* 58:705–715 [PubMed: 956397]
13. Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L (2010) In vivo labeling with $2\text{H}_2\text{O}$ reveals a human neutrophil lifespan of 5.4 days. *Blood* 116:625–627 [PubMed: 20410504]
14. Umansky V, Blattner C, Gebhardt C, Utikal J (2016) The role of myeloid-derived suppressor cells (MDSC) in cancer progression. *Vaccines (Basel)* 4:E36 [PubMed: 27827871]
15. Gabrilovich DI (2017) Myeloid-derived suppressor cells. *Cancer Immunol Res* 5:3–8 [PubMed: 28052991]
16. Cowland JB, Borregaard N (2016) Granulopoiesis and granules of human neutrophils. *Immunol Rev* 273:11–28 [PubMed: 27558325]
17. Lawrence SM, Corriden R, Nizet V (2018) The ontogeny of a neutrophil: mechanisms of granulopoiesis and homeostasis. *Microbiol Mol Biol Rev* 82:e00057–e00017 [PubMed: 29436479]
18. Yamanaka R, Barlow C, Lekstrom-Himes J, Castilla LH, Liu PP, Eckhaus M, Decker T, Wynshaw-Boris A, Xanthopoulos KG (1997) Impaired granulopoiesis, myelodysplasia, and early lethality in CCAAT/ enhancer binding protein epsilon-deficient mice. *Proc Natl Acad Sci U S A* 94:13187–13192 [PubMed: 9371821]
19. Fiedler K, Brunner C (2012) The role of transcription factors in the guidance of granulopoiesis. *Am J Blood Res* 2:57–65 [PubMed: 22432088]
20. Furze RC, Rankin SM (2008) Neutrophil mobilization and clearance in the bone marrow. *Immunology* 125:281–288 [PubMed: 19128361]
21. Beyrau M, Bodkin JV, Nourshargh S (2012) Neutrophil heterogeneity in health and disease: a revitalized avenue in inflammation and immunity. *Open Biol* 2:120134 [PubMed: 23226600]
22. Eash KJ, Means JM, White DW, Link DC (2009) CXCR4 is a key regulator of neutrophil release from the bone marrow under basal and stress granulopoiesis conditions. *Blood* 113:4711–4719 [PubMed: 19264920]
23. Strydom N, Rankin SM (2013) Regulation of circulating neutrophil numbers under homeostasis and in disease. *J Innate Immun* 5:304–314 [PubMed: 23571274]
24. Furze RC, Rankin SM (2008) The role of the bone marrow in neutrophil clearance under homeostatic conditions in the mouse. *FASEB J* 22:3111–3119 [PubMed: 18509199]
25. Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM (2003) Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* 19:583–593 [PubMed: 14563322]
26. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER (2010) Neutrophil kinetics in health and disease. *Trends Immunol* 31:318–324 [PubMed: 20620114]
27. Ocana A, Nieto-Jimenez C, Pandiella A, Templeton AJ (2017) Neutrophils in cancer: prognostic role and therapeutic strategies. *Mol Cancer* 16:137 [PubMed: 28810877]
28. Leiding JW (2017) Neutrophil evolution and their diseases in humans. *Front Immunol* 8:1009 [PubMed: 28894446]
29. Hsieh MM, Everhart JE, Byrd-Holt DD, Tisdale JF, Rodgers GP (2007) Prevalence of neutropenia in the U.S. population: age, sex, smoking status, and ethnic differences. *Ann Intern Med* 146:486–492 [PubMed: 17404350]
30. Manz MG, Boettcher S (2014) Emergency granulopoiesis. *Nat Rev Immunol* 14:302–314 [PubMed: 24751955]
31. Witter AR, Okunnu BM, Berg RE (2016) The essential role of neutrophils during infection with the intracellular bacterial pathogen *Listeria monocytogenes*. *J Immunol* 197:1557–1565 [PubMed: 27543669]

32. Pillay J, Ramakers BP, Kamp VM, Loi AL, Lam SW, Hietbrink F, Leenen LP, Tool AT, Pickkers P, Koenderman L (2010) Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. *J Leukoc Biol* 88:211–220 [PubMed: 20400675]
33. Lustberg MB (2012) Management of neutropenia in cancer patients. *Clin Adv Hematol Oncol* 10:825–826 [PubMed: 23271355]
34. Uribe-Querol E, Rosales C (2015) Neutrophils in cancer: two sides of the same coin. *J Immunol Res* 2015:983698 [PubMed: 26819959]
35. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, Nair VS, Xu Y, Khuong A, Hoang CD, Diehn M, West RB, Plevritis SK, Alizadeh AA (2015) The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med* 21:938–945 [PubMed: 26193342]
36. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, Tannock IF, Amir E (2014) Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 106:dju124 [PubMed: 24875653]
37. Boyum A (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest Suppl* 97:77–89 [PubMed: 4179068]
38. Brandau S, Moses K, Lang S (2013) The kinship of neutrophils and granulocytic myeloid-derived suppressor cells in cancer: cousins, siblings or twins? *Semin Cancer Biol* 23:171–182 [PubMed: 23459190]
39. Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, Damti P, Lumbroso D, Polyansky L, Sionov RV, Ariel A, Hovav AH, Henke E, Fridlender ZG, Granot Z (2015) Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Rep* 10:562–573 [PubMed: 25620698]
