SPANDIDOS PUBLICATIONS

Dual roles of neutrophil extracellular traps in tumors: From pro-metastatic mechanisms to immunotherapeutic strategies (Review)

MINGXIN GUO[1*], HAN WANG[2*], JIAQI ZENG[1], NING WU[1],
ZHIQIANG HU[1], AO XUE[3] and TING DAI[1]

[1]Department of Pharmacy, The Affiliated Yixing Hospital of Jiangsu University, Yixing, Jiangsu 214200, P.R. China;
[2]Graduate School, The Second Affiliated Hospital of Hainan Medical University, Haikou, Hainan 570311, P.R. China;
[3]Jiangsu CM Clinical Innovation Center of Degenerative Bone and Joint Disease,
Wuxi Affiliated Hospital of Nanjing University of Chinese Medicine, Wuxi, Jiangsu 214071, P.R. China

Received October 9, 2025; Accepted April 2, 2026

DOI: 10.3892/ol.2026.15614

Abstract. Neutrophil extracellular traps (NETs) serve a dual role in tumor progression. On the one hand, they promote tumor invasion and metastasis by facilitating cancer cell adhesion to the endothelium, supporting vascular remodeling and establishing pre-metastatic niches. On the other hand, NETs exert potent immunosuppressive effects that foster tumor immune evasion and contribute to immunotherapy resistance. Specifically, NETs create physical barriers within the tumor microenvironment, directly suppress effector immune cells, and degrade critical molecules such as interferon-γ, leading to immune exhaustion and dysfunction. The present review comprehensively summarizes the biology of NETs and their molecular mechanisms in tumor metastasis, with a particular focus on NET-mediated immunosuppression and its causal link to immunotherapy resistance. This review further discusses emerging strategies that disrupt NET formation or function, which may reverse their immunosuppressive impact and restore antitumor immunity, thereby enhancing the efficacy of current immunotherapies.

Contents

1. Introduction

As the most abundant leukocyte population in the innate immune system, neutrophils orchestrate host antimicrobial defense through phagocytic elimination of invading pathogens, targeted microbial destruction via proteolytic enzyme degranulation and strategic modulation of inflammatory cascades through coordinated chemokine/cytokine signaling (1,2). Notably, neutrophils can deploy neutrophil extracellular traps (NETs), which are chromatin scaffolds decorated with cytotoxic proteins and proteases to immobilize pathogens through physical containment and biochemical neutralization. Emerging evidence demonstrates that NETs actively remodel the tumor microenvironment (TME) by facilitating oncogenic transformation, promoting metastatic dissemination and subverting antitumor immunity through coordinated molecular cascades (3).

While NETs may induce cancer cell apoptosis in specific contexts, the prevalence of experimental evidence establishes their critical pro-tumorigenic effects in accelerating local tumor progression, facilitating distant metastasis and conferring therapeutic resistance through matrix remodeling and immunosuppressive niche formation (4). Neutrophils drive oncogenesis through multifaceted mechanisms, inducing genomic instability via sustained release of reactive oxygen species (ROS) and proteolytic enzymes, while directly stimulating cancer cell proliferation through paracrine growth factor signaling. Furthermore, tumor-associated neutrophils (TANs) establish immunosuppressive networks by suppressing

---

Correspondence to: Dr Ao Xue, Jiangsu CM Clinical Innovation Center of Degenerative Bone and Joint Disease, Wuxi Affiliated Hospital of Nanjing University of Chinese Medicine, 8 Zhongnan West Road, Binhu, Wuxi, Jiangsu 214071, P.R. China
E-mail: aoxue15@163.com

Professor Ting Dai, Department of Pharmacy, The Affiliated Yixing Hospital of Jiangsu University, 1588 Xincheng Road, Yixing, Jiangsu 214200, P.R. China
E-mail: staff1582@yxph.com

[*]Contributed equally

Key words: neutrophil extracellular traps, neoplasm metastasis, immunity, immunotherapy

cytotoxic T cell activity through programmed cell death ligand 1 (PD-L1) expression and arginase-1 (ARG1)-mediated nutrient depletion, thereby orchestrating tumor immune evasion (5). This functional duality stems from neutrophil heterogeneity wherein innate immune cells dynamically adapt to microenvironmental cues to exhibit distinct phenotypic and functional states. Their intrinsic plasticity not only drives NET formation, but also enables multifaceted tumor interactions through diverse molecular modalities, ultimately dictating context-dependent pro- or antitumor outcomes (6). The present article presents a systematic review of the biogenic mechanisms underlying NET formation and their regulatory roles in the metastatic cascade of tumors. Building on current research, the present study explores targeted intervention strategies, offering a theoretical framework and potential pathways for translational research aimed at enhancing tumor immunotherapy responses through the regulation of NETs.

