Neutrophils in cancer development and progression: Roles, mechanisms, and implications (Review)

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Abstract. Neutrophils are predominant immune cells that protect the host from microbial infection. The roles of neutrophils in tumor have long been ignored due to their short life span and terminal differentiation phenotype. In recent years, emerging evidence indicates that neutrophils have phenotypic and functional plasticity. Neutrophils eliminate malignant cells by releasing the antimicrobial and cytotoxic contents in their granules or secreting immune mediators to recruit and activate other antitumor effector cells. On the contrary, tumor derived factors can convert neutrophils into a pro-tumor phenotype. Neutrophils have been shown to facilitate tumorigenesis, promote tumor growth and metastasis, stimulate tumor angiogenesis, and mediate immunosuppression. The number of neutrophils in blood and tumor tissues of cancer patients is associated with disease progression and patient outcome. In this review, we summarize the recent progress of neutrophils in the pathogenesis of cancer with an emphasis on neutrophil polarization. Better understanding of the mechanisms that regulate the dichotomy of neutrophils will not only shed light on their roles in cancer but also provide new approaches for cancer diagnosis and treatment.

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Abbreviations: ACEis, angiotensin converting enzyme inhibitors; AGTR1, angiotensin II type 1 receptor antagonist; ALOX5, arachidonic acid 5-lipoxygenase; ARG1, arginase 1; AR, androgen receptor; BLT1, leukotriene B4 receptor-1; CAC, colitis-associated colon cancer; CCL, chemokine (C-C motif) ligand; CS, crystalline silica; CXCR, chemokine (C-X-C motif) receptor; DC, dendritic cells; DEN, diethylnitrosamine; EMT, epithelial-to-mesenchymal transition; ERβ, estrogen receptor β; FcR, Fc receptors; G-CSF, granulocyte colony-stimulating factor; G-MDSCs, granulocytic myeloid-derived suppressor cells; HA, hyaluronan; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; HMGB1, high mobility group box-1 protein; HNSCC, head and neck squamous cell carcinoma; H_2O_2, hydrogen peroxide; HOCl, oxidanthypochlorous acid; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IRS-1, insulin receptor substrate-1; LDNs, low density neutrophils; LTB4, leukotriene B4; MDSCs, myeloid derived suppressor cells; MIF, macrophage migration inhibitory factor; MM, multiple myeloma; MMP-9, matrix metalloproteinase 9; MPO, myeloperoxidase; MSCs, mesenchymal stem cells; NE, neutrophil elastase; NETs, neutrophil extracellular traps; NLR, neutrophil/lymphocyte ratio; NK, natural killer; NO, nitric oxide; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; OSM, oncostatin M; PDA, pancreatic ductal adenocarcinoma; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; RAGE, receptor for advanced glycation end products; RCC, renal cell carcinoma; ROS, reactive oxygen species; TAN, tumor-associated neutrophils; TGF, transforming growth factor; TLR, Toll-like receptor; Tsp-1, thrombospondin-1; VEGF, vascular endothelial growth factor

Key words: neutrophils, cancer, polarization, diagnosis, therapy

Contents

1. Introduction

Neutrophils are innate immune cells that protect the host from infection by eliminating the invading pathogens. In recent

years, these cells have been shown to play important roles in other pathological conditions including cancer. Neutrophils make up a significant portion of the inflammatory cell infiltrate in cancer, whereby they show high functional plasticity and display both antitumor and pro-tumor activities (1). The antitumor effects of neutrophils are related to their cytotoxicity and the regulation of antitumor immune responses, which has been denominated as N1 neutrophils. In addition, tumor derived signals can induce a pro-tumor phenotype in neutrophils, which supports tumor growth and metastasis (N2 neutrophils). N2 polarized neutrophils promote the proliferation, migration, and invasion of tumor cells, stimulate angiogenesis, as well as mediate immunosuppression (2,3). Moreover, increased number of neutrophils in blood and tumors has been linked to poor clinical outcome. Strategies designed to inhibit the pro-tumor activities of neutrophils have shown promising anticancer effects. In this review, we summarize the recent findings on the functional roles of neutrophils in cancer. We mainly focus on the molecular mechanisms that modulate the phenotypic and functional plasticity of neutrophils. The diagnostic value and therapeutic potential of neutrophils in cancer is also discussed.

2. The antitumor roles of neutrophils

The antitumor activities of neutrophils are supported by several lines of evidence. Neutrophils limit tumor growth and metastasis through distinct mechanisms including direct and antibody-dependent cytotoxic activity as well as the activation of other innate and adaptive immune cells such as T cells, B cells, natural killer (NK) cells, and dendritic cells (DCs).