40. Pillay J, Tak T, Kamp VM, Koenderman L (2013) Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. *Cell Mol Life Sci* 70:3813–3827 [PubMed: 23423530]
41. Raber PL, Thevenot P, Sierra R, Wyczzechowska D, Halle D, Ramirez ME, Ochoa AC, Fletcher M, Velasco C, Wilk A, Reiss K, Rodriguez PC (2014) Subpopulations of myeloid-derived suppressor cells impair T cell responses through independent nitric oxide-related pathways. *Int J Cancer* 134:2853–2864 [PubMed: 24259296]
42. Greten TF, Manns MP, Korangy F (2011) Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol* 11:802–807 [PubMed: 21237299]
43. Magcwebaba T, Dorhoi A, du Plessis N (2019) The emerging role of myeloid-derived suppressor cells in tuberculosis. *Front Immunol* 10:917 [PubMed: 31114578]
44. Kolahian S, Oz HH, Zhou B, Griessinger CM, Rieber N, Hartl D (2016) The emerging role of myeloid-derived suppressor cells in lung diseases. *Eur Respir J* 47:967–977 [PubMed: 26846830]
45. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, Passegue E, Werb Z (2015) Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci U S A* 112:E566–E575 [PubMed: 25624500]
46. Eruslanov EB, Singhal S, Albelda SM (2017) Mouse versus human neutrophils in cancer: a major knowledge gap. *Trends Cancer* 3:149–160 [PubMed: 28718445]
47. Mouchemore KA, Anderson RL, Hamilton JA (2018) Neutrophils, G-CSF and their contribution to breast cancer metastasis. *FEBS J* 285:665–679 [PubMed: 28834401]
48. Shaul ME, Fridlender ZG (2017) Neutrophils as active regulators of the immune system in the tumor microenvironment. *J Leukoc Biol* 102:343–349 [PubMed: 28264904]
49. Dale DC, Boxer L, Liles WC (2008) The phagocytes: neutrophils and monocytes. *Blood* 112:935–945 [PubMed: 18684880]
50. Peyron P, Maridonneau-Parini I, Stegmann T (2001) Fusion of human neutrophil phagosomes with lysosomes in vitro: involvement of tyrosine kinases of the Src family and inhibition by mycobacteria. *J Biol Chem* 276:35512–35517 [PubMed: 11463789]
51. Jankowski A, Scott CC, Grinstein S (2002) Determinants of the phagosomal pH in neutrophils. *J Biol Chem* 277:6059–6066 [PubMed: 11744729]

52. Winterbourn CC, Kettle AJ, Hampton MB (2016) Reactive oxygen species and neutrophil function. *Annu Rev Biochem* 85:765–792 [PubMed: 27050287]
53. Cadet J, Wagner JR (2013) DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harb Perspect Biol* 5:a012559 [PubMed: 23378590]
54. Cooke MS, Evans MD, Dizdaroglu M, Lunec J (2003) Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* 17:1195–1214 [PubMed: 12832285]
55. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, Herber DL, Schneck J, Gabrilovich DI (2007) Altered recognition of antigen is a mechanism of CD8⁺ T cell tolerance in cancer. *Nat Med* 13:828–835 [PubMed: 17603493]
56. Parekh A, Das S, Parida S, Das CK, Dutta D, Mallick SK, Wu PH, Kumar BNP, Bharti R, Dey G, Banerjee K, Rajput S, Bharadwaj D, Pal I, Dey KK, Rajesh Y, Jena BC, Biswas A, Banik P, Pradhan AK, Das SK, Das AK, Dhara S, Fisher PB, Wirtz D, Mills GB, Mandal M (2018) Multi-nucleated cells use ROS to induce breast cancer chemo-resistance in vitro and in vivo. *Oncogene* 37:4546–4561 [PubMed: 29743594]
57. Liou GY, Storz P (2010) Reactive oxygen species in cancer. *Free Radic Res* 44:479–496 [PubMed: 20370557]
58. Dallegri F, Ottonello L, Ballestrero A, Dapino P, Ferrando F, Patrone F, Sacchetti C (1991) Tumor cell lysis by activated human neutrophils: analysis of neutrophil-delivered oxidative attack and role of leukocyte function-associated antigen 1. *Inflammation* 15:15–30 [PubMed: 1647368]
59. Gershkovitz M, Fainsod-Levi T, Zelter T, Sionov RV, Granot Z (2019) TRPM2 modulates neutrophil attraction to murine tumor cells by regulating CXCL2 expression. *Cancer Immunol Immunother* 68:33–43 [PubMed: 30251149]
60. Finisguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AA, Wauters E, Walmsley S, Prenen H, Granot Z, Casazza A, Mazzone M (2015) MET is required for the recruitment of anti-tumoural neutrophils. *Nature* 522:349–353 [PubMed: 25985180]
61. Powell DR, Huttenlocher A (2016) Neutrophils in the tumor microenvironment. *Trends Immunol* 37:41–52 [PubMed: 26700397]
62. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, Cassatella MA (2000) The neutrophil as a cellular source of chemokines. *Immunol Rev* 177:195–203 [PubMed: 11138776]
63. Tecchio C, Scapini P, Pizzolo G, Cassatella MA (2013) On the cytokines produced by human neutrophils in tumors. *Semin Cancer Biol* 23:159–170 [PubMed: 23410636]