2. Literature search strategy

To ensure a comprehensive and unbiased coverage of the literature on NETs in tumor progression and immunotherapy, a systematic search strategy was implemented.

Search engines and databases. Literature was retrieved from the following databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://www.webofscience.com/) and Embase (https://www.embase.com/)and Cochrane Library (https://www.cochranelibrary.com/).

Search terms. Key terms and Boolean operators were combined to maximize sensitivity and specificity. The core concepts were: 'Neutrophil extracellular traps' or 'NETs' or 'NETosis' and 'tumor' or 'cancer' or 'neoplasm' or 'metastasis' or 'oncology'. The subtopics were: And 'immunotherapy' or 'immune checkpoint inhibitor' or 'tumor microenvironment' or 'pro-metastatic' or 'therapeutic resistance'.

Criteria. The inclusion criteria were: i) Original research articles, reviews and meta-analyses; ii) studies exploring NETs in tumor biology, metastasis or immunotherapy; iii) preclinical and clinical studies; and iv) English-language publications. The exclusion criteria were: i) Non-peer-reviewed articles; ii) studies unrelated to NETs in cancer; and iii) duplicate datasets or overlapping analyses.

3. Introduction to NETs

Basic composition and functions of NETs. In 1966, Takei *et al* (7) first reported that phorbol-12-myristate-13-acetate (PMA; a phorbol ester and protein kinase C activator) induces unique phenotypic changes in neutrophils distinct from classical apoptosis and necrosis, and this observation is now recognized as an early precursor to the formal characterization of NETs decades later. In 2004, Brinkmann *et al* (8) reported the discovery of NETs and extracellular chromatin-protein complexes that exhibit antimicrobial activity through pathogen entrapment and elimination. In this process, the activated neutrophil nuclear membrane dissolves, followed by disintegration of the plasma membrane, release of nuclear contents, decondensation of chromatin and release of granulin-loaded chromatin into the extracellular space (9). The molecular mechanisms underlying NET formation remain unclear. The primary mechanisms of NET formation are categorized into neutrophil lysis and non-lysis, as shown in Fig. 1.

The release of chromatin by neutrophils was initially found to depend on ROS (10). In the lytic pathway, NADPH oxidase (NOX)-dependent ROS generation initiates sequential membrane disintegration, beginning with nuclear envelope rupture, followed by granule membrane permeabilization. Myeloperoxidase (MPO) and neutrophil elastase (NE) from azurophilic granules subsequently translocate to the nucleus where they cooperatively mediate citrullinated histone H3 (citH3) proteolysis. Concurrently, ROS activate peptidyl arginine deiminase 4 (PAD4), catalyzing histone citrullination through arginine-to-citrulline conversion. This post-translational modification neutralizes histone-DNA electrostatic interactions, culminating in chromatin decondensation and extracellular expulsion of nuclear DNA complexed with granular proteins, thereby forming lytic NETs (11). In patients with chronic granulomatous disease, a defect in the gene encoding NOX impairs the ability of cells to synthesize ROS, thereby preventing the formation of NETs by neutrophils. Additionally, neither NOX inhibitors nor NADPH-deficient mice produce NETs (12). PAD4 overexpression in osteosarcoma cell lines converts intracellular histones into citrullinated histones, inducing chromatin decondensation and release (13). The NOX-independent NETs pathway is involved in mitochondrial ROS generation and calcium-dependent PAD4 activation. Upon stimulation, cytoplasmic PAD4 translocates to the nucleus following calcium ion influx, where H3 is citrullinated. This post-translational modification induces chromatin decondensation through charge neutralization, enabling the co-release of nuclear DNA and granular proteins via vesicular extrusion, thereby forming non-lytic NETs and preserving neutrophil membrane integrity (14).

In non-lytic NETs, neutrophils respond to microbial pathogens or endogenous danger signals through coordinated receptor activation. The engagement of platelet adhesion molecules or interactions between complement receptors/Toll-like receptors (TLRs) and pathogen-associated molecular patterns trigger calcium-dependent PAD4 activation. This enzymatic cascade induces histone citrullination-mediated chromatin decondensation, followed by vesicular extrusion of nuclear DNA-protein complexes via nuclear envelope blebbing, forming intact membrane-encased NETs that preserve cellular viability (15). Currently, immunofluorescence staining and western blotting are commonly used to analyze the expression levels and colocalization of different components of extracellular DNA and NETs in tissues. Alternatively, extracellular DNA content and presence can be confirmed by specifically binding a dye such as SYTOX Green to DNA and measuring fluorescence or using a spectrophotometer.

