Role of tumor-associated neutrophils in lung cancer (Review)

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Abstract. Lung cancer is the most common malignancy and the leading cause of cancer mortality worldwide; therefore, it is very important to understand the mechanism of its occurrence and progression. It has reported that inflammation is linked to the incidence of various malignancies. Neutrophils not only participate in the inflammatory response, but are also involved in the composition of the tumor microenvironment. Tumor-associated neutrophils (TANs) are infiltrating neutrophils in tumors that directly promote tumor development and progression. Moreover, they regulate the immune microenvironment and affect the therapeutic efficacy and prognosis of lung cancer. In the present review, the role of TANs in lung cancer development/progression and the underlying molecular signaling are evaluated, as well as the possibility of TANs as a potential therapeutic target for lung cancer intervention.

Contents

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1. Introduction

Lung cancer remains the leading cause of cancer incidence and mortality globally, with an estimated 2 million new diagnoses and 1.8 million deaths in 2019 (1). Non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancer cases, with a 5-year survival rate of only 16% (2,3) despite recent advancements in targeted therapy and immune checkpoint inhibitors (4,5). Metastasis is a major cause of death in patients with malignant tumors, accounting for ~90% of cancer-associated deaths. Tumor metastasis has been reported to depend on the formation of a pre-metastatic niche (PMN) (6,7). The PMN specifically refers to the microenvironment in which the primary tumor foci are prepared for distant dissemination and colonization of tumor cells. The features of this microenvironment include inflammation, immunosuppression, angiogenesis/vascular permeability, organophilicity, reprogramming and lymphangiogenesis (8-10). An early study reported that lung cancer metastasis was highly correlated with leukocytosis (11). It has been reported that tumor-associated neutrophils (TANs), which are major components in tumor-associated inflammation, support lung cancer cell

growth, invasion, angiogenesis and cancer cell metastasis, and are associated with a poor prognosis (12-14). Therefore, the present study reviewed the role and mechanisms of TANs in lung cancer development and progression, as well as TANs as a potential therapeutic target for lung cancer.

2. TANs

Neutrophils account for 50-70% of all white blood cells in the circulation and serve an important role in the inflammatory process, which is regarded as the first line of defense against infection (15). Neutrophils in the blood circulation are usually dormant and will be activated once they migrate into the tissues (16). TANs are infiltrating neutrophils within tumors. The chemokines produced by tumors attract the neutrophils from the blood circulation, which enter the tumor tissue through the blood vessel wall and then form TANs. The spleen is a recently discovered reservoir of mononuclear cells, and a large number of TAN precursor cells migrate from the spleen to the tumor stroma (17). Furthermore, splenectomy has been reported to decrease the number of primary tumor infiltrating TANs, leading to a reduced number or size of metastases (18). Once TANs are activated in the tumor microenvironment, they appear to increase the complexity of the inflammatory environment through a mechanism that involves the attraction of other leukocytes. TANs secrete a large amount of interleukin-8 (IL-8), which promotes neutrophil survival and recruits more neutrophils (19). TANs serve a major role in numerous aspects of tumor development, such as malignant transformation, tumor progression, extracellular matrix modification, angiogenesis, cell migration and immunosuppression (20-22). Numerous studies have reported that neutrophils promote tumor progression by degrading the stroma, immunosuppression, stimulating tumor cell proliferation, increasing metastasis and promoting angiogenesis (20,23). The enrichment of neutrophils in tumors was also reported to be associated with lymph node metastasis, tumor differentiation and grade, and tumor stage. These findings indicated that tumors and the tumor microenvironment regulated neutrophil recruitment and that TAN feedback regulated tumor progression (24,25). Furthermore, clinical studies have reported that patients with lung cancer had high levels of neutrophils in both the tumor and the circulation (26-28). The higher neutrophil/lymphocyte ratios were associated with higher recurrence rates following operations, less effective treatment responses and worse prognoses compared with the lower ratios (29-32).

3. TAN phenotype

TANs have a high functional plasticity, which not only inhibits cancer growth but also stimulates cancer progression. Previous studies have reported that the phenotypes of TANs are similar to the phenotypes of tumor-associated macrophages, with both the 'N1' type that inhibits tumor growth and the 'N2' type that promotes tumor growth and malignant metastasis (33,34). N1 neutrophils can produce and release cytotoxic mediators such as reactive oxygen species (ROS) and myeloperoxidase (MPO), to kill tumor cells directly or inhibit tumor cell proliferation. N1 neutrophils have been reported to inhibit tumor metastasis and infiltration in breast cancer by releasing C-C motif chemokine

ligand 2 (CCL2) and promoting the production of ROS; in gastric carcinoma, a high density of N1 type neutrophils also inhibits lymph node metastasis (35,36). However, N2 neutrophils participate in tumor proliferation, invasion and metastasis through the synthesis and secretion of proteases. Studies have reported that N2 type neutrophil elastase (NE), matrix metallopeptidase-9 (MMP-9) and MMP-2 promote tumor growth and angiogenesis, which results in distant metastasis (37-41). It was also reported that TANs maintained some functional plasticity and could be 'alternately activated' when exposed to different tumor microenvironments (21,42). For example, transforming growth factor-β (TGF-β) induced a pro-tumor phenotype (N2-TAN) (42,43), whereas interferon-β (IFN-β) or inhibition of TGF-β signaling led to an antitumor (N1-TAN) phenotype (41,44). Further studies reported that administration of TGF-β could convert TANs from an antitumor (N1) phenotype into a more permissible (N2) phenotype, whereas blocking TGF-β transformed TANs from the N2 phenotype to the N1 phenotype in mouse models of mesothelioma and lung cancer (45,46). IFN-γ and granulocyte macrophage colony-stimulating factor (GM-CSF) were reported to be key factors in the differentiation process of TANs; IFN-γ had a dose-dependent specific effect on neutrophil differentiation, inducing a hybrid phenotype with immune-stimulating T cell characteristics at low doses, whereas a hybrid phenotype induced by programmed death-ligand 1 (PD-L1) expression with inhibition of T cell response was stimulated at high doses (47). In the early stage of tumor development, TANs mainly manifest as the N1 type and serve an antitumor role. However, in the late stages of tumor development, TANs primarily function as the N2 type and promote tumor development, invasion and metastasis (19). Furthermore, the phenotype and function of TANs are different in different locations and tumor stages. Studies using animal experiments reported that mice injected with circulating TANs (cTANs) demonstrated more pulmonary metastatic nodules, which suggested that the increase in cTANs enhanced tumor metastasis (48). Compared with circulating peripheral blood neutrophils, neutrophils absorbed into lung tumors exhibit an activated phenotype (19). It was reported that there was no difference in the number of neutrophils entering tumors between the early and late stage of lung cancer in a mouse model (49). However, neutrophils, in the early stage of tumor development, are almost completely located at the edge of the tumor. It was only during the later stages that neutrophils were discovered scattered among the tumor cells. Moreover, TANs in early stage tumors demonstrate greater cytotoxicity to tumor cells by producing higher levels of tumor necrosis factor (TNF)-α, nitric oxide (NO) and hydrogen peroxide (49). These results provide a new concept for tumor intervention, namely, the inhibition of cancer progression by finding strategies to activate N1 neutrophils and/or convert N2 to N1 neutrophils (Fig. 1).