64. Zhang F, Wang H, Wang X, Jiang G, Liu H, Zhang G, Wang H, Fang R, Bu X, Cai S, Du J (2016) TGF-beta induces M2-like macrophage polarization via SNAIL-mediated suppression of a pro-inflammatory phenotype. *Oncotarget* 7:52294–52306 [PubMed: 27418133]
65. Shrivastava R, Asif M, Singh V, Dubey P, Ahmad Malik S, Lone MU, Tewari BN, Baghel KS, Pal S, Nagar GK, Chattopadhyay N, Bhaduria S (2019) M2 polarization of macrophages by Oncostatin M in hypoxic tumor microenvironment is mediated by mTORC2 and promotes tumor growth and metastasis. *Cytokine* 118:130–143 [PubMed: 29625858]
66. Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau CS, Verstegen NJM, Ciampricotti M, Hawinkels L, Jonkers J, de Visser KE (2015) IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* 522:345–348 [PubMed: 25822788]
67. Queen MM, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL (2005) Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. *Cancer Res* 65:8896–8904 [PubMed: 16204061]
68. Pang Y, Gara SK, Achyut BR, Li Z, Yan HH, Day CP, Weiss JM, Trinchieri G, Morris JC, Yang L (2013) TGF-beta signaling in myeloid cells is required for tumor metastasis. *Cancer Discov* 3:936–951 [PubMed: 23661553]
69. Elaskalani O, Razak NB, Falasca M, Metharom P (2017) Epithelial-mesenchymal transition as a therapeutic target for overcoming chemoresistance in pancreatic cancer. *World J Gastrointest Oncol* 9:37–41 [PubMed: 28144398]
70. Dumitru CA, Fechner MK, Hoffmann TK, Lang S, Brandau S (2012) A novel p38-MAPK signaling axis modulates neutrophil biology in head and neck cancer. *J Leukoc Biol* 91:591–598 [PubMed: 22262799]

71. Galdiero MR, Varricchi G, Loffredo S, Bellevisine C, Lansione T, Ferrara AL, Iannone R, di Somma S, Borriello F, Clery E, Triassi M, Troncone G, Marone G (2018) Potential involvement of neutrophils in human thyroid cancer. *PLoS One* 13:e0199740 [PubMed: 29953504]
72. Tsuda Y, Fukui H, Asai A, Fukunishi S, Miyaji K, Fujiwara S, Teramura K, Fukuda A, Higuchi K (2012) An immunosuppressive subtype of neutrophils identified in patients with hepatocellular carcinoma. *J Clin Biochem Nutr* 51:204–212 [PubMed: 23170048]
73. Mishalian I, Bayuh R, Eruslanov E, Michaeli J, Levy L, Zolotarov L, Singhal S, Albelda SM, Granot Z, Fridlender ZG (2014) Neutrophils recruit regulatory T-cells into tumors via secretion of CCL17—a new mechanism of impaired antitumor immunity. *Int J Cancer* 135:1178–1186 [PubMed: 24501019]
74. Yan HH, Jiang J, Pang Y, Achyut BR, Lizardo M, Liang X, Hunter K, Khanna C, Hollander C, Yang L (2015) CCL9 induced by TGFbeta signaling in myeloid cells enhances tumor cell survival in the premetastatic organ. *Cancer Res* 75:5283–5298 [PubMed: 26483204]
75. Eash KJ, Greenbaum AM, Gopalan PK, Link DC (2010) CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest* 120:2423–2431 [PubMed: 20516641]
76. Li TJ, Jiang YM, Hu YF, Huang L, Yu J, Zhao LY, Deng HJ, Mou TY, Liu H, Yang Y, Zhang Q, Li GX (2017) Interleukin-17-producing neutrophils link inflammatory stimuli to disease progression by promoting angiogenesis in gastric cancer. *Clin Cancer Res* 23:1575–1585 [PubMed: 27620275]
77. Zhang H, Chen J (2018) Current status and future directions of cancer immunotherapy. *J Cancer* 9:1773–1781 [PubMed: 29805703]
78. de Oliveira S, Reyes-Aldasoro CC, Candel S, Renshaw SA, Mulero V, Calado A (2013) Cxcl8 (IL-8) mediates neutrophil recruitment and behavior in the zebrafish inflammatory response. *J Immunol* 190:4349–4359 [PubMed: 23509368]
79. Sokol CL, Luster AD (2015) The chemokine system in innate immunity. *Cold Spring Harb Perspect Biol* 7:a016303 [PubMed: 25635046]
80. Akgul C, Moulding DA, Edwards SW (2001) Molecular control of neutrophil apoptosis. *FEBS Lett* 487:318–322 [PubMed: 11163351]
81. Novitskiy SV, Pickup MW, Gorska AE, Owens P, Chytil A, Aakre M, Wu H, Shyr Y, Moses HL (2011) TGF-beta receptor II loss promotes mammary carcinoma progression by Th17 dependent mechanisms. *Cancer Discov* 1:430–441 [PubMed: 22408746]
82. Bodogai M, Moritoh K, Lee-Chang C, Hollander CM, Sherman-Baust CA, Wersto RP, Araki Y, Miyoshi I, Yang L, Trinchieri G, Biragyn A (2015) Immunosuppressive and prometastatic functions of myeloid-derived suppressive cells rely upon education from tumor-associated B cells. *Cancer Res* 75:3456–3465 [PubMed: 26183924]
83. Bottoni U, Trapasso F (2009) The role of G-CSF in the treatment of advanced tumors. *Cancer Biol Ther* 8:1744–1746 [PubMed: 19652549]
84. Aliper AM, Frieden-Korovkina VP, Buzdin A, Roumiantsev SA, Zhavoronkov A (2014) A role for G-CSF and GM-CSF in nonmyeloid cancers. *Cancer Med* 3:737–746 [PubMed: 24692240]