Neutrophil heterogeneity in NETs. TANs serve dual regulatory roles in tumor progression through distinct subpopulations, exhibiting both anticarcinogenic and carcinogenic functions (16). The accumulation of neutrophils within solid tumors is associated with a poor clinical prognosis, reflecting phenotypic heterogeneity in mediating both anti-metastatic and pro-metastatic activities (17). Notably, CD62L$^+$ neutrophils exhibit enhanced NET formation capacity, a process that is amplified by tumor-secreted high-mobility group

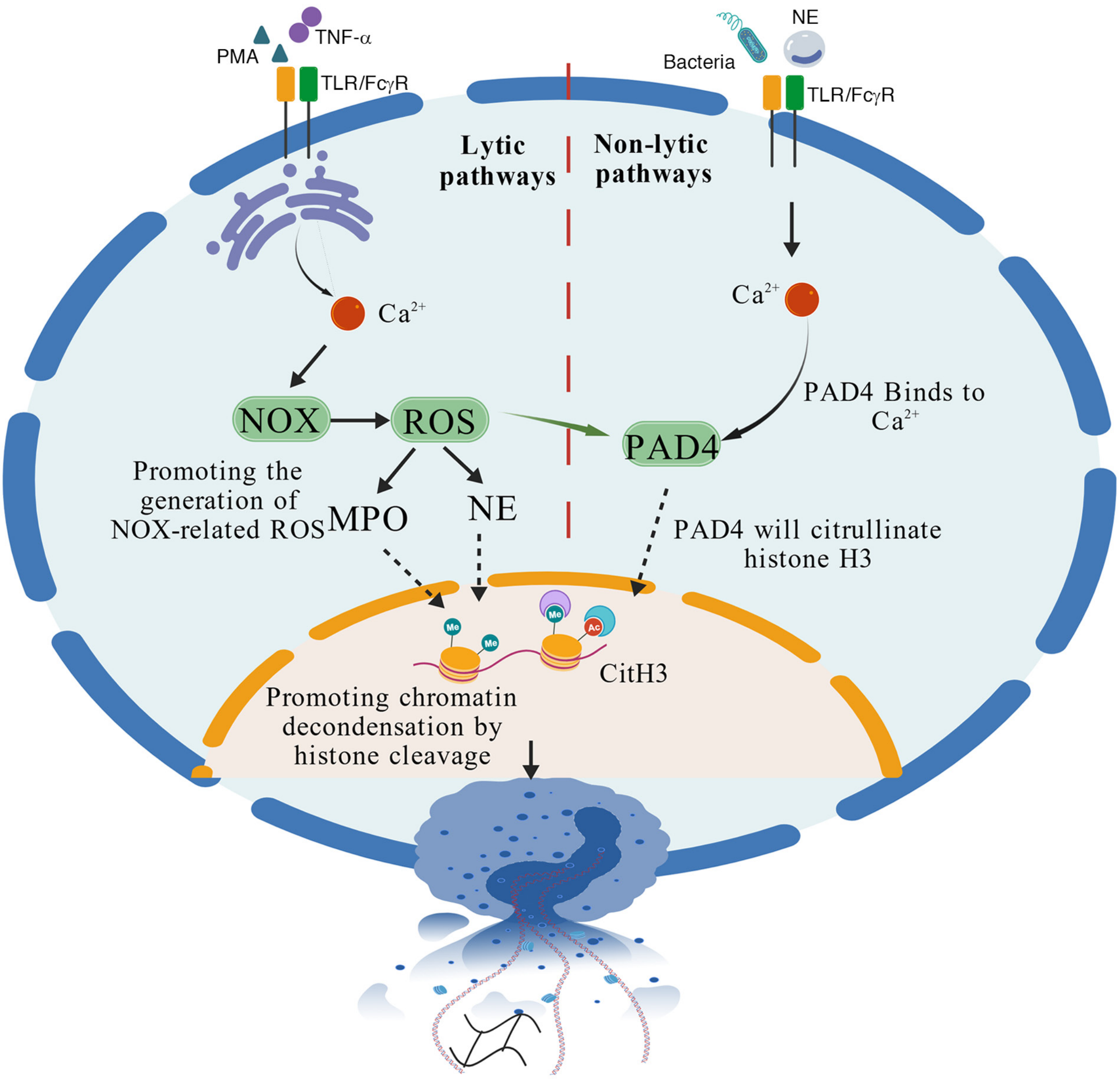

Figure 1. Molecular mechanisms of NET formation. NET formation is triggered by stimuli such as PMA, TNF-α and elevated intracellular Ca^{2+} levels, which activate NOX to produce ROS. Increased Ca^{2+} also promotes PAD4 activation, leading to CitH3. MPO and neutrophil elastase further facilitate chromatin decondensation through histone cleavage, ultimately resulting in NET release. Figure made using BioGDP.com. NOX, NADPH oxidase; citH3 citrullinated histone H3; NETs, neutrophil extracellular traps; PMA, phorbol-12-myristate-13-acetate; ROS, reactive oxygen species; PAD4, peptidyl arginine deiminase 4; MPO, myeloperoxidase; NE, neutrophil elastase; FcγR, Fc gamma receptor; TLR, Toll-like receptor.

box 1 (HMGB1) via the TLR2 signaling axis in both the circulatory system and metastatic lung tissues of patients with triple-negative breast cancer (TNBC) (18). Pharmacological inhibition of the HMGB1-CD62L neutrophil-NETs cascade can markedly suppress TNBC lung metastasis. Although tumor-derived granulocyte colony-stimulating factor is necessary, it is insufficient to mobilize immature low-density neutrophils that promote hepatic metastasis. By contrast, mature high-density neutrophils exhibit protective effects against liver metastasis (19).

Immature low-density neutrophils exhibit enhanced global bioenergetic capacity through mitochondrial-dependent adenosine triphosphate (ATP) production, enabling them to perform pro-metastatic functions such as NET formation under nutrient-deprived conditions (20). Furthermore, neutrophils depend on glutamate and proline catabolism to sustain mitochondrial metabolism during glucose deprivation, thereby maintaining persistent NET formation. This metabolic adaptation underscores the notable metabolic plasticity of distinct pro-metastatic neutrophil subpopulations, ultimately promoting hepatic metastasis (21).