4. Neutrophilic extracellular traps (NETs)

NETs are released by neutrophils in response to extracellular pathogens and are typically composed of densified fibrous chromatin and polymerized histones, MPO and numerous cytoplasmic proteins that promote disease progression and transmission (50,51). Previous studies have reported that
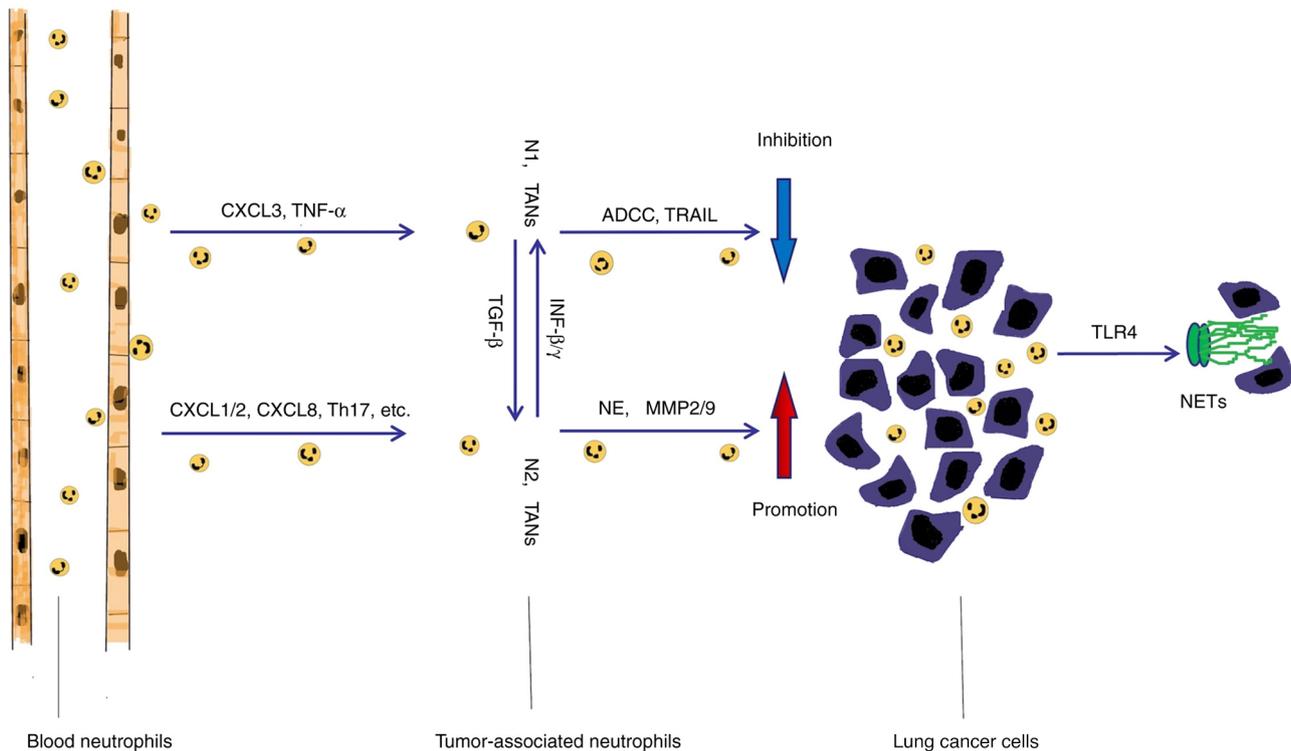
Figure 1. Circulating neutrophils enter the tumor tissue under the action of chemokines to form TANs. TANs demonstrate the 'N1' phenotype that inhibits tumor growth and the 'N2' phenotype that promotes tumor growth and metastasis. TGF-β promotes the production of N2 TANs, whereas the presence of IFN-β/γ or the inhibition of TGF-β induces the production of N1 TANs. N1 TANs inhibit lung cancer growth through ADCC and TRAIL. N2 TANs secrete NE, MMP-9 and MMP-2 to trigger tumor growth. Lung cancer cells activate TLR4, which stimulates the formation of NETs, which leads to tumor progression. TANs, tumor-associated neutrophils; TGF-β, transforming growth factor-β; IFN, interferon; ADCC, antibody-dependent cell-mediated cytotoxicity; Th17, T-helper cell 17; TRAIL, TNF-related apoptosis-inducing ligand; NE, neutrophil elastase; MMP, matrix metallopeptidase; TLR4, Toll like receptor 4; NETs, neutrophil extracellular traps; CXCL, C-X-C motif chemokine ligand.

NETs are not only involved in the inflammatory process, but also promote the adhesion of circulating tumor cells and the occurrence of micro-metastasis (33,52). Compared with that in healthy control and early stage tumor groups, the Net levels were reported to be significantly increased in patients with advanced cancer of the esophagus, stomach, pancreas and lung, which was related to TNM staging, supported metastasis and was negatively correlated with prognosis (53-57). Furthermore, the molecules released from Lewis lung cancer cells were found to activate toll-like receptor 4 (TLR4), promote the formation of NETs, increase the adhesion of cancer cells and promote lung cancer progression (58-60). NETs also function as a cancer cell adhesion matrix to promote tumor progression (61,62). The capture of tumor cells by NETS has no cytotoxic effect on the cells, rather it enhances tumor proliferation, invasion and metastasis by triggering the expression of tumor IL-8 (63). NETs have also been reported to trigger metastatic potential through the internalization of captured tumor cells and the activation of TLR4/9-COX2 signaling, mediating cell death resistance and enhancing invasion (64). Elevated COX2 expression levels are associated with higher metastatic behavior, including protective cell death, the induction of invasion, the stimulation of angiogenesis and the suppression of immune surveillance (65-67). Furthermore, NETs promote tumor micro-metastases by the activation of cancer-related fibroblasts (68). A previous study reported that tumor-secreted protease cathepsin C in breast cancer induced lung metastasis

by the regulation of neutrophil recruitment and the formation of NETs (69) (Fig. 1).

5. Mechanism of neutrophil recruitment

TANs can be differentiated on the basis of their activation and cytokine status, and their effect on the growth of N1 and N2 TAN tumor cells. N1 TANs are characterized by high expression levels of TNFα, CCL3 and intercellular adhesion molecule (ICAM)-1, and low expression levels of argininase axis proteins, whereas N2 neutrophils are typified by the upregulation of chemokines, including CCL2, CCL3, CCL4, CCL8, CCL12, CCL17, C-X-C motif chemokine ligand (CXCL)1, CXCL2, IL-8/CXCL8 and CXCL16 (33). A previous study reported that the serum CXCL1 level was significantly upregulated in tumor-bearing mice with Lewis lung carcinoma (3LL) cells and that the depletion of CXCL1 in 3LL cells significantly inhibited neutrophil infiltration, which led to decreased tumor growth *in vivo* (70). These results indicated that CXCL1 was involved in TAN infiltration of lung cancer and promoted tumor growth. CXCR2 and its ligands (i.e., CXCL1-3, CXCL5, CXCL7 and CXCL8) were reported to be responsible for the recruitment of neutrophils under normal physiological conditions and to participate in the mobilization of TANs (71). Furthermore, oxidative sterols derived from Lewis lung cancer cells serve a critical role in the promotion of neutrophil recruitment to tumor tissues by