85. Dorsam B, Bosl T, Reiners KS, Barnert S, Schubert R, Shatnyeva O, Zigrino P, Engert A, Hansen HP, von Strandmann EP (2018) Hodgkin lymphoma-derived extracellular vesicles change the secretome of fibroblasts toward a CAF phenotype. *Front Immunol* 9:1358 [PubMed: 29967610]
86. Metcalf D (1989) The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. *Nature* 339:27–30 [PubMed: 2469962]
87. Alves JJP, De Medeiros Fernandes TAA, De Araujo JMG, Cobucci RNO, Lanza DCF, Bezerra FL, Andrade VS, Fernandes JV (2018) Th17 response in patients with cervical cancer. *Oncol Lett* 16:6215–6227 [PubMed: 30405758]
88. Patil RS, Shah SU, Shrikhande SV, Goel M, Dikshit RP, Chiplunkar SV (2016) IL17 producing gammadeltaT cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. *Int J Cancer* 139:869–881 [PubMed: 27062572]
89. Borregaard N, Cowland JB (1997) Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 89:3503–3521 [PubMed: 9160655]
90. Felix K, Gaida MM (2016) Neutrophil-derived proteases in the microenvironment of pancreatic cancer—active players in tumor progression. *Int J Biol Sci* 12:302–313 [PubMed: 26929737]

91. Okada Y (2017) In: Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR (eds) Kelley and Firestein's textbook of rheumatology, 10th edn Elsevier, Philadelphia
92. DiCamillo SJ, Yang S, Panchenko MV, Toselli PA, Naggar EF, Rich CB, Stone PJ, Nugent MA, Panchenko MP (2006) Neutrophil elastase-initiated EGFR/MEK/ERK signaling counteracts stabilizing effect of autocrine TGF-beta on tropoelastin mRNA in lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 291:L232–L243 [PubMed: 16473861]
93. Yang R, Zhong L, Yang XQ, Jiang KL, Li L, Song H, Liu BZ (2016) Neutrophil elastase enhances the proliferation and decreases apoptosis of leukemia cells via activation of PI3K/Akt signaling. *Mol Med Rep* 13:4175–4182 [PubMed: 27035679]
94. Lerman I, Hammes SR (2018) Neutrophil elastase in the tumor microenvironment. *Steroids* 133:96–101 [PubMed: 29155217]
95. Lerman I, Garcia-Hernandez ML, Rangel-Moreno J, Chiriboga L, Pan C, Nastiuk KL, Krolewski JJ, Sen A, Hammes SR (2017) Infiltrating myeloid cells exert protumorigenic actions via neutrophil elastase. *Mol Cancer Res* 15:1138–1152 [PubMed: 28512253]
96. Foekens JA, Ries C, Look MP, Gippner-Steppert C, Klijn JG, Jochum M (2003) Elevated expression of polymorphonuclear leukocyte elastase in breast cancer tissue is associated with tamoxifen failure in patients with advanced disease. *Br J Cancer* 88:1084–1090 [PubMed: 12671709]
97. Wada Y, Yoshida K, Hihara J, Konishi K, Tanabe K, Ukon K, Taomoto J, Suzuki T, Mizuiri H (2006) Sivelestat, a specific neutrophil elastase inhibitor, suppresses the growth of gastric carcinoma cells by preventing the release of transforming growth factor-alpha. *Cancer Sci* 97:1037–1043 [PubMed: 16918998]
98. Caruso JA, Hunt KK, Keyomarsi K (2010) The neutrophil elastase inhibitor elafin triggers rb-mediated growth arrest and caspase-dependent apoptosis in breast cancer. *Cancer Res* 70:7125–7136 [PubMed: 20823156]
99. Kerros C, Tripathi SC, Zha D, Mehrens JM, Sergeeva A, Philips AV, Qiao N, Peters HL, Katayama H, Sukhumalchandra P, Ruisaard KE, Perakis AA, St John LS, Lu S, Mittendorf EA, Clise-Dwyer K, Herrmann AC, Alatrash G, Toniatti C, Hanash SM, Ma Q, Molldrem JJ (2017) Neutropilin-1 mediates neutrophil elastase uptake and cross-presentation in breast cancer cells. *J Biol Chem* 292:10295–10305 [PubMed: 28468826]
100. Yui S, Osawa Y, Ichisugi T, Morimoto-Kamata R (2014) Neutrophil cathepsin G, but not elastase, induces aggregation of MCF-7 mammary carcinoma cells by a protease activity-dependent cell-oriented mechanism. *Mediat Inflamm* 2014:971409
101. Morimoto-Kamata R, Yui S (2017) Insulin-like growth factor-1 signaling is responsible for cathepsin G-induced aggregation of breast cancer MCF-7 cells. *Cancer Sci* 108:1574–1583 [PubMed: 28544544]
102. Akizuki M, Fukutomi T, Takasugi M, Takahashi S, Sato T, Harao M, Mizumoto T, Yamashita J (2007) Prognostic significance of immunoreactive neutrophil elastase in human breast cancer: long-term follow-up results in 313 patients. *Neoplasia* 9:260–264 [PubMed: 17401466]
103. Ho AS, Chen CH, Cheng CC, Wang CC, Lin HC, Luo TY, Lien GS, Chang J (2014) Neutrophil elastase as a diagnostic marker and therapeutic target in colorectal cancers. *Oncotarget* 5:473–480 [PubMed: 24457622]
104. Wilson TJ, Nannuru KC, Futakuchi M, Sadanandam A, Singh RK (2008) Cathepsin G enhances mammary tumor-induced osteolysis by generating soluble receptor activator of nuclear factor-kappaB ligand. *Cancer Res* 68:5803–5811 [PubMed: 18632634]