The heterogeneous distribution of NETs within solid tumors is partially mediated by interleukin-8 (IL-8)-dependent mechanisms. IL-8 orchestrates neutrophil recruitment to tumor sites and triggers NETs extrusion (22). In colorectal cancer (CRC), exosomal KRAS mutations drive tumor-associated NET formation by augmenting IL-8 production, thereby accelerating disease progression (23). By contrast, NETs promote tumor cell proliferation and lymphatic spread in diffuse large B-cell lymphoma (DLBCL) through mechanistically distinct pathways. DLBCL-secreted IL-8 engages neutrophil C-X-C chemokine receptor 2 (CXCR2), activating

Src/p38/extracellular signal-regulated kinase (ERK) signaling cascades to induce NETs (24). The resultant NETs stimulate TLR9 signaling in DLBCL cells, concurrently activating the nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT3) and p38 pathways to drive oncogenesis. Therapeutic strategies targeting NETs dissolution, IL-8-CXCR2 axis blockade and TLR9 inhibition show potent antitumor efficacy in this context.

4. NETs affect tumor occurrence and development

Beyond their established roles in chronic inflammation and autoimmune disorders, accumulating evidence implicates NETs in tumorigenic processes (25). Following tumor initiation, NETs not only stimulate cancer cell proliferation by activating signaling pathways, providing dynamic support for tumor growth, but also enhance mitochondrial biogenesis in cancer cells, increasing ATP production and supplying essential energy for tumor progression (26).

Elevated levels of circulating MPO-DNA complexes and tumor-associated citH3 have been consistently observed in patients with hepatocellular carcinoma (HCC), with markedly higher NETs deposition in intrahepatic and pulmonary metastatic lesions than in primary tumors (27,28). A previous study reported that preoperative serum levels of MPO-DNA and tumor citH3 in patients with liver cancer are negatively associated with both overall survival and recurrence-free survival (29). NETs have been shown to promote cell proliferation and invasion in CRC. Yang *et al* (30) demonstrated that compared with the normal control group, patients with CRC, particularly those with liver metastases, exhibited elevated levels of MPO-DNA in their serum and increased citH3 levels in their tissues.