3. The direct cytotoxicity of neutrophils

Neutrophils produce a number of antimicrobial mediators that have potential tumoricidal activity, including reactive oxygen species (ROS), myeloperoxidase (MPO), hydrogen peroxide (H_2O_2), and proteases. Neutrophils from healthy donors have potent cytotoxicity against tumor cells (4). The administration of neutrophils from healthy donors reduces experimental tumor growth and extends the survival of tumor-bearing animals (5). After stimulation with cytokines, neutrophils release ROS to trigger oxidative damage and consequent apoptotic cell death in melanoma cells (6). In addition, neutrophils could inhibit the metastatic potential of tumor cells. Granot and colleagues demonstrated that neutrophils generate H_2O_2 to suppress metastatic seeding of breast cancer cells in the lungs of mice (7), suggesting that neutrophils could prevent tumor metastasis via the generation of cytotoxic substances (8).

4. The antibody-dependent cell cytotoxicity of neutrophils

Neutrophils are critical effector cells that mediate the antitumor effects of mAb-mediated immunotherapy. Antibody-targeting cells could be destroyed by immune cells that express Fc receptors (FcR). Neutrophils express the family members of FcγR. The interactions between neutrophils and mAb through FcR induce the release of tumoricidal mediators (9,10). In several tumor models mAb-induced tumor reduction is abolished in mice with depleted neutrophils. In FcR-deficient mice, the transfer of normal neutrophils or transgenic expression of FcR restore the antitumor effects of mAb, suggesting that neutrophils are required for effective, mAb-induced cancer immunotherapy.

5. The recruitment and activation of innate and adaptive immune cells by neutrophils

In addition to direct and antibody-dependent cytotoxic effects on tumor cells, neutrophils could also recruit and activate immune cells to elicit antitumor immune responses (11-15). Neutrophils release a wide array of factors including cytokines, chemokines, and proteases that have promoting roles in the proliferation and cytokine production of T cells. Neutrophils isolated from the surgically resected human lung cancer tissues could stimulate T cell proliferation and IFN-γ release (16). Neutrophils could efficiently process and present antigens to directly stimulate immune response. Moreover, TLR-stimulated neutrophils induce enhanced cytotoxicity and cytokine production in NK cells and trigger the maturation of dendritic cells, promoting T cell proliferation and IFN-γ production (17).

6. The pro-tumor roles of neutrophils

There is mounting evidence showing that neutrophils are critically involved in the development and progression of cancer (18). Neutrophils play important roles in neoplastic transformation, tumor growth and metastasis, angiogenesis, and the modulation of immunosuppression (Fig. 1).

7. Neutrophils and tumorigenesis

The accumulation of genetic instability is associated with increased cancer risk. Neutrophils release genotoxic substances to inflict DNA damage on epithelial cells and initiate carcinogenic response (19-22). Exposure to activated neutrophils increases the number of replication errors in colon epithelial cells (23). In colitis-associated colon cancer (CAC) mouse model, depletion of neutrophils markedly reduces the number and size of tumors, indicating a crucial role for neutrophils in the initiation and progression of CAC (24-26). Lakritz *et al* demonstrate that neutrophils are critical for mammary tumor development because systemic depletion of neutrophils entirely inhibits tumorigenesis (27). Wilson and colleagues demonstrated that neutrophils stimulate the production of ROS and telomere DNA damage in hepatocytes and promote diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) (28). Yan *et al* further demonstrated the promoting role of neutrophils in hepatocarcinogenesis by using a zebrafish model (29).

8. Neutrophils and tumor growth

Neutrophils generate and release a wide spectrum of factors to support tumor cell growth *in vitro* and *in vivo* (30). Neutrophil elastase (NE) was able to enter into tumor cells to degrade insulin receptor substrate-1 (IRS-1), resulting in increased interaction between PI3K and PDGFR and accelerated tumor cell proliferation (31,32). Neutrophils could promote tumor