105. Chakrabarti S, Zee JM, Patel KD (2006) Regulation of matrix metalloproteinase-9 (MMP-9) in TNF-stimulated neutrophils: novel pathways for tertiary granule release. *J Leukoc Biol* 79:214–222 [PubMed: 16275891]
106. Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69:562–573 [PubMed: 16405877]
107. Pal-Ghosh S, Blanco T, Tadvalkar G, Pajoohesh-Ganji A, Parthasarathy A, Zieske JD, Stepp MA (2011) MMP9 cleavage of the beta4 integrin ectodomain leads to recurrent epithelial erosions in mice. *J Cell Sci* 124:2666–2675 [PubMed: 21750188]

108. Lin M, Jackson P, Tester AM, Diaconu E, Overall CM, Blalock JE, Pearlman E (2008) Matrix metalloproteinase-8 facilitates neutrophil migration through the corneal stromal matrix by collagen degradation and production of the chemotactic peptide Pro-Gly-Pro. *Am J Pathol* 173:144–153 [PubMed: 18556780]
109. Gordon GM, Ledee DR, Feuer WJ, Fini ME (2009) Cytokines and signaling pathways regulating matrix metalloproteinase-9 (MMP-9) expression in corneal epithelial cells. *J Cell Physiol* 221:402–411 [PubMed: 19626678]
110. Hollborn M, Stathopoulos C, Steffen A, Wiedemann P, Kohen L, Bringmann A (2007) Positive feedback regulation between MMP-9 and VEGF in human RPE cells. *Invest Ophthalmol Vis Sci* 48:4360–4367 [PubMed: 17724228]
111. Finke J, Ko J, Rini B, Rayman P, Ireland J, Cohen P (2011) MDSC as a mechanism of tumor escape from sunitinib mediated anti-angiogenic therapy. *Int Immunopharmacol* 11:856–861 [PubMed: 21315783]
112. Li H, Qiu Z, Li F, Wang C (2017) The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. *Oncol Lett* 14:5865–5870 [PubMed: 29113219]
113. Yousef EM, Tahir MR, St-Pierre Y, Gaboury LA (2014) MMP-9 expression varies according to molecular subtypes of breast cancer. *BMC Cancer* 14:609 [PubMed: 25151367]
114. Mehner C, Hockla A, Miller E, Ran S, Radisky DC, Radisky ES (2014) Tumor cell-produced matrix metalloproteinase 9 (MMP-9) drives malignant progression and metastasis of basal-like triple negative breast cancer. *Oncotarget* 5:2736–2749 [PubMed: 24811362]
115. PeUikainen JM, Ropponen KM, Kataja VV, Kellokoski JK, Eskelinen MJ, Kosma VM (2004) Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. *Clin Cancer Res* 10:7621–7628 [PubMed: 15569994]
116. Gutierrez-Fernandez A, Fueyo A, Folgueras AR, Garabaya C, Pennington CJ, Pilgrim S, Edwards DR, Holliday DL, Jones JL, Span PN, Sweep FC, Puente XS, Lopez-Otin C (2008) Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. *Cancer Res* 68:2755–2763 [PubMed: 18413742]
117. Thirkettle S, Decock J, Arnold H, Pennington CJ, Jaworski DM, Edwards DR (2013) Matrix metallo-proteinase 8 (collagenase 2) induces the expression of interleukins 6 and 8 in breast cancer cells. *J Biol Chem* 288:16282–16294 [PubMed: 23632023]
118. Bockelman C, Beilmann-Lehtonen I, Kaprio T, Koskensalo S, Tervahartiala T, Mustonen H, Stenman UH, Sorsa T, Haglund C (2018) Serum MMP-8 and TIMP-1 predict prognosis in colorectal cancer. *BMC Cancer* 18:679 [PubMed: 29929486]
119. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. *Science* 303:1532–1535 [PubMed: 15001782]
120. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A (2007) Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol* 176:231–241 [PubMed: 17210947]
121. Erpenbeck L, Schon MP (2017) Neutrophil extracellular traps: protagonists of cancer progression? *Oncogene* 36:2483–2490 [PubMed: 27941879]
122. Pilsczek FH, Salina D, Poon KK, Fahey C, Yipp BG, Sibley CD, Robbins SM, Green FH, Surette MG, Sugai M, Bowden MG, Hussain M, Zhang K, Kubes P (2010) A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*. *J Immunol* 185:7413–7425 [PubMed: 21098229]
123. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU (2009) Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ* 16:1438–1444 [PubMed: 19609275]
124. van der Windt DJ, Sud V, Zhang H, Varley PR, Goswami J, Yazdani HO, Tohme S, Loughran P, O'Doherty RM, Minervini MI, Huang H, Simmons RL, Tsung A (2018) Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. *Hepatology* 68:1347–1360 [PubMed: 29631332]

125. Tohme S, Yazdani HO, Al-Khafaji AB, Chidi AP, Loughran P, Mowen K, Wang Y, Simmons RL, Huang H, Tsung A (2016) Neutrophil extracellular traps promote the development and progression of liver metastases after surgical stress. *Cancer Res* 76:1367–1380 [PubMed: 26759232]
126. Oklu R, Sheth RA, Wong KHK, Jahromi AH, Albadawi H (2017) Neutrophil extracellular traps are increased in cancer patients but does not associate with venous thrombosis. *Cardiovasc Diagn Ther* 7:S140–S1S9 [PubMed: 29399517]