Research on gastric cancer (GC) has shown that, compared with normal individuals, a notable number of NETs are deposited in the tumor tissue of patients with GC. Additionally, the levels of human MPO-DNA and cell-free DNA in the serum of patients with stage III/IV GC with metastasis are markedly higher than those in patients with stage I/II GC (31). Xia *et al* (32) also reported that the levels of NETs in the peripheral blood, ascites and abdominal tissues, as well as serum MPO-DNA, were markedly elevated in patients with GC with postoperative abdominal infection compared with those without infection. Additionally, it has been reported that NETs are enriched around metastases of head and neck squamous cell carcinoma and pancreatic cancer (33). Furthermore, elevated serum NET levels can be used to predict the risk of liver metastasis in patients with early breast cancer (34). Consequently, NETs have become a current research hotspot for tumor metastasis. The mechanisms by which NETs promote tumor metastasis are summarized in Table I.

NETs induce epithelial-mesenchymal transition (EMT) in cancer cells. EMT is a process by which polarized epithelial cells acquire motile mesenchymal characteristics under specific physiological or pathological conditions, granting cancer cells the ability to migrate and invade (35). NETs have been shown to contribute to cancer metastasis by activating EMT (31). NETs can promote EMT by regulating the expression of EMT-related molecules (E-cadherin, N-cadherin, Snail and ZEB1) in primary tumors, thereby enhancing the migratory and invasive capacities of cancer cell and contributing to tumor metastasis.

Research on GC has shown that NETs can promote EMT in GC cells, thereby enhancing their malignant and invasive potential. Zhu *et al* (31) demonstrated that, compared with the conditioned medium induced by PMA alone, GC cells treated simultaneously with DNase-1 or PAD4 inhibitors exhibited increased expression of the epithelial marker E-cadherin, decreased expression of the stromal marker vimentin and reduced cell migration. The same study also revealed that intraperitoneal injection of DNase-1 and PAD4 inhibitors resulted in marked downregulation of citH3 levels in GC tissues compared with the control group, along with an increase in E-cadherin expression and a decrease in vimentin expression. Xia *et al* (32) also demonstrated that co-culturing NETs extracted from the peripheral blood of patients with postoperative infection complications of GC with control GC cells induced EMT, thereby promoting the migratory and invasive capabilities of the cells. It has been reported that that purified NETs can promote the expression of EMT transcription factors *in vitro* (33). Additionally, the expression levels of vimentin and fibronectin increase, whereas the expression levels of E-cadherin and epithelial cell adhesion molecule proteins decrease, leading to the induction of pseudopodia formation and enhanced cell motility (15).

During NET formation, HMGB1 is released from the nuclei of neutrophils and participates in DNA decondensation and chromatin extrusion. HMGB1 not only structurally helps to form the network of NETs but also enhances the inflammatory response of NETs through interactions with other molecules, such as interleukin-1β (34). Guan *et al* (27) reported that HMGB1 induces EMT in pancreatic cancer cells through NETs, enhancing their migratory and invasive capabilities, thereby promoting liver metastasis of pancreatic cancer. Additionally, NETs have been shown to promote EMT in breast cancer cells, as evidenced by the loss of epithelial cell-cell adhesion, downregulation of E-cadherin, upregulation of vimentin, acquisition of a spindle-like fibroblastoid morphology and increased migratory capacity (36). Collectively, these studies suggest that NETs facilitate tumor metastasis by regulating cancer cell EMT, thereby enhancing the migratory and invasive abilities of cancer cells.

NETs trap and protect cancer cells from damage. NETs capture cancer cells and provide a physical barrier that serves as the initial step in tumor metastasis. NETs can also act as an immune barrier to protect cancer cells. A study reported that integrin-β1 is expressed in both cancer cells and NETs, mediating the interaction between them. This interaction facilitates the capture of circulating cancer cells by NETs (37). Yang *et al* (28) found that NETs can capture HCC cells but do not have a notable cytotoxic effect; instead, NETs are internalized into HCC cells, activating TLR4/9-COX2 signaling, which induces drug resistance and enhances invasive potential. Additionally, NETs can capture CRC cells, promoting their dissemination to the liver and facilitating liver metastasis of CRC (30). CXCR1 and CXCR2 agonists can induce NET formation, which encases circulating cancer cells and protects them from cytotoxic $CD8^+$ T and natural killer (NK) cells

Table I. Mechanisms of NETs promoting tumor metastasis.