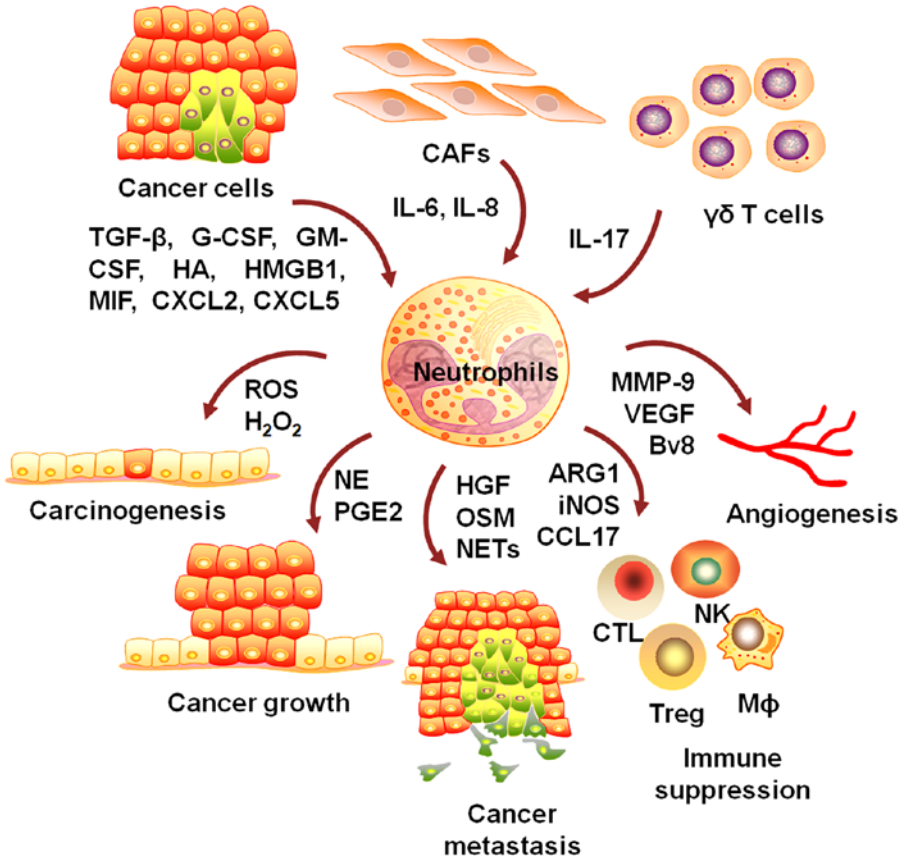

Figure 1. The roles of neutrophils in cancer development and progression. Neutrophils are recruited, expanded, and N2 polarized by tumor derived factors. Neutrophils promote tumorigenesis by inducing genomic instability. Neutrophils could enhance tumor growth via the production of soluble factors and proteinases. Neutrophils promote tumor metastasis by acting as carriers for tumor cells, inducting EMT in tumor cells, and establishing pre-metastatic niche. Neutrophils produce a wide spectrum of pro-angiogenic factors to stimulate tumor angiogenesis. Neutrophils inhibit the proliferation and function of effector T cells and NK cells and recruit regulatory T cells and macrophages to promote tumor growth and metastasis.

cell proliferation through COX-2-mediated prostaglandin E2 (PGE2) synthesis (33,34). Antonio *et al* suggested that acute wound (such as tumor biopsy) induces rapid recruitment of neutrophils to interact with nearby neoplastic cells, leading to increased proliferation of the neoplastic cells through PGE2 (35). Neutrophils enhanced the proliferation of glioblastoma-initiating cells through the upregulation of S100A4 expression (36). Neutrophils could promote the proliferation of renal cell carcinoma (RCC) cells via modulating androgen receptor (AR)/c-Myc signals (37). Moreover, neutrophils from B-cell lymphoma patients induce stromal cell activation to promote the growth of germinal center B-cell lymphoma cells (38). Neutrophils were able to promote multiple myeloma (MM) survival from doxorubicin and melphalan by secretion of soluble factors (39).

9. Neutrophils and tumor metastasis

Neutrophils play a key role in cancer metastasis (40). Neutrophils could promote tumor metastasis by increasing the migratory and invasive potential of tumor cells, degrading extracellular matrix, and promoting the colonization of tumor cells (41-43).

Neutrophils recruited by LPS-induced inflammation could release proteinases such as catheptin G and elastase to degrade thrombospondin-1 (Tsp-1) and facilitate lung metastasis (44). When stimulated with GM-CSF from breast cancer cells, neutrophils release a high level of oncostatin M (OSM), which in turn promotes the detachment of breast cancer cells (45). Wu *et al* demonstrate that hyaluronan (HA) from tumor cells activates neutrophils, which in turn effectively enhances the motility of tumor cells via a cell contact-dependent mechanism (46). Macrophage migration inhibitory factor (MIF) from human head and neck squamous cell carcinoma (HNSCC) cells could activate neutrophils, which in turn enhances the migratory properties of HNSCC cells (47). Moreover, G-CSF from breast cancer cells expand and mobilize neutrophils to release of Bv8, resulting in the promotion of metastasis (48).