127. Richardson JJR, Hendrickse C, Gao-Smith F, Thickett DR (2017) Neutrophil extracellular trap production in patients with colorectal cancer in vitro. *Int J Inflam* 2017:4915062 [PubMed: 28828191]
128. Berger-Achituv S, Brinkmann V, Abed UA, Kuhn LI, Ben-Ezra J, Elhasid R, Zychlinsky A (2013) A proposed role for neutrophil extracellular traps in cancer immunoediting. *Front Immunol* 4:48 [PubMed: 23508552]
129. Sangaletti S, Tripodo C, Vitali C, Portararo P, Guarnotta C, Casalini P, Cappetti B, Miotti S, Pinciroli P, Fuligni F, Fais F, Piccaluga PP, Colombo MP (2014) Defective stromal remodeling and neutrophil extracellular traps in lymphoid tissues favor the transition from autoimmunity to lymphoma. *Cancer Discov* 4:110–129 [PubMed: 24189145]
130. Joyce JA, Pollard JW (2009) Microenvironmental regulation of metastasis. *Nat Rev Cancer* 9:239–252 [PubMed: 19279573]
131. Nguyen DX, Bos PD, Massague J (2009) Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 9:274–284 [PubMed: 19308067]
132. Comen EA (2012) Tracking the seed and tending the soil: evolving concepts in metastatic breast cancer. *Discov Med* 14:97–104 [PubMed: 22935206]
133. Valastyan S, Weinberg RA (2011) Tumor metastasis: molecular insights and evolving paradigms. *Cell* 147:275–292 [PubMed: 22000009]
134. Vanharanta S, Massague J (2013) Origins of metastatic traits. *Cancer Cell* 24:410–421 [PubMed: 24135279]
135. Spicer JD, McDonald B, Cools-Lartigue JJ, Chow SC, Giannias B, Kubes P, Ferri LE (2012) Neutrophils promote liver metastasis via Mac-1-mediated interactions with circulating tumor cells. *Cancer Res* 72:3919–3927 [PubMed: 22751466]
136. Leach J, Morton JP, Sansom OJ (2019) Neutrophils: homing in on the myeloid mechanisms of metastasis. *Mol Immunol* 110:69–76 [PubMed: 29269005]
137. Benson DD, Meng X, Fullerton DA, Moore EE, Lee JH, Ao L, Silliman CC, Barnett CC Jr (2012) Activation state of stromal inflammatory cells in murine metastatic pancreatic adenocarcinoma. *Am J Physiol Regul Integr Comp Physiol* 302:R1067–R1075 [PubMed: 22422663]
138. Dumitru CA, Lang S, Brandau S (2013) Modulation of neutrophil granulocytes in the tumor microenvironment: mechanisms and consequences for tumor progression. *Semin Cancer Biol* 23:141–148 [PubMed: 23485549]
139. Deryugina EI, Zajac E, Juncker-Jensen A, Kupriyanova TA, Welter L, Quigley JP (2014) Tissue-infiltrating neutrophils constitute the major in vivo source of angiogenesis-inducing MMP-9 in the tumor microenvironment. *Neoplasia* 16:771–788 [PubMed: 25379015]
140. Ardi VC, Kupriyanova TA, Deryugina EI, Quigley JP (2007) Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis. *Proc Natl Acad Sci U S A* 104:20262–20267 [PubMed: 18077379]
141. Nozawa H, Chiu C, Hanahan D (2006) Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci U S A* 103:12493–12498 [PubMed: 16891410]
142. Yu PF, Huang Y, Han YY, Lin LY, Sun WH, Rabson AB, Wang Y, Shi YF (2017) TNFalpha-activated mesenchymal stromal cells promote breast cancer metastasis by recruiting CXCR2(+) neutrophils. *Oncogene* 36:482–490 [PubMed: 27375023]
143. De Larco JE, Wuertz BR, Furcht LT (2004) The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. *Clin Cancer Res* 10:4895–4900 [PubMed: 15297389]

144. Hu P, Shen M, Zhang P, Zheng C, Pang Z, Zhu L, Du J (2015) Intratumoral neutrophil granulocytes contribute to epithelial-mesenchymal transition in lung adenocarcinoma cells. *Tumour Biol* 36:7789–7796 [PubMed: 25944163]
145. Wu Y, Zhao Q, Peng C, Sun L, Li XF, Kuang DM (2011) Neutrophils promote motility of cancer cells via a hyaluronan-mediated TLR4/PI3K activation loop. *J Pathol* 225:438–447 [PubMed: 21826665]
146. Zhang J, Qiao X, Shi H, Han X, Liu W, Tian X, Zeng X (2016) Circulating tumor-associated neutrophils (cTAN) contribute to circulating tumor cell survival by suppressing peripheral leukocyte activation. *Tumour Biol* 37:5397–5404 [PubMed: 26563367]
147. Choi JW, Kim JK, Yang YJ, Kim P, Yoon KH, Yun SH (2015) Urokinase exerts antimetastatic effects by dissociating clusters of circulating tumor cells. *Cancer Res* 75:4474–4482 [PubMed: 26527605]
148. Fabisiewicz A, Grzybowska E (2017) CTC clusters in cancer progression and metastasis. *Med Oncol* 34:12 [PubMed: 28012133]
149. Morimoto-Kamata R, Mizoguchi S, Ichisugi T, Yui S (2012) Cathepsin G induces cell aggregation of human breast cancer MCF-7 cells via a 2-step mechanism: catalytic site-independent binding to the cell surface and enzymatic activity-dependent induction of the cell aggregation. *Mediat Inflamm* 2012:456462