CXCR2 (72). TANs participate in the tumor microenvironment through secretion of PD-L1, CXCR4, CCR5, Adam 17 and NOS2, and are reported to have an immunosuppressive effect in T-cell proliferation assays. Tumors with overexpression of CXCL5 have reduced frequencies of lung metastasis and neutrophil depletion reverses this effect (73). Tumor suppressor chemokines CXCL8 and CXCL6 are also involved in neutrophil infiltration (74). Recently, the CXCL8-CXCR1/2 axis was reported to serve an important role in the occurrence of solid tumors, including cancer of the lung, colon and breast. CXCL8 was reported to promote tumor growth and metastasis through increased MMP-2 activity (75,76). Changes in cell adhesion molecules (CD62L and CD54) and CXC chemokine receptors (CXCR1 and CXCR2) are related to leukocyte activation, chemotaxis enhancement and trans endothelial migration (77,78). Circulating neutrophils were reported to be attracted to tumor tissues from blood vessels by chemokines, which bind to the G protein coupled receptors CXCR1 and CXCR2 on the surface of neutrophils (34,79). Moreover, it has been reported that neutrophils and T helper cell 17 (Th17) cells can stimulate each other in a chemokine/cytokine-dependent manner. Th17-produced cytokines, including IL-17A and IL-17F, can indirectly induce the recruitment of neutrophils, and active neutrophils release CCL2 and CCL20 chemokines to induce chemotaxis of Th17 cells. Furthermore, activated Th17 cells can directly attract neutrophils by releasing bioactive CXCL8 (80). IL-17C was also reported to promote neutrophil recruitment, tumor-related inflammation, and tumor proliferation and growth (81).

6. Regulatory mechanism of TANs on tumor angiogenesis/epithelial-mesenchymal transition (EMT)

Angiogenesis is essential for tumor metastasis. Previous studies have reported that TANs support tumor metastasis by generating angiogenic factors and stromal degrading enzymes to drive tumor angiogenesis (82,83). TANs are an important source of MMP-9 in NSCLC (84,85). Furthermore, in a tumor xenotransplantation model, the upregulation of Bv8 (also known as activin-2) in neutrophils induced by granulocyte colony-stimulating factor (G-CSF) was reported to promote tumor angiogenesis (86). It has also been reported that G-CSF promotes the recruitment of neutrophils to tumors, which express Bv8 leading to induction of angiogenesis and resistance to anti-vascular endothelial growth factor (VEGF) therapy (87,88). EMT serves an important role in tumor invasion and metastasis (89,90). Several molecular pathways that mediate EMT in cancer cells have been reported, such as the TGF-β, Ras, Notch and Wnt/β-catenin cascades (91). In previous studies, the formation of vascular mimicry (VM) was induced by cancer-associated fibroblast (CAFs) in both *in vitro* and *in vivo* experiments. Notch 2-Jagged 1 cell-cell contact between cancer cells and CAFs contributes to VM network formation. Intercellular adhesion molecule-2 contributes to VM-mediated neutrophil infiltration. VM networks not only change the phenotype of neutrophils from the tumor-suppressing N1 type to the tumor-promoting N2 type, but also provide a vital channel for the infiltration of neutrophils. VM networks serve a crucial role in the promotion of lung cancer metastasis and chemoresistance to

angiostatic therapy (92-94). Tumor cells and mesenchymal cells secrete chemokines, which bind to CXCR1 and CXCR2, the G-protein coupled receptors expressed on the surface of neutrophils, promoting tumor progression (34,79). TANs were also reported to promote the EMT of lung adenocarcinoma cells *in vitro* and enhance their migration activity (91,95).

7. TAN regulation of T cells

As well as acting directly on tumor cells, TANs regulate T lymphocytes to control tumor growth. It has been previously reported that granulocyte myeloid inhibitory cells are able to inhibit CD8^{+} T cell proliferation and activation. The infiltrating neutrophils in tumor tissues express higher levels of MPO, Fas/Fas ligand and PD-L1, which are involved in the TAN-mediated inhibition of CD4^{+} and CD8^{+} T cells (70). Michaeli *et al* (96) reported that TANs promote immunosuppression by strongly inducing CD8^{+} T cell apoptosis, which leads to tumor progression. The mechanism by which TANs induce CD8^{+} T cell death involves the TNF signaling pathway and NO production. In the early stage of lung cancer, TANs are not immunosuppressive, rather they stimulate the T-cell response and enhance the immune response of cytotoxic T lymphocytes, which leads to the inhibition of tumor development (19,97). Activated T cells lead to significant upregulation of CD54, CD86, OX40L and 4-1BBL costimulatory molecules on the surface of neutrophils, which further support T-cell proliferation in a positive feedback loop. In the case of lung cancer, a subset of TANs, at the early stage of tumor development, stimulates T-cell activation by secreting GM-CSF. Moreover, TANs produce classical pro-inflammatory cytokines, such as GM-CSF and IL-6, which serve as positive regulators of T cells, leading to tumor suppression (98). Neutrophils can also secrete cytokines, including TNFR and cathepsin G, which act directly on T cells and enhance the overall adaptive immune antitumor response (99). Furthermore, TANs have been reported to support the adaptive antitumor immune response by secreting chemokines such as CXCL9 or CXCL10 to recruit T cells to tumor sites (46) (Fig. 2).