Neutrophils could induce epithelial-to-mesenchymal transition (EMT) in tumor cells, which significantly increases the migratory and invasive capacity of tumor cells (49-52). Neutrophils increase bladder cancer cell invasion through the modulation of androgen receptor (AR)/MMP13 signals (53). In addition, neutrophils could promote renal cell carcinoma cell migration and invasion via the activation of VEGFa/HIF2α and estrogen receptor β signals (54). Moreover, neutrophils could also diminish immune protection to promote metastasis. Coffelt *et al* demonstrate that gamma delta (γδ) T cell-derived IL-17 induce G-CSF-dependent expansion and activation of

neutrophils, which inhibits cytotoxic $CD8^+$ T lymphocytes and helps establish metastases (55).

Neutrophils may serve as a carrier to assist tumor cell extravasation. Tumor-elicited neutrophils bind to tumor cells and facilitate tumor cell migration, which is dependent on the expression of intercellular adhesion molecule-1 (ICAM-1) on tumor cells and CD11b on neutrophils (56,57). *In vivo*, neutrophils regulate lung metastasis through physical interaction and anchoring of circulating tumor cells to endothelium (58). Neutrophils promote cancer cell adhesion within liver sinusoids, however, neutrophil depletion impairs the formation of liver metastasis (59,60).

10. Neutrophils and tumor angiogenesis

Neutrophils synthesize and release a number of molecules to activate endothelial cells and induce angiogenesis (61,62). Shojaei *et al* suggest that tumor derived G-CSF upregulates Bv8 expression, which mobilizes neutrophils to promote angiogenesis (63). MMP-9 is implicated in VEGF activation to induce and maintain angiogenesis. Neutrophils are found to be the major sources of MMP-9 (64-66). Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent stimulator of angiogenesis (67). Tumor infiltrating neutrophil-derived MMP-9 coordinately regulate tumor angiogenesis and tumor cell intravasation. Specific inhibition of neutrophil accumulation results in the coordinated inhibition of tumor angiogenesis and intravasation (68).

11. Neutrophils and tumor immunosuppression

Tumor-elicited neutrophils could inhibit the proliferation of T cells through the release of arginase 1 (ARG1) and the modulation of PD-L1/PD-1 signaling (69-72). Neutrophils isolated from the circulation of tumor-bearing mice contribute to the survival of tumor cells by suppressing peripheral leukocyte activation (73). A subset of neutrophils with low density is enriched in the peripheral blood of cancer patients and display immature phenotype similar to that of MDSCs (74). Fridlender and colleagues have compared tumor-associated neutrophils (TANs) with granulocytic myeloid-derived suppressor cells (G-MDSCs) by using transcriptomic analysis and found that the two cell populations are significantly different in their mRNA profiles, pointing out the differences between TANs and MDSCs (75). Hypoxia within the primary tumor sites induce increased infiltration of immunosuppressive neutrophils into the lung, where these cells compromise NK cell cytotoxicity, resulting in a reduced antitumor response that allows metastasis formation (76). Neutrophils inhibit NK cell function to increase the intraluminal survival of tumor cells, facilitating tumor cell extravasation and metastatic dissemination (77). In addition, neutrophils isolated from murine tumor tissues secrete significant amounts of CCL17 to progressively attract Tregs during tumor development (78). CCL17 secretion is relevant to the number of tumor infiltrating neutrophils in human lung cancer patients, suggesting that neutrophils may suppress antitumor immunity and promote tumor growth by regulating Tregs. Zhou *et al* demonstrated that TANs recruit macrophages and Treg cells to promote HCC growth, progression, and resistance to sorafenib (79). The expansion of myeloid cells contributes to tumor progression. Using a multistage mouse model of breast cancer, Casbon *et al* demonstrate that the invasive breast cancer reprograms early differentiation of myeloid cells in the bone marrow to generate immunosuppressive neutrophils (80).

12. Neutrophil extracellular traps in cancer

Neutrophil extracellular traps (NETs) is a web-like structure to trap and kill invading microorganisms (81). The contribution of NETs to tumor has recently been demonstrated (82-84). Cancer-associated thrombosis is linked to a poor prognosis and represents the second-leading cause of death in cancer patients. Using a murine model of chronic myelogenous leukemia, Demers *et al* demonstrated that cancers predispose the release of NETs to cause cancer-associated thrombosis (85,86). Cools-Lartigue *et al* have shown that circulating tumor cells are trapped within NETs *in vitro* under both static and dynamic conditions. In a murine model of lung cancer, deposition of NETs and consequent trapping of circulating lung carcinoma cells are associated with increased hepatic metastasis (87). Neutrophils isolated from mouse models of pancreatic ductal adenocarcinoma (PDA) have shown an increased ability to form NETs (88). Guglietta *et al* showed that increased circulating lipopolysaccharide induces upregulation of complement C3a receptor on neutrophils and activation of the complement cascade, which leads to NETosis and N2 polarization of neutrophils, inducing coagulation and promoting spontaneous intestinal tumorigenesis (89). Moreover, neutrophil extracellular traps have been shown to promote the development and progression of liver metastases after surgical stress (90).