150. Jadhav S, Bochner BS, Konstantopoulos K (2001) Hydrodynamic shear regulates the kinetics and receptor specificity of polymorphonuclear leukocyte-colon carcinoma cell adhesive interactions. *J Immunol* 167:5986–5993 [PubMed: 11698478]
151. Spiegel A, Brooks MW, Houshyar S, Reinhardt F, Ardolino M, Fessler E, Chen MB, Krall JA, DeCock J, Zervantonakis IK, Iannello A, Iwamoto Y, Cortez-Retamozo V, Kamm RD, Pittet MJ, Raulet DH, Weinberg RA (2016) Neutrophils suppress intraluminal NK cell-mediated tumor cell clearance and enhance extravasation of disseminated carcinoma cells. *Cancer Discov* 6:630–649 [PubMed: 27072748]
152. McDonald B, Spicer J, Giannais B, Fallavollita L, Brodt P, Ferri LE (2009) Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. *Int J Cancer* 125:1298–1305 [PubMed: 19431213]
153. Reticker-Flynn NE, Bhatia SN (2015) Aberrant glycosylation promotes lung cancer metastasis through adhesion to galectins in the metastatic niche. *Cancer Discov* 5:168–181 [PubMed: 25421439]
154. Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, Giannais B, Bourdeau F, Kubes P, Ferri L (2013) Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. *J Clin Invest*. 10.1172/JCI67484
155. Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, Schott AF, Kinugasa-Katayama Y, Lee Y, Won NH, Nakasone ES, Hearn SA, Kuttner V, Qiu J, Almeida AS, Perurena N, Kessenbrock K, Goldberg MS, Egeblad M (2016) Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. *Sci Transl Med* 8:361ra138
156. Najmeh S, Cools-Lartigue J, Rayes RF, Gowing S, Vourtzoumis P, Bourdeau F, Giannais B, Berube J, Rousseau S, Ferri LE, Spicer JD (2017) Neutrophil extracellular traps sequester circulating tumor cells via beta1-integrin mediated interactions. *Int J Cancer* 140:2321–2330 [PubMed: 28177522]
157. Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, Manova-Todorova K, Leversha M, Hogg N, Seshan VE, Norton L, Brogi E, Massague J (2012) A CXCL1 paracrine network links cancer chemoresistance and metastasis. *Cell* 150:165–178 [PubMed: 22770218]
158. Steele CW, Karim SA, Leach JDG, Bailey P, Upstill-Goddard R, Rishi L, Foth M, Bryson S, McDaid K, Wilson Z, Eberlein C, Candido JB, Clarke M, Nixon C, Connelly J, Jamieson N, Carter CR, Balkwill F, Chang DK, Evans TRJ, Strathdee D, Biankin AV, Nibbs RJB, Barry ST, Sansom OJ, Morton JP (2016) CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. *Cancer Cell* 29:832–845 [PubMed: 27265504]
159. Wang D, Sun H, Wei J, Cen B, DuBois RN (2017) CXCL1 is critical for premetastatic niche formation and metastasis in colorectal cancer. *Cancer Res* 77:3655–3665 [PubMed: 28455419]

160. Seubert B, Grunwald B, Kobuch J, Cui H, Schelter F, Schaten S, Siveke JT, Lim NH, Nagase H, Simonavicius N, Heikenwalder M, Reinheckel T, Sleeman JP, Janssen KP, Knolle PA, Kruger A (2015) Tissue inhibitor of metalloproteinases (TIMP)-1 creates a premetastatic niche in the liver through SDF-1/CXCR4-dependent neutrophil recruitment in mice. *Hepatology* 61:238–248 [PubMed: 25131778]
161. Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, Stolz DB, Land SR, Marconcini LA, Kliment CR, Jenkins KM, Beaulieu KA, Mouded M, Frank SJ, Wong KK, Shapiro SD (2010) Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. *Nat Med* 16:219–223 [PubMed: 20081861]
162. Lim SY, Gordon-Weeks A, Allen D, Kersemans V, Beech J, Smart S, Muschel RJ (2015) Cd11b(+) myeloid cells support hepatic metastasis through down-regulation of angiopoietin-like 7 in cancer cells. *Hepatology* 62:521–533 [PubMed: 25854806]
163. Gordon-Weeks AN, Lim SY, Yuzhalin AE, Jones K, Markelc B, Kim KJ, Buzzelli JN, Fokas E, Cao Y, Smart S, Muschel R (2017) Neutrophils promote hepatic metastasis growth through fibroblast growth factor 2-dependent angiogenesis in mice. *Hepatology* 65:1920–1935 [PubMed: 28133764]
164. Ham B, Wang N, D'Costa Z, Fernandez MC, Bourdeau F, Auguste P, Illemann M, Eefsen RL, Hoyer-Hansen G, Vainer B, Evrard M, Gao ZH, Brodt P (2015) TNF receptor-2 facilitates an immunosuppressive microenvironment in the liver to promote the colonization and growth of hepatic metastases. *Cancer Res* 75:5235–5247 [PubMed: 26483205]
165. Lorente D, Mateo J, Templeton AJ, Zafeiriou Z, Bianchini D, Ferraldeschi R, Bahl A, Shen L, Su Z, Sartor O, de Bono JS (2015) Baseline neutrophil-lymphocyte ratio (NLR) is associated with survival and response to treatment with second-line chemotherapy for advanced prostate cancer independent of baseline steroid use. *Ann Oncol* 26:750–755 [PubMed: 25538172]