8. Additional mechanisms of neutrophils for facilitating lung cancer progression

NE is a neutrophil-derived protease with broad substrate and neutrophil specificity (100). NE promotes lung cancer growth in the LOX-Stop-Stop-K-ras mouse model. This effect of NE is not through the degradation of the extracellular matrix, but rather by direct action on the tumor cells (37). NE is not only involved in the occurrence of lung cancer, but also induces distant metastasis. Wislez *et al* (101) studied the role of neutrophils in the metastasis of bronchoalveolar carcinoma and reported that human lung adenocarcinoma cells interact with neutrophils. The neutrophils promoted the abduction of cancer cells through secretion of NE, which suggested that NE is involved in tumor metastasis (101,102). NE also accelerates the lung tumor growth through mediation of the degradation of IRS-1. In addition to NE, matrix metalloproteinases (MMPs) are important proteolytic enzymes released by neutrophils. MMPs degrade the extracellular matrix to promote neovascularization and tumor metastasis (40,103).
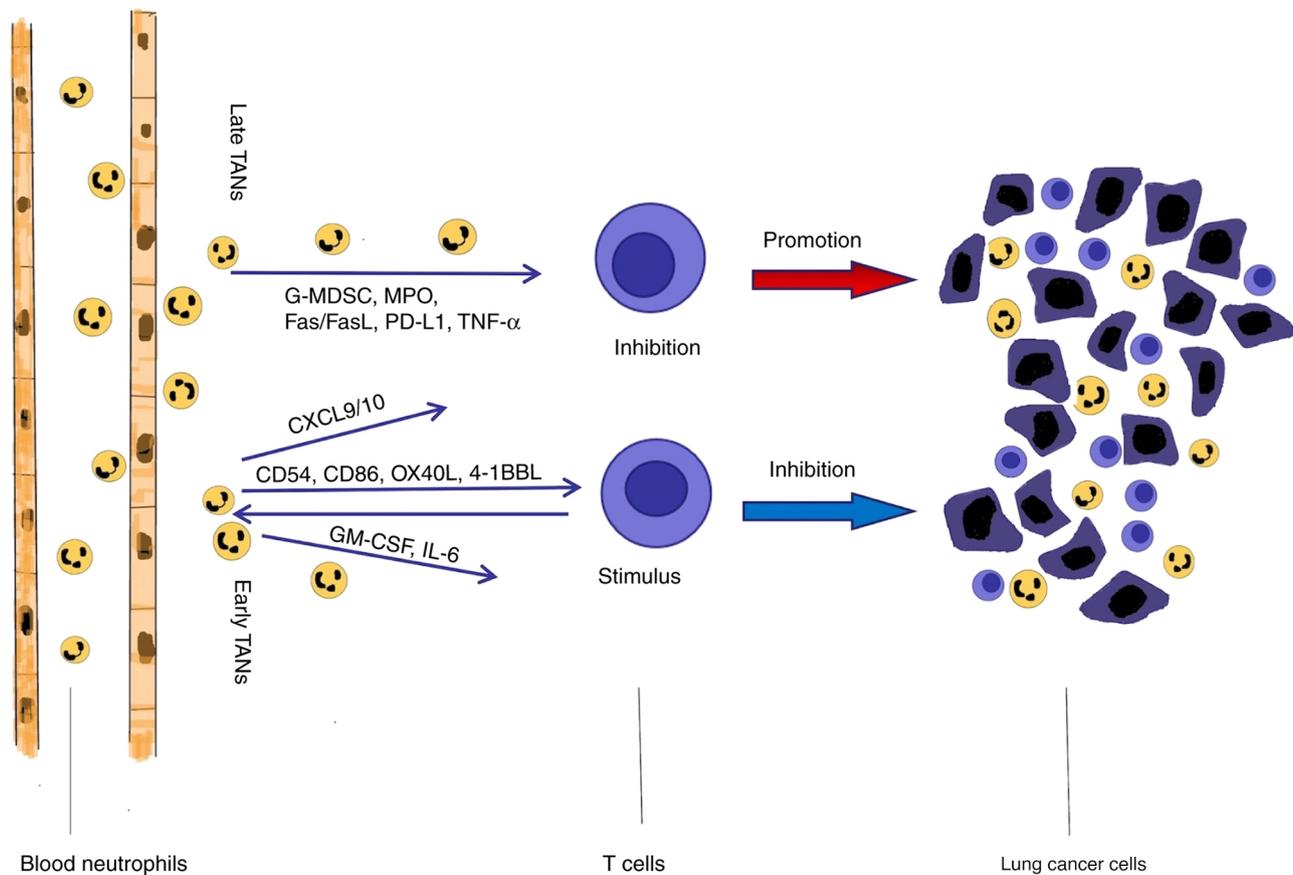
Figure 2. In the early stage of lung cancer, TANs produce a large amount of CD54, CD86, OX40L and 4-1BBL, which co-stimulate the molecules on the surface of neutrophils through interaction with activated T cells. GM-CSF and IL-6 secreted from TANs stimulate T-cell activation, and CXCL9 or CXCL10 produced by TANs facilitates recruitment of T cells. This positive feedback regulation promotes T-cell proliferation/activation, which results in tumor inhibition. In advanced lung cancer, TANs produce G-MDSC, MPO, Fas/FasL, PD-L1 and TNF-α, which inhibit CD4$^+$ and CD8$^+$ T cells, leading to tumor progression. TANs, tumor-associated neutrophils; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; CXCL, C-X-C motif chemokine ligand; G-MDSC, granulocyte myeloid inhibitory cells; MPO, myeloperoxidase; FasL, Fas ligand; PD-L1, programmed death-ligand 1; TNF-α, tumor necrosis factor-α.

9. TAN inhibition of lung cancer

Contrary to the aforementioned tumor-promoting effect of TANs, a number of studies using animal tumor models have reported the antitumor and anti-metastatic functions of neutrophils. A subset of TANs exhibit antitumor presenting cell (APC) characteristics in early stage human lung cancer (103). IFN-γ and GM-CSF partially promote the APC characteristics of immature neutrophils by downregulation of the transcription factor Ikaros (104). TANs directly inhibit tumor cell proliferation through antibody-dependent cell-mediated cytotoxicity. The antibodies recognize tumors through the Fc receptor on the surface of TANs, releasing cytotoxic mediators, which results in cytotoxicity and the killing of tumor cells. TANs also directly produce cytotoxic mediators such as ROS and MPO, and NADP oxidase, to eliminate tumor cells. TANs have also been reported to release TNF-related apoptosis-inducing ligand, which results in the apoptosis of tumor cells (105-107). Previous studies have reported that type I IFN can change the phenotype of TANs to antitumor characteristics and prolong the life span of neutrophils in both mice and humans. In the absence of IFN-β, TANs exhibit pro-tumor characteristics, such as the low expression of NETs, decreased tumor cytotoxicity, and the low expression of ICAM-1 and TNF-α. In both a mouse melanoma model and patients with melanoma, IFN-β has been reported to induce the polarization of N1 neutrophils towards the antitumor type N1 TAN (41). IFN-β can inhibit the expression of angiogenic factors such as VEGF and MMP-9 in neutrophils during tumor invasion (108). In the LLC lung cancer model, the lung metastasis rate of IFFNAR1(-/-) mice with impaired type I interferon signal was reported to be higher than that of the control group. Formation of the pre-metastatic niche and reduced neutrophil toxicity to tumor cells enhanced the metastatic process in IFFNAR1(-/-) mice (109). Furthermore, IFN-β serves a major role in regulating neutrophil production and their longevity in the primary tumor (110,111). The antitumor effect of neutrophils in the tumor microenvironment in solid tumors varies with tumor type, tumor progression stage and treatment type. Although numerous clinical data suggest that neutrophil infiltration leads to poor prognoses in solid tumors, some tumor therapies depend on functioning neutrophils to be effective. Therefore, controlling neutrophil phenotype could be an effective treatment option, even though the factors that mediate neutrophil polarization are still not entirely clear.

10. TANs and the prognosis of lung cancer

Chronic inflammation is not only related to the onset and progression of lung cancer, but also negatively affects the chemotherapeutic response and prognosis of patients. Previous studies reported that the density of tumor-associated CD66b neutrophils in NSCLC was an adverse prognostic factor and a marker of systemic blood inflammation (112). Inflammation was also reported to interfere with the effect of immunotherapy. In patients with NSCLC, low neutrophil absolute value and high lymphocyte and eosinophil counts were reported to be positively correlated with nivolumab therapeutic outcome. Furthermore, higher neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are negatively correlated with overall survival (OS) and progression-free survival (PFS) times, as well as with a poor response to nivolumab in patients with metastatic NSCLC. However, low NLR and PLR are associated with longer PFS times (30,113,114). Furthermore, elevated white blood cell counts are associated with survival in those patients with NSCLC suitable for resection. In a study involving patients with early stage (stage I to III) NSCLC, high CD66b neutrophil density was reported to have a small effect on OS, but was associated with the incidence of recurrence after surgical resection (32). CD66b positive neutrophils were reported to be elevated in 50% of NSCLCs and the increase in CD66b-positive cells was associated with a higher cumulative incidence of relapse (CIR) (median CIR, 51 months in patients with low CD66b-positive cell density vs. 36 months in patients with high CD66b-positive cell density) and tended to have worse OS time (median OS, 57 months for patients with low CD66b-positive cell density vs. 54 months for high CD66b-positive cell density group). Furthermore, Rakaee *et al* (115) reported the relative subtype-specific prognostic significance of TANs in patients with early NSCLC; the presence of CD66b TANs in squamous cell carcinoma was described as a positive prognostic factor, whereas in adenocarcinoma it was reported as a negative prognostic factor. However, a different study reported that high neutrophil counts were associated with a poor prognosis in patients with squamous cell carcinoma, but not adenocarcinoma (26). Moreover, high neutrophil counts were reported to be associated with a poor prognosis in patients with NSCLC who were treated with immune checkpoint inhibitors (116).