13. The recruitment, expansion, and polarization of neutrophils in cancer

The origin of the infiltrating neutrophils in tumor has not been well characterized. Cortez-Retamozo *et al* demonstrated that the spleen is an important origin of tumor associated neutrophils. The precursors of neutrophils relocate from the spleen to the tumor stroma during tumor progression. Removal of the spleen reduces the number of the infiltrating neutrophils and delays tumor growth (91). A large number of molecules from tumor cells have been shown to recruit neutrophils. IL-8 is one of the potent neutrophil chemoattractants. Tumor cells with IL-8 overexpression recruit more neutrophils and display increased metastatic potential (92). IL-17 recruits blood neutrophils into the peritumoral stroma of hepatocellular carcinoma by inducing expression of chemokines in epithelial cells (66). Wu *et al* also suggest that tumor-infiltrating DCs induce the activation of IL-17 producing γδT cells to promote the accumulation and expansion of immunosuppressive neutrophils in colon cancer (93).

Tumor-derived oxysterols could recruit neutrophils to favor tumor growth by promoting angiogenesis and immunosuppression (94,95). CXCL5 has a direct chemoattractant effect on neutrophils. CXCL5 overexpression is positively correlated with neutrophil infiltration in hepatocellular carcinoma and intrahepatic cholangiocarcinoma patients (96,97). UV irradiation-damaged epidermal keratinocytes release high mobility

group box 1 (HMGB1) to recruit and activate neutrophils by interacting with toll-like receptor 4 (TLR4), which stimulates angiogenesis and promotes the ability of melanoma cells to metastasize (98). Leukotriene B4 (LTB4), an inflammation mediator, induces the recruitment of neutrophils via interaction with BLT1 on neutrophils (30). Neutrophils from HNSCC patients display a significantly reduced apoptosis compared to those from healthy donors, which may be associated with the secretion of MIF by HNSCC cells (99). Hypoxia induces an HIF-1α-dependent activation of NF-κB to inhibit neutrophil apoptosis (100). Li *et al* demonstrated that the prolonged survival of neutrophils in tumor is associated with increased autophagy. Neutrophils in HCC intratumoral regions undergo increased autophagy and display long-lived phenotypes and sustained production of pro-metastatic factors (101). IFN-β negatively regulates the survival and recruitment of neutrophils. In the absence of endogenous IFN-β the life span of neutrophils from blood and tumors of IFN-β deficient mice is remarkably prolonged (102,103). On the contrary, MET is required for the recruitment of antitumor neutrophils (104). Met deletion in mouse neutrophils enhances tumor growth and metastasis.

Tumor-derived factors could modify the phenotype and function of myeloid cells (105,106). Neutrophils are polarized to N1 and N2 phenotypes in cancer. In general, the N1 and N2 polarized neutrophils could be distinguished based on their phenotype and function. The N1 polarized neutrophils are short-lived cells with mature phenotype and display high cytotoxicity and immunostimulating activity. The N2 polarized neutrophils are long-lived cells with immature phenotype and show low cytotoxicity but high pro-angiogenic, pro-metastatic, and immunosuppressive activities.

The potent drivers of neutrophil polarization have recently been demonstrated. Inhibition of TGF-β increases the expression of chemokines that recruit neutrophils, resulting in an influx of neutrophils that has strong cytotoxic activity to tumor cells. Following TGF-β blockade, depletion of these neutrophils significantly attenuates antitumor effects of treatment and reduces $CD8^+$ T cell activation. In contrast, in control tumors, neutrophil depletion decreases tumor growth and results in more activated $CD8^+$ T cells within tumor, suggesting that tumor associated neutrophils are driven by TGF-β to acquire N2 protumoral phenotype. In contrast, TGF-β inhibition induces an antitumor N1 phenotype (107). The anti- and pro-tumor functions of neutrophils imply its diversity and plasticity. Sagiv *et al* have identified a heterogeneous subset of low density neutrophils (LDNs) that progressively accumulate in tumors. LDNs consist of both immature MDSCs and mature cells that are derived from HDNs in a TGF-β-dependent mechanism (108). The plasticity of neutrophils has been determined in mouse tumor models at different time points during tumor progression. Neutrophils are mainly located at the peritumoral tissues at early stage of tumor development while these cells are found scattered in tumor cells at later stage. Neutrophils isolated from tumors at early stage are more cytotoxic toward tumor cells and produce higher levels of NO and H_2O_2. In established tumors, these functions are decreased and these cells acquire a more protumorigenic phenotype, suggesting the critical role of tumor niche in modulating neutrophil phenotype and function. In line with this phenotype, only depletion of neutrophils at later stage of tumor development inhibits tumor growth, indicating the functional changes in neutrophils with tumor progression (109).