166. Gonda K, Shibata M, Sato Y, Washio M, Takeshita H, Shigeta H, Ogura M, Oka S, Sakuramoto S (2017) Elevated neutrophil-to-lymphocyte ratio is associated with nutritional impairment, immune suppression, resistance to S-1 plus cisplatin, and poor prognosis in patients with stage IV gastric cancer. *Mol Clin Oncol* 7:1073–1078 [PubMed: 29285377]
167. Suzuki R, Takagi T, Hikichi T, Konno N, Sugimoto M, Watanabe KO, Nakamura J, Waragai Y, Kikuchi H, Takasumi M, Watanabe H, Ohira H (2016) Derived neutrophil/lymphocyte ratio predicts gemcitabine therapy outcome in unresectable pancreatic cancer. *Oncol Lett* 11:3441–3445 [PubMed: 27123132]
168. Mimica X, Acevedo F, Oddo D, Ibanez C, Medina L, Kalergis A, Camus M, Sanchez C (2016) Neutrophil/ lymphocyte ratio in complete blood count as a mortality predictor in breast cancer. *Rev Med Chil* 144:691–696 [PubMed: 27598486]
169. Doi H, Nakamatsu K, Anami S, Fukuda K, Inada M, Tatebe H, Ishikawa K, Kanamori S, Monzen H, Nishimura Y (2019) Neutrophil-to-lymphocyte ratio predicts survival after whole-brain radiotherapy in non-small cell lung cancer. *In Vivo* 33:195–201 [PubMed: 30587623]
170. Zhao L, Li T, Yang Y, Zhang Y, Li W, Han L, Shang Y, Lin H, Ren X, Gao Q (2019) Clinical value of neutrophil-to-lymphocyte ratio as a predictor of prognosis of RetroNectin((R))-activated cytokine-induced killer cell therapy in advanced non-small-cell lung cancer. *Immunotherapy* 11:273–282 [PubMed: 30547699]
171. Quigley JP, Deryugina EI (2012) Combating angiogenesis early: potential of targeting tumor-recruited neutrophils in cancer therapy. *Future Oncol* 8:5–8 [PubMed: 22149028]
172. Gargiulo P, Dietrich D, Herrmann R, Bodoky G, Ruhstaller T, Scheithauer W, Glimelius B, Berardi S, Pignata S, Brauchli P (2019) Predicting mortality and adverse events in patients with advanced pancreatic cancer treated with palliative gemcitabine-based chemotherapy in a multicentre phase III randomized clinical trial: the APC-SAKK risk scores. *Ther Adv Med Oncol* 11:1758835918818351 [PubMed: 30636977]
173. Gurluler E, Tumay LV, Guner OS, Kucukmetin NT, Hizli B, Zorluoglu A (2014) Oncostatin-M as a novel biomarker in colon cancer patients and its association with clinicopathologic variables. *Eur Rev Med Pharmacol Sci* 18:2042–2047 [PubMed: 25027345]
174. De Soyza A, Pavord I, Elborn JS, Smith D, Wray H, Puu M, Larsson B, Stockley R (2015) A randomised, placebo-controlled study of the CXCR2 antagonist AZD5069 in bronchiectasis. *Eur Respir J* 46:1021–1032 [PubMed: 26341987]

175. Bertini R, Allegretti M, Bizzarri C, Moriconi A, Locati M, Zampella G, Cervellera MN, Di Cioccio V, Cesta MC, Galliera E, Martinez FO, Di Bitondo R, Troiani G, Sabbatini V, D'Anniballe G, Anacardio R, Cutrin JC, Cavalieri B, Mainiero F, Strippoli R, Villa P, Di Girolamo M, Martin F, Gentile M, Santoni A, Corda D, Poli G, Mantovani A, Ghezzi P, Colotta F (2004) Noncompetitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. *Proc Natl Acad Sci U S A* 101:11791–11796 [PubMed: 15282370]
176. Gaffen SL, Jain R, Garg AV, Cua DJ (2014) The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat Rev Immunol* 14:585–600 [PubMed: 25145755]
177. Suda K, Kitagawa Y, Ozawa S, Miyasho T, Okamoto M, Saikawa Y, Ueda M, Yamada S, Tasaka S, Funakoshi Y, Hashimoto S, Yokota H, Maruyama I, Ishizaka A, Kitajima M (2007) Neutrophil elastase inhibitor improves postoperative clinical courses after thoracic esophagectomy. *Dis Esophagus* 20:478–486 [PubMed: 17958722]
178. Hawes MC, Wen F, Elquza E (2015) Extracellular DNA: a bridge to cancer. *Cancer Res* 75:4260–4264 [PubMed: 26392072]

Fig. 1.1.

Neutrophil-released factors in the tumor microenvironment. The neutrophils in the tumor microenvironment may facilitate cancer progression through secretion of proteases such as NE and CG, chemokines, and cytokines (which leads to the recruitment of other pro-tumor immune cells or T cell immunosuppression), together with the release of RNS/ROS. Neutrophils also facilitate cancer progression through formations of NETs

Fig. 1.2.

The potential mechanism regarding IL17-induced chemokine/cytokine secretion. IL17 enhanced expression of ERK signaling in multiple cell types, including cancer cells, which results in upregulation of cytokines and chemokines such as CXCR2 ligands. The upregulation of CXCR2 ligands results in positive neutrophils mobilization to the tumor sites

Fig. 1.3. Putative mechanism(s) regarding neutrophils facilitated cancer progression. The neutrophils can facilitate cancer progression by multiple mechanisms, including metastasis