11. Limitations of TAN research and TANs as a potential therapeutic target in lung cancer

Neutrophils have recently emerged as an important factor in the occurrence and development of lung cancer. However, a number of the current studies on the role of neutrophils in lung cancer are based on animal experiments and most of the clinical data reported were obtained from the separation of neutrophils from peripheral blood. Data on the phenotype and function of TANs in patients with lung cancer remain limited and mostly come from patients with early disease. The potential of TANs as a therapeutic target for cancer still requires further study. The change in the phenotype and function of TANs should be one of key research topics in lung cancer, which could lead to the development of a new immunotherapeutic strategy. As TGF-β induces the N2 phenotype and inhibits the N1 antitumor phenotype of TANs, blocking TGF-β could be a potential therapeutic approach. Furthermore, neutralization of chemokines to impair recruitment of neutrophils to the tumor could be another effective therapeutic strategy. For instance, blockage of the CXCL-8/CXCR-1/CXCR-2 axis using neutralizing antibodies could inhibit the function of N2 TANs. Therefore, further studies are required to gain a detailed understanding of TANs and lung cancer, and to evaluate the modulation of TANs as a novel antitumor therapy in lung cancer.

12. Conclusions

There is increasing evidence demonstrating that TANs infiltrate lung cancer tissue. TANs exhibit plasticity between the antitumor N1 and pro-tumor N2 phenotypes, which is determined by the levels of related signaling factors in the tumor microenvironment. The present review evaluated the role of TANs in the onset and progression of lung cancer and the underlying mechanisms, including the direct and indirect effect of TANs on tumor cells, with emphasis on the role of TANs in lung cancer progression. The literature strongly indicated that TANs are crucial factors in lung cancer and could be a new immunotherapeutic target in this disease.

Availability of data and materials

Not applicable.

Authors' contributions

JZ wrote the manuscript and reviewed the literature. HL assisted in collection and review of the literature. WW and SJ provided guidance, and revised and corrected the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Rosen R and Karachaliou N: Large-scale screening for somatic mutations in lung cancer. Lancet 387: 1354-1356, 2016.
3. Liu L, Wang X, Ji Z, Wang J, Bi N, Hui Z, Lyu J, Liang J, Zhou Z, Feng Q, et al: Outcome of concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer patients. Zhonghua Zhong Liu Za Zhi 37: 863-867, 2015 (In Chinese).
4. Moro-Sibilot D, Smit E, de Castro Carpeño J, Lesniewski-Kmak K, Aerts J, Villatoro R, Kraaij K, Nacerddine K, Dyachkova Y, Smith KT, et al: Outcomes and resource use of non-small cell lung cancer (NSCLC) patients treated with first-line platinum-based chemotherapy across Europe: FRAME prospective observational study. Lung Cancer 88: 215-222, 2015.
5. Lu H and Jiang Z: Advances in antibody therapeutics targeting small-cell lung cancer. Adv Clin Exp Med 27: 1317-1323, 2018.
6. Bielenberg DR and Zetter BR: The contribution of angiogenesis to the process of metastasis. Cancer J 21: 267-273, 2015.
7. Nemeth JA, Cher ML, Zhou Z, Mullins C, Bhagat S and Trikha M: Inhibition of alpha(v)beta3 integrin reduces angiogenesis, bone turnover, and tumor cell proliferation in experimental prostate cancer bone metastases. Clin Exp Metastasis 20: 413-420, 2003.
8. Liu Y and Cao X: Characteristics and significance of the pre-metastatic niche. Cancer Cell 30: 668-681, 2016.
9. Quail DF and Joyce JA: Microenvironmental regulation of tumor progression and metastasis. Nat Med 19: 1423-1437, 2013.
10. Sceneay J, Smyth MJ and Möller A: The pre-metastatic niche: Finding common ground. Cancer Metastasis Rev 32: 449-464, 2013.
11. Shoenfeld Y, Tal A, Berliner S and Pinkhas J: Leukocytosis in non hematological malignancies-a possible tumor-associated marker. J Cancer Res Clin Oncol 111: 54-58, 1986.
12. Tecchio C, Scapini P, Pizzolo G and Cassatella MA: On the cytokines produced by human neutrophils in tumors. Semin Cancer Biol 23: 159-170, 2013.
13. Houghton AM: The paradox of tumor-associated neutrophils. Cell Cycle 9: 1732-1737, 2010.
14. Gregory A and Houghton AM: Tumor-associated neutrophils: New targets for cancer therapy. Cancer Research 71: 2411-2416, 2011.
15. Kim J and Bae JS: Tumor-Associated macrophages and neutrophils in tumor microenvironment. Mediators Inflamm 2016: 6058147, 2016.
16. Borregaard N: Neutrophils, from marrow to microbes. Immunity 33: 657-670, 2010.
17. Cortez-Retamozo V, Etzrodt M, Newton A, Rauch PJ, Chudnovskiy A, Berger C, Ryan RJ, Iwamoto Y, Marinelli B, Gorbatov R, et al: Origins of tumor associated macrophages and neutrophils. Proc Natl Acad Sci USA 109: 2491-2496, 2012.
18. Stöth M, Freire Valls A, Chen M, Hidding S, Knipper K, Shen Y, Klose J, Ulrich A, Ruiz de Almodovar C, Schneider M and Schmidt T: Splenectomy reduces lung metastases and tumoral and metastatic niche inflammation. Int J Cancer 145: 2509-2520, 2019.
19. Eruslanov EB, Bhojnagarwala PS, Quatromoni JG, Stephen TL, Ranganathan A, Deshpande C, Akimova T, Vachani A, Litzky L, Hancock WW, et al: Tumor-associated neutrophils stimulate T cell responses in early-stage human lung cancer. J Clin Invest 124: 5466-5480, 2014.
20. Piccard H, Muschel RJ and Opdenakker G: On the dual roles and polarized phenotypes of neutrophils in tumor development and progression. Crit Rev Oncol Hematol 82: 296-309, 2012.
21. Sionov RV, Fridlender ZG and Granot Z: The multifaceted roles neutrophils play in the tumor microenvironment. Cancer Microenviron 8: 125-158, 2015.
22. Coffelt SB, Wellenstein MD and de Visser KE: Neutrophils in cancer: Neutral no more. Nat Rev Cancer 16: 443-446, 2016.
23. Houghton AM: The paradox of tumor-associated neutrophils: Fueling tumor growth with cytotoxic substances. Cell Cycle 9: 1732-1737, 2010.
24. Shen M, Hu P, Donskov F, Wang G, Liu Q and Du J: Tumorassociated neutrophils as a new prognostic factor in cancer: A systematic review and meta-analysis. PLoS One 9: e98259, 2014.
25. Uribe-Querol E and Rosales C: Neutrophils in cancer: Two sides of the same coin. J Immunol Res 2015: 983698, 2015.
26. Liu X, Wu S, Yang Y, Zhao M, Zhu G and Hou Z: The prognostic landscape of tumor-infiltrating immune cell and immunomodulators in lung cancer. Biomed Pharmacother 95: 55-61, 2017.
27. Shaul ME, Eyal O, Guglietta S, Aloni P, Zlotnik A, Forkosh E, Levy L, Weber LM, Levin Y, Pomerantz A, et al: Circulating neutrophil subsets in advanced lung cancer patients exhibit unique immune signature and relate to prognosis. FASEB J 34: 4204-4218, 2020.
28. Chen Y, Chen H, Mao B, Zhou Y, Shi X, Tang L, Jiang H, Wang G and Zhuang W: Transcriptional characterization of the tumor immune microenvironment and its prognostic value for locally advanced lung adenocarcinoma in a Chinese population. Cancer Manag Res 11: 9165-9173, 2019.
29. Sebastian NT, Raj R, Prasad R, Barney C, Brownstein J, Grecula J, Haglund K, Xu-Welliver M, Williams TM and Bazan JG: Association of Pre- and Posttreatment neutrophil-lymphocyte ratio with recurrence and mortality in locally advanced non-small cell lung cancer. Front Oncol 10: 598873, 2020.
30. Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, Templeton AJ and Früh M: Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Lung Cancer 111: 176-181, 2017.
31. Zhao K, Wang C, Shi F, Huang Y, Ma L, Li M and Song Y: Combined prognostic value of the SUVmax derived from FDG-PET and the lymphocyte-monocyte ratio in patients with stage IIIB-IV non-small cell lung cancer receiving chemotherapy. BMC Cancer 21: 66, 2021.
32. Ilie M, Hofman V, Ortholan C, Bonnetaud C, Coëlle C, Mouroux J and Hofman P: Predictive clinical outcome of the intratumoral CD66b-positive neutrophil-to-CD8-positive T-cell ratio in patients with resectable nonsmall cell lung cancer. Cancer 118: 1726-1737, 2012.
33. Fridlender ZG and Albelda SM: Tumor-associated neutrophils: Friend or foe? Carcinogenesis 33: 949-955, 2012.
34. Dumitru CA, Lang S and Brandau S: Modulation of neutrophil granulocytes in the tumor microenvironment: Mechanisms and consequences for tumor progression. Semin Cancer Biol 23: 141-148, 2013.
35. Abe H, Morikawa T, Saito R, Yamashita H, Seto Y and Fukayama M: In Epstein-Barr virus-associated gastric carcinoma a high density of CD66b-positive tumor-associated neutrophils is associated with intestinal-type histology and low frequency of lymph node metastasis. Virchows Arch 468: 539-548, 2016.
36. López-Lago MA, Posner S, Thodima VJ, Molina AM, Motzer RJ and Chaganti RS: Neutrophil chemokines secreted by tumor cells mount a lung antimetastatic response during renal cell carcinoma progression. Oncogene 32: 1752-1760, 2013.
37. Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, Stolz DB, Land SR, Marconcini LA, Kliment CR, et al: Neutrophil Elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med 16: 219-223, 2010.
38. Deryugina EI, Zajac E, Juncker-Jensen A, Kupriyanova TA, Welter L and Quigley JP: Tissue-infiltrating neutrophils constitute the major in vivo source of angiogenesis-inducing MMP-9 in the tumor microenvironment. Neoplasia 16: 771-788, 2014.
39. Deryugina EI and Quigley JP: Tumor angiogenesis: MMP-mediated induction of intravasation- and metastasis-sustaining neovasculature. Matrix Biol 44-46: 94-112, 2015.
40. Grunwald B, Vandooren J, Locatelli E, Fiten P, Opdenakker G, Proost P, Krüger A, Lellouche JP, Israel LL, Shenkman L and Comes Franchini M: Matrix metalloproteinase-9 (MMP-9) as an activator of nanosystems for targeted drug delivery in pancreatic cancer. J Control Release 239: 39-48, 2016.
41. Andzinski L, Kasnitz N, Stahnke S, Wu CF, Gereke M, von Köckritz-Blickwede M, Schilling B, Brandau S, Weiss S and Jablonska J: Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. Int J Cancer 138: 1982-1993, 2016.
42. Wang N, Wang Q, Chi J, Xiang F, Lin M, Wang W, Wei F and Feng Y: Carcinoembryonic antigen cell adhesion molecule 1 inhibits the antitumor effect of neutrophils in tongue squamous cell carcinoma. Cancer Sci 110: 519-529, 2019.
43. Krstic J and Santibanez JF: Transforming growth factor-beta and matrix metalloproteinases: Functional interactions in tumor Stroma-infiltrating myeloid cells. ScientificWorldJournal 2014: 521754, 2014.
44. Kim Y, Lee D, Lee J, Lee S and Lawler S: Role of tumor-associated neutrophils in regulation of tumor growth in lung cancer development: A mathematical model. PLoS One 14: e0211041, 2019.