In the absence of endogenous IFN-β, mice develop a fast-growing tumor accompanied with increased infiltration of neutrophils which produce a large amount of VEGF and MMP-9 to promote tumor angiogenesis and metastasis. *In vitro* treatment with recombinant IFN-β inhibit the activation of STAT3 pathway and the upregulation of VEGF and MMP-9 genes in tumor infiltrating neutrophils. In addition, the transplantation of neutrophils from control mice into IFN-β-deficient mice retards tumor growth, suggesting that IFN-β may be a factor that maintain the N1 polarization of neutrophils. The conversion of neutrophil phenotype and function may occur in the bone marrow of tumor-bearing mice (110).

In addition to tumor cells, the microenvironmental cells also participate in the regulation of neutrophil biology in cancer. Tumor-resident mesenchymal stem cell (MSC)-derived IL-6 induced N2 polarized activation of neutrophils (111). Intriguingly, Hu *et al* demonstrated that TNFα-primed mouse MSCs could program neutrophils into an immunosuppressive and tumor-promoting phenotype (112). Moreover, in response to tumor derived IL-1β signal, tumor infiltrating γδ T cells release IL-17 to recruit, expand, and activate neutrophils to promote cancer metastasis. Taken together, these findings indicate that neutrophils are polarized during tumor progression by the signals from tumor milieu (Table I).

14. Targeting neutrophils for cancer diagnosis and therapy

Cancer-related inflammation plays a key role in tumor progression. The increased neutrophil infiltration in tumor is associated with poor outcome in renal cell carcinoma (113), head and neck squamous cell carcinoma (114), melanoma (115), lung carcinoma (116,117), colorectal carcinoma (118), gastric carcinoma (119), cholangiocarcinoma (97), hepatocellular carcinoma (120), tongue squamous cell carcinoma (121), and esophageal squamous cell carcinoma (122,123). High intratumoral neutrophil is positively correlated with lymph node metastasis, tumor grade, and tumor stage. High densities of neutrophils in tumor are identified as an independent risk factor for poor prognosis (124). In addition, a high neutrophil-to-lymphocyte ratio (NLR) has also been suggested as a poor prognostic indicator in cancer (125). Moreover, the high numbers of neutrophils and NLR in cancer patients are associated with poor response to chemotherapy and immunotherapy (126).

The idea of targeting neutrophils represents a new approach for cancer therapy (127). Several strategies have been proposed to inhibit their recruitment, interfere with their survival, or reprogram them into N1 antitumor phenotype (Fig. 2). 'Reeducation' to activate the antitumor potential of cells or elimination of tumor promoting cells is a new strategy undergoing preclinical and clinical evaluation. Since tumor derived factors contribute to phenotypic and functional plasticity of neutrophils in cancer, modulation of tumor milieu can lead to reeducation of neutrophils. The conversion of pro-tumor activity of neutrophils into antitumor potential with appropriate stimulation and modulation provides new opportunities for cancer therapy. Sun *et al* have recently shown that

Table I. Factors that mediate neutrophil recruitment, expansion, polarization, and pro-tumoral function.