45. Shaul ME, Levy L, Sun J, Mishalian I, Singhal S, Kapoor V, Horng W, Fridlender G, Albelda SM and Fridlender ZG: Tumor-associated neutrophils display a distinct N1 profile following TGF-β modulation: A transcriptomics analysis of pro-vs. Antitumor TANs. Oncoimmunology 5: e1232221, 2016.

46. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS and Albelda SM: Polarization of tumor-associated neutrophil phenotype by TGF-beta: 'N1' versus 'N2' TAN. Cancer Cell 16: 183-194, 2009.

47. Saha S and Biswas SK: Tumor-Associated neutrophils show phenotypic and functional divergence in human lung cancer. Cancer Cell 30: 11-13, 2016.

48. Zhang J, Qiao X, Shi H, Han X, Liu W, Tian X and Zeng X: Circulating tumor-associated neutrophils (cTAN) contribute to circulating tumor cell survival by suppressing peripheral leukocyte activation. Tumour Biol 37: 5397-5404, 2016.

49. Mishalian I, Bayuh R, Levy L, Zolotarov L, Michaeli J and Fridlender ZG: Tumor-associated neutrophils (TAN) develop pro-tumorigenic properties during tumor progression. Cancer Immunol Immunother 62: 1745-1756, 2013.

50. McDonald B, Urrutia R, Yipp BG, Jenne CN and Kubes P: Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. Cell Host Microbe 12: 324-333, 2012.

51. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, Brinkmann V, Jungblut PR and Zychlinsky A: Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog 5: e1000639, 2009.

52. Spicer JD, McDonald B, Cools-Lartigue JJ, Chow SC, Giannias B, Kubes P and Ferri LE: Neutrophils promote liver metastasis via Mac-1-mediated interactions with circulating tumor cells. Cancer Res 72: 3919-3927, 2012.

53. Rayes RF, Mouhanna JG, Nicolau I, Bourdeau F, Giannias B, Rousseau S, Quail D, Walsh L, Sangwan V, Bertos N, *et al*: Primary tumors induce neutrophil extracellular traps with targetable metastasis promoting effects. JCI Insight 5: e128008, 2019.

54. Erpenbeck L and Schön MP: Neutrophil extracellular traps: Protagonists of cancer progression? Oncogene 36: 2483-2490, 2017.

55. Richardson JJR, Hendrickse C, Gao-Smith F and Thickett DR: Neutrophil extracellular trap production in patients with colorectal cancer in vitro. Int J Inflam 2017: 4915062, 2017.

56. Jin W, Xu HX, Zhang SR, Li H, Wang WQ, Gao HL, Wu CT, Xu JZ, Qi ZH, Li S, *et al*: Tumor-Infiltrating nets predict postsurgical survival in patients with pancreatic ductal adenocarcinoma. Ann Surg Oncol 26: 635-643, 2019.

57. Thålin C, Lundström S, Seignez C, Daleskog M, Lundström A, Henriksson P, Helleday T, Phillipson M, Wallén H and Demers M: Citrullinated histone H3 as a novel prognostic blood marker in patients with advanced cancer. PLoS One 13: e0191231, 2018.

58. Zhou J, Yang Y, Gan T, Li Y, Hu F, Hao N, Yuan B, Chen Y and Zhang M: lung cancer cells release high mobility group box 1 and promote the formation of neutrophil extracellular traps. Oncol Lett 18: 181-188, 2019.