Factors	Cancer type	Function	Species	Refs.
CXCL5	HCC	Recruit neutrophils to promote cancer growth and metastasis	Human, mouse	(96,136)
IL-17	Breast cancer, HCC	Recruit neutrophils to promote tumor growth and metastasis	Mouse	(55,66,137)
HMGB1	Melanoma	Recruit neutrophils to promote tumor angiogenesis and metastasis	Mouse	(98)
Oxysterol	Lung cancer	Recruit neutrophils to favor tumor growth	Mouse	(94)
GM-CSF	Breast cancer	Recruit neutrophils to promote tumor angiogenesis and metastasis	Human	(45)
HGF	Lung cancer	Recruit neutrophils to promote tumor metastasis	Human, mouse	(138)
IL-8	Lung cancer, skin cancer	Recruit neutrophils to promote tumor initiation and progression	Human, zebrafish	(51,92
G-CSF	Breast cancer	Recruit and expand neutrophils to promote tumor growth and metastasis	Mouse	(48,80)
HA	HCC	Recruit neutrophils to promote tumor angiogenesis and metastasis	Human	(46)
IL-6	HCC, gastric cancer	Recruit neutrophils to promote tumor growth and metastasis	Human, mouse	(110,111)
CXCL2	Colon cancer	Recruit neutrophils to promote tumor growth and metastasis	Mouse	(24)
TGF-β	Lung cancer	Polarize neutrophils to an N2 phenotype to promote tumor growth	Mouse	(107)
MIF	Head and neck cancer	Recruit neutrophils to promote tumor cell migration	Human	(47)
LTB4	Lung cancer	Recruit neutrophils to promote tumor growth	Mouse	(30)
a2NTD	Breast cancer	Recruit neutrophils to promote tumor cell invasion	Human	(139)
PGE2	Lung cancer, skin cancer	Promote tumor cell proliferation	Human, zebrafish	(33,35)
NE	Lung cancer, pancreatic cancer	Promote tumor cell proliferation and tumor cell dyshesion	Mouse	(31,49)
OSM	Breast cancer	Promote tumor angiogenesis and metastasis	Human	(45)
Arg-1	Lung cancer	Promote tumor immunosuppression	Human	(70)
CCL17	Lung cancer, HCC	Promote tumor immunosuppression	Mouse	(78,79)
PD-L1	HCC	Promote tumor immunosuppression	Human	(71)
NET	CML, lung cancer	Promote tumor-associated thrombosis and tumor metastasis	Human	(85,87)
MMP-9	HCC, lung cancer, pancreatic cancer	Promotes tumor angiogenesis	Human, mouse	(64,65)
IL-1β	Colon cancer	Promote tumor initiation and progression	Mouse	(25)

priming with TNF-α and IFN-γ could convert the potential of neutrophils from tumor-promoting to tumor-suppressing through the activation of NK cells (128). TGF-β signaling regulates neutrophil N2 polarization. Depletion of the receptor for TGF-β decreases the production of arginase 1 and iNOS in neutrophils, which in turn increases IFN-γ expression in $CD8^+$ T cells and inhibits tumor metastasis (129). IFN-β regulates the N1 polarization of neutrophils. In mice, the treatment with low dose of IFN-β induces antitumor activation of neutrophils (130). The chemokine receptor CXCR2 is a key mediator of neutrophil recruitment. CXCR2 inhibitor attenuates neutrophil recruitment and profoundly suppresses tumor growth (131,132). Moreover, pharmacological inhibition of ALOX5, a leukotriene-generating enzyme, inhibits the recruitment of pro-metastatic neutrophils and reduces lung metastasis (133). A recent study from Shrestha *et al* indicates that the inhibitors for angiotensin converting enzyme (ACEis) and the antagonists for angiotensin II type 1 receptor (AGTR1) could attenuate