59. Najmeh S, Cools-Lartigue J, Giannias B, Spicer J and Ferri LE: Simplified human neutrophil extracellular traps (NETs) isolation and handling. J Vis Exp: 52687, 2015 doi: 10.3791/52687.

60. Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, Giannias B, Bourdeau F, Kubes P and Ferri L: Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. J Clin Invest 123: 3446-3458, 2013.

61. Monti M, De Rosa V, Iommelli F, Carriero MV, Terlizzi C, Camerlingo R, Belli S, Fonti R, Di Minno G and Del Vecchio S: Neutrophil extracellular traps as an adhesion substrate for difffferent tumor cells expressing RGD-binding integrins. Int J Mol Sci 19: 2350, 2018.

62. Kanamaru R, Ohzawa H, Miyato H, Yamaguchi H, Hosoya Y, Lefor AK, Sata N and Kitayama J: Neutrophil extracellular traps generated by low density neutrophils obtained from peritoneal lavage fluid mediate tumor cell growth and attachment. J Vis Exp 138: 58201, 2018.

63. Yang L, Liu L, Zhang R, Hong J, Wang Y, Wang J, Zuo J, Zhang J, Chen J and Hao H: IL-8 mediates a positive loop connecting increased neutrophil extracellular traps (Nets) and colorectal cancer liver metastasis. J Cancer 11: 4384-4396, 2020.

64. Yang LY, Luo Q, Lu L, Zhu WW, Sun HT, Wei R, Lin ZF, Wang XY, Wang CQ, Lu M, *et al*: Increased neutrophil extracellular traps promote metastasis potential of hepatocellular carcinoma via provoking tumorous inflammatory response. J Hematol Oncol 13: 3, 2020.

65. Kern MA, Haugg AM, Koch AF, Schilling T, Breuhahn K, Walczak H, Fleischer B, Trautwein C, Michalski C, Schulze-Bergkamen H, *et al*: yclooxygenase-2 inhibition induces apoptosis signaling via death receptors and mitochondria in hepatocellular carcinoma. Cancer Res 66: 7059-7066, 2006.

66. Leng J, Han C, Demetris AJ, Michalopoulos GK and Wu T: Cyclooxygenase-2 promotes hepatocellular carcinoma cell growth through Akt activation: Evidence for Akt inhibition in celecoxib-induced apoptosis. Hepatology 38: 756-768, 2003.

67. Xu L, Stevens J, Hilton MB, Seaman S, Conrads TP, Veenstra TD, Logsdon D, Morris H, Swing DA, Patel NL, *et al*: COX-2 inhibition potentiates antiangiogenic cancer therapy and prevents metastasis in preclinical models. Sci Transl Med 6: 242ra84, 2014.

68. Takesue S, Ohuchida K, Shinkawa T, Otsubo Y, Matsumoto S, Sagara A, Yonenaga A, Ando Y, Kibe S, Nakayama H, *et al*: Neutrophil extracellular traps promote liver micrometastasis in pancreatic ductal adenocarcinoma via the activation of cancer-associated fibroblasts. Int J Oncol 56: 596-605, 2020.

69. Xiao Y, Cong M, Li J, He D, Wu Q, Tian P, Wang Y, Yang S, Liang C, Liang Y, *et al*: Cathepsin C promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. Cancer Cell 39: 423-437.e7, 2020.

70. Yuan M, Zhu H, Xu J, Zheng Y, Cao X and Liu Q: Tumor-Derived CXCL1 promotes lung cancer growth via recruitment of tumor-associated neutrophils. J Immunol Res 2016: 6530410, 2016.

71. Eash KJ, Greenbaum AM, Gopalan PK and Link DC: CXCR2 and CXCR4 antagonistically regulate neutrophil traffificking from murine bone marrow. J Clin Investig 120: 2423-2431, 2010.

72. Raccosta L, Fontana R, Maggioni D, Lanterna C, Villablanca EJ, Paniccia A, Musumeci A, Chiricozzi E, Trincavelli ML, Daniele S, *et al*: The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils. J Exp Med 210: 1711-1728, 2013.

73. Forsthuber A, Lipp K, Andersen L, Ebersberger S, Graña-Castro, Ellmeier W, Petzelbauer P, Lichtenberger BM and Loewe R: CXCL5 as regulator of neutrophil function in cutaneous melanoma. J Invest Dermatol 139: 186-194, 2019.

74. Viola A, Sarukhan A, Bronte V and Molon B: The pros and cons of chemokines in tumor immunology. Trends Immunol 33: 496-504, 2012.

75. Huang SY, Mills L, Mian B, Tellez C, McCarty M, Yang XD, Gudas JM and Bar-Eli M: Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. Am J Pathol 161: 125-134, 2002.

76. Luca M, Huang S, Gershenwald JE, Singh RK, Reich R and Bar-Eli M: Expression of interleukin-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis. Am J Pathol 151: 1105-1113, 1997.

77. Pignatti P, Moscato G, Casarini S, Delmastro M, Poppa M, Brunetti G, Pisati P and Balbi B: Downmodulation of CXCL8/IL-8 receptors on neutrophils after recruitment in the airways. J Allergy Clin Immunol 115: 88-94, 2005.

78. Fortunati E, Kazemier KM, Grutters JC, Koenderman L and Van den Bosch VJ: Human neutrophils switch to an activated phenotype after homing to the lung irrespective of inflammatory disease. Clin Exp Immunol 155: 559-566, 2009.

79. Tazzyman S, Niaz H and Murdoch C: Neutrophil-mediated tumour angiogenesis: Subversion of immune responses to promote tumour growth. Semin Cancer Biol 23: 149-158, 2013.

80. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, Cosmi L, Lunardi C, Annunziato F, Romagnani S and Cassatella MA: Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 115: 335-343, 2010.

81. Jungnickel C, Schmidt LH, Bittigkoffer L, Wolf L, Wolf A, Ritzmann F, Kamyschnikow A, Herr C, Menger MD, Spieker T, *et al*: IL-17C mediates the recruitment of tumor-associated neutrophils and lung tumor growth. Oncogene 36: 4182-4190, 2017.

82. Shojaei F, Singh M, Thompson JD and Ferrara N: Role of Bv8 in neutrophildependent angiogenesis in a transgenic model of cancer progression. Proc Natl Acad Sci 105: 2640-2645, 2008.

83. Tazawa H, Okada F, Kobayashi T, Tada M, Mori Y, Une Y, Sendo F, Kobayashi M and Hosokawa M: Infiltration of neutrophils is required for acquisition of metastatic phenotype of benign murine fibrosarcoma cells: Implication of inflammation-associated carcinogenesis and tumor progression. Am J Pathol 163: 2221-2232, 2003.

84. Vannitamby A, Seow HJ, Anderson G, Vlahos R, Thompson M, Steinfort D, Irving LB and Bozinovski S: Tumour-associated neutrophils and loss of epithelial PTEN can promote corticosteroid-insensitive MMP-9 expression in the chronically inflamed lung microenvironment. Thorax 72: 1140-1143, 2017.

85. Coussens LM, Tinkle CL, Hanahan D and Werb Z: MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 103: 481-490, 2000.

86. Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, Blanchard D, Bais C, Peale FV, van Bruggen N, et al: Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature 450: 825-831, 2007.

87. Qu X, Zhuang G, Yu L, Meng G and Ferrara N: Induction of Bv8 expression by granulocyte colony-stimulating factor in CD11b+Gr1+ cells: Key role of Stat3 signaling. J Biol Chem 287: 19574-19584, 2012.

88. Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, Yu L, Ross J, Korsisaari N, Cao T, et al: Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. Proc Natl Acad Sci USA 107: 21248-21255, 2010.

89. Kalluri R and Weinberg RA: The basics of epithelial-mesenchymal transition. J Clin Invest 119: 1420-1428, 2009.

90. Xu J, Lamouille S and Derynck R: TGF-beta-induced epithelial to mesenchymal transition. Cell Res 19: 156-172, 2009.

91. Hu P, Shen M, Zhang P, Zheng C, Pang Z, Zhu L and Du J: Intratumoral neutrophil granulocytes contribute to epithelial-mesenchymal transition in lung adenocarcinoma cells. Tumor Boil 36: 7789-7796, 2015.

92. Tsai YM, Wu KL, Liu YW, Chang AW, Huang YC, Chang CY, Tsai PH, Liao SH, Hung JY and Hsu YL: Cooperation between cancer and fibroblasts in vascular mimicry and N2-type neutrophil recruitment via Notch2-Jagged1 interaction in lung cancer. Front Oncol 11: 696931, 2021.

93. Patel S, Fu S, Mastio J, Dominguez GA, Purohit A, Kossenkov A, Lin C, Alicea-Torres K, Sehgal M, Nefedova Y, et al: Unique pattern of neutrophil migration and function during tumor progression. Nat Immunol 19: 1236-1247, 2018.

94. Shao B, Zhao X, Liu T, Zhang Y, Sun R, Dong X, Liu F, Zhao N, Zhang D, Wu L, et al: LOXL2 promotes vasculogenic mimicry and tumour aggressiveness in hepatocellular carcinoma. J Cell Mol Med 23: 1363-1374, 2019.

95. Mayer C, Darb-Esfahani S, Meyer AS, Hübner K, Rom J, Sohn C, Braicu I, Sehouli J, Hänsch GM and Gaida MM: Neutrophil granulocytes in ovarian cancer-induction of epithelial-to-mesenchymal-transition and tumor cell migration. J Cancer 7: 546-554, 2016.

96. Michaeli J, Shaul ME, Mishalian I, Hovav AH, Levy L, Zolotriov L, Granot Z and Fridlender ZG: Tumor-associated neutrophils induce apoptosis of non-activated CD8 T-cells in a TNFα and NO-dependent mechanism, promoting a tumor-supportive environment. Oncoimmunology 6: e1356965, 2017.

97. Singhal S, Bhojnagarwala PS, O'Brien S, Moon EK, Garfall AL, Rao AS, Quatromoni JG, Stephen TL, Litzky L, Deshpande C, et al: Origin and role of a subset of tumor-associated neutrophils with antigen-presenting cell features in early-stage human lung cancer. Cancer Cell 30: 120-135, 2016.

98. Shaul ME and Fridlender ZG: Tumour-associated neutrophils in patients with cancer. Nat Rev Clin Oncol 16: 601-620, 2019.

99. Sica A, Porta C, Morlacchi S, Banfi S, Strauss L, Rimoldi M, Totaro MG and Riboldi E: Origin and functions of tumor-associated myeloid cells (TAMCs). Cancer Microenviron 5: 133-149, 2012.

100. Lee WL and Downey GP: Leukocyte elastase: Physiological functions and role in acute lung injury. Am J Respir Crit Care Med 164: 896-904, 2001.

101. Wislez M, Antoine M, Rabbe N, Gounant V, Poulot V, Lavolé A, Fleury-Feith J and Cadranel J: Neutrophils promote aerogenous spread of lung adenocarcinoma with bronchiolo alveolar carcinoma features. Clin Cancer Res 13: 3518-3527, 2007.

102. Gong L, Cumpian AM, Caetano MS, Ochoa CE, De la Garza MM, Lapid DJ, Mirabolfathinejad SG, Dickey BF, Zhou Q and Moghaddam SJ: Promoting effect of neutrophils on lung tumorigenesis is mediated by CXCR2 and neutrophil elastase. Mol Cancer 12: 154, 2013.

103. Liang W, Li Q and Ferrara N: Metastatic growth instructed by neutrophil-derived transferrin. Proc Natl Acad Sci USA 115: 11060-11065, 2018.

104. O'Brien S, Thomas RM, Wertheim GB, Zhang F, Shen H and Wells AD: Ikaros imposes a barrier to CD8+ T cell differentiation by restricting autocrine IL-2 production. J Immunol 11: 5118-5129, 2014.

105. Wang W, Erbe AK, Hank JA, Morris ZS and Sondel PM: NK cell-mediated antibody dependent cellular cytotoxicity in cancer immunotherapy. Front Immunol 6: 368, 2015.

106. Amarante-Mendes GP and Gfiffith TS: Therapeutic applications of TRAIL receptor agonists in cancer and beyond. Pharmacol Ther 155: 117-131, 2015.

107. Granot Z, Henke E, Comen EA, King TA, Norton L and Benezra R: Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell 20: 300-314, 2011.

108. Jablonska S, Leschner K and Westphal S: Neutrophils responsive to endogenous IFN-β regulate tumor angiogenesis and growth in a mouse tumor model. J Clin Invest 120: 1151-1164, 2010.

109. Wu CF, Andzinski L, Kasnitz N, Kröger A, Klawonn F, Lienenklaus S, Weiss S and Jablonska J: The lack of type I interferon induces neutrophil-mediated pre-metastatic niche formation in the mouse lung. Int J Cancer 137: 837-847, 2015.

110. Andzinski L, Wu CF, Lienenklaus S, Kröger A, Weiss S and Jablonska J: Delayed apoptosis of tumor associated neutrophils in the absence of endogenous IFN-β. Int J Cancer 136: 572-583, 2015.

111. Jablonska J, Wu CF, Andzinski L, Leschner S and Weiss S: CXCR2-mediated tumor associated neutrophil recruitment is regulated by IFN-beta. Int J Cancer 134: 1346-1358, 2014.

112. Carus A, Ladekarl M, Hager H, Pilegaard H, Nielsen PS and Donskov F: Tumor-associated neutrophils and macrophages in non-small cell lung cancer: No immediate impact on patient outcome. Lung Cancer 81: 130-137, 2013.

113. Tanizaki J, Haratani K, Hayashi H, Chiba Y, Nakamura Y, Yonesaka K, Kudo K, Kaneda H, Hasegawa Y, Tanaka K, et al: Peripheral blood biomarkers associated with clinical outcome in non-small cell lung cancer patients treated with nivolumab. J Thorac Oncol 13: 97-105, 2018.

114. Taniguchi Y, Tamiya A, Isa SI, Nakahama K, Okishio K, Shiroyama T, Suzuki H, Inoue T, Tamiya M, Hirashima T, et al: Predictive factors for poor progression-free survival in patients with non-small cell lung cancer treated with nivolumab. Anticancer Res 37: 857-5862, 2017.

115. Rakaee M, Busund LT, Paulsen EE, Richardsen E, Al-Saad S, Andersen S, Donnem T, Bremnes RM and Kilvaer TK: Prognostic effect of intratumoral neutrophils across histological subtypes of non-small cell lung cancer. Oncotarget 7: 72184-72196, 2016.

116. Kubo S, Kobayashi N, Somekawa K, Hirata M, Kamimaki C, Aiko H, Katakura S, Teranishi S, Watanabe K, Hara YU, et al: Identification of biomarkers for non-small-cell lung cancer patients treated with an immune checkpoint inhibitor. Anticancer Res 40: 3889-3896, 2020.