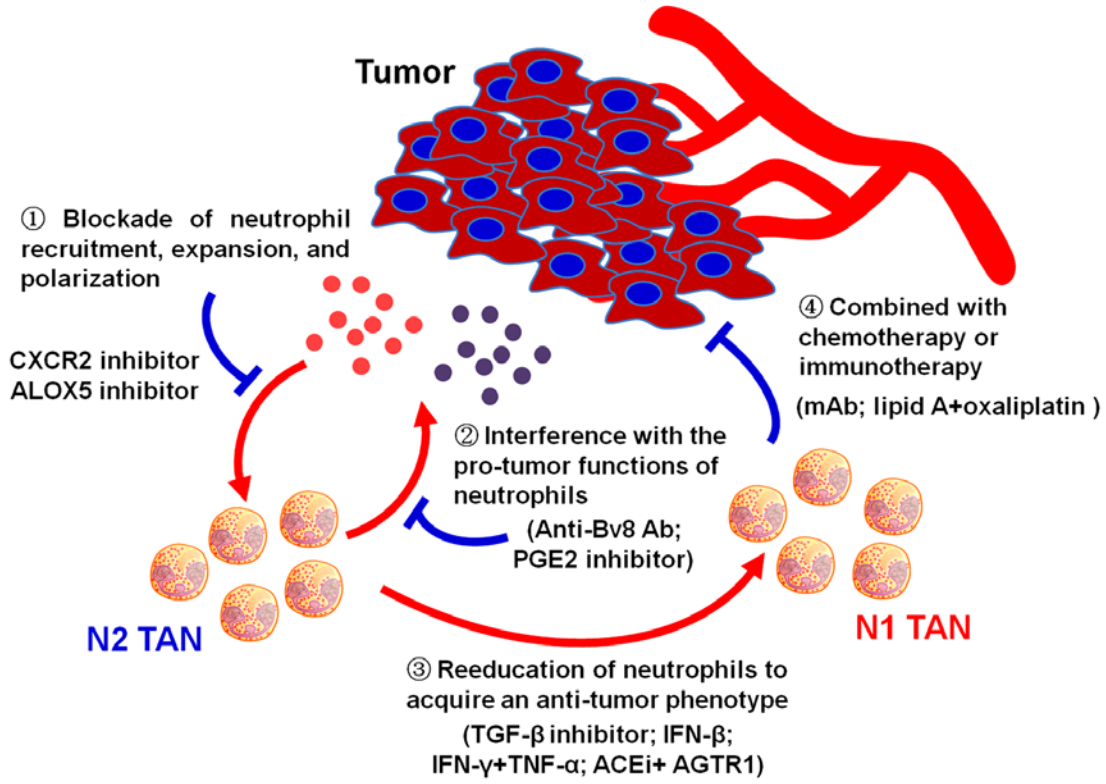

Figure 2. Targeting tumor-associated neutrophils for cancer therapy. Several neutrophil-targeting approaches have been developed and shown antitumor effects in experimental and clinical settings. Pharmacological blockade of tumor derived factors and downstream signaling pathways abrogate the recruitment, expansion, and polarization of neutrophils. Selective interference with the pro-tumoral functions of neutrophils represents an alternative cancer treatment approach. Reeducation of neutrophils from a tumor-supporting phenotype to a tumor-suppressive phenotype also have therapeutic potential. These strategies, when combined with conventional anticancer strategy such as chemotherapy or new anticancer strategy such as immunotherapy would probably show more effective therapeutic effects.

Table II. The clinical value of tumor-associated neutrophils in cancer.

Cancer type	Clinical significance	Refs.
Hepatocellular carcinoma (HCC)	The presence of intratumoral neutrophils is an independent prognostic indicator for overall survival and cumulative recurrence	(96,120)
Cholangiocarcinoma	Increased number of intratumoral $CD66b^+$ neutrophils predicts poor survival and high risk of recurrence	(97)
Colorectal carcinoma (CRC)	High intratumoral neutrophil is associated with shorter survival	(118)
Esophageal squamous cell carcinoma (ESCC)	Increased intratumoral neutrophils is associated with decreased disease-free survival and overall survival	(122,123)
Gastric carcinoma (GC)	Lower density of intratumoral neutrophils suggests a better prognosis	(119)
Head and neck squamous cell carcinoma (HNSCC)	Strong presence of intratumoral neutrophils represent a negative prognostic factor for HNSCC patients with advanced disease	(114)
Lung cancer	Elevated levels of neutrophils correlates with poor prognosis in lung cancer patients	(116,117)
Melanoma	Neutrophil infiltration is independently associated with poor prognosis	(115)
Renal cell carcinoma (RCC)	The presence of intratumoral neutrophils is an independent prognostic factor for cancer specific survival and overall survival	(113)
Tongue squamous cell carcinoma (TSCC)	High neutrophil density is associated with lymph node metastasis, higher clinical stage and tumor recurrence	(121)
Glioma	Neutrophil infiltration is correlated with glioma grade and tumor progression	(36)

tumor growth via the polarization of neutrophils toward an antitumoral phenotype (134).

15. Concluding remarks

The roles of neutrophils in the pathogenesis of cancer have recently become an intense research area. Neutrophils have both pro-tumor and antitumor activities. Neutrophils are frequently recruited to local tumor sites, whereby neutrophils can be polarized towards distinct phenotypes by tumor derived signals. In turn, neutrophils suppress or promote tumor development and progression by cell contact-dependent mechanism or secretion of soluble factors. Herein, we summarize the roles of neutrophils in cancer and their potential as cancer diagnosis biomarker and therapy target. Although early studies indicate that neutrophils have direct cytotoxicity against tumor cells and regulate the functions of innate and adaptive immune cells, more recent reports have shown that neutrophils promote tumor development and progression by enhancing tumor cell growth and metastasis, stimulating tumor angiogenesis, and mediating immunosuppression. Previous studies have mainly focused on experimental animal models of cancer (135), however, more studies are needed to elucidate the cellular and molecular mechanisms that modulate the phenotype and function of neutrophils in human tumor, such as recruitment to the tumor site, prolonged survival and enhanced release of tumor-promoting factors. In addition, further studies are needed to elucidate the relationship between heterogeneous neutrophil subsets. Moreover, novel strategies to reeducate the tumor-promoting neutrophils to activate the host's innate and adaptive immune responses will provide new approaches for tumor therapy.

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