Mode of action	Mechanism
Induce EMT in tumor cells	Enhanced angiogenesis, EMT-associated cell migration, matrix metalloproteinase-mediated extracellular matrix degradation and NET-induced cell trapping
NETs trap tumor cells and provide protection against damage	NETs wrap around tumor cells to form a physical shield, creating an immunologically privileged niche that physically impedes lymphocyte infiltration. This steric hindrance prevents immune effector cell such as CD8$^+$ T cells and NK cells from accessing and eliminating tumor cells
NETs stimulate an inflammatory response and promote tumor cell migration	Circulating tumor cells are captured, and inflammation is induced through the TLR4/9-COX2 signaling pathway, enhancing tumor cell survival, invasion and metastatic potential
NETs promote the formation of a premetastatic niche, facilitating tumor spread	The local microenvironment of the target organ is remodeled to create conditions more conducive to the colonization and growth of circulating tumor cells
NETs trigger the activation of dormant tumor cells, promoting their reawakening	NETs-associated proteases, NE and MMP-9, sequentially cleave laminin in the ECM; proteolytically remodeled laminin activates integrin α3β1 signaling in dormant cancer cells, inducing cell cycle re-entry and proliferation. NETs-mediated ECM remodeling activates β1-integrin/ILK signaling and YAP-dependent transcription via mechano-transduction, further promoting the reawakening of dormant tumor cells.
NETs facilitate the invasion of tumor cells into blood vessels, aiding in their spread to distant sites	Downregulation of VE-cadherin expression disrupts the integrity of endothelial cell junctions, increases vascular permeability and promotes the extravascular penetration and migration of tumor cells

EMT, epithelial-to-mesenchymal transition; NETs, neutrophil extracellular traps; TLR4, Toll-like receptor 4; COX2, cyclooxygenase 2; VE, vascular endothelial.; YAP, yes-associated protein; ILK, integrin-linked kinase; ECM, extracellular matrix.

by preventing immune cell contact with cancer cells (38). Alfaro *et al* (39) reported that the binding of tumor-derived IL-8 to CXCR2 attracts bone marrow-derived suppressor cells (MDSCs), thereby directly suppressing tumor immunity. IL-17, secreted by T helper type 17 (Th17) cells, serves a crucial role in the progression of pancreatic ductal adenocarcinoma. IL-17 recruits neutrophils and promotes the formation of NETs, which can reduce CD8$^+$ T cells in the TME and interfere with immune checkpoint blockade (ICB) (40). NETs can also capture CRC cells to promote their dissemination in the liver, leading to liver metastasis (30). In summary, NETs capture cancer cells and provide a physical barrier without exerting cytotoxic effects on cancer cells, thereby preventing cancer cell damage. Additionally, they can inhibit immune cells from attacking cancer cells, ultimately promoting tumor metastasis.

NETs stimulate an inflammatory response and promote tumor migration. A study showed that NETs capture circulating cancer cells and induce inflammatory responses via the TLR-cyclooxygenase 2 signaling pathway, which enhances cancer cell survival, invasion and metastatic potential (28). Tumor-derived cathepsin C (CTSC) can activate protein 3 (PR3) on the surface of neutrophils, promoting the processing of IL-1β. The CTSC/PR3/IL-1β axis upregulates the expression of IL-6 and chemokines, thereby recruiting neutrophils to metastatic lesions. It also promotes p38 activation and ROS production, leading to NET formation. These NETs then support the growth of breast cancer cells in the lung by downregulating platelet-reactive proteins (41). Zha *et al* (42) found that HMGB1 secreted by NETs can bind to the receptor for advanced glycation end products, activating the NF-κB signaling pathway, which enhances IL-8 secretion, recruits neutrophils and promotes NET formation through the phosphoinositide 3-kinase/protein kinase B axis. This ultimately supports the proliferation and invasion of glioblastoma cells. IL-8 derived from CRC recruits neutrophils to the liver where they form NETs. NETs capture disseminated cancer cells, enabling circulating cancer cells to colonize the liver and stimulate IL-8 secretion in metastatic CRC cells. This creates a feedback loop that recruits more neutrophils and promotes the migration and invasion of CRC cells (30). In a study on pancreatic ductal adenocarcinoma, it was found that the interaction between collagen I and discoidin domain receptor 1 induces the secretion of CXCL5, which promotes neutrophil infiltration and NET formation, thereby enhancing cancer cell invasion and metastasis (43).

NETs promote the formation of the 'premetastatic niche'. The premetastatic niche theory suggests that soluble factors released by the primary tumor travel to specific distant organs where they reshape the local microenvironment of the target tissue, making it more conducive to the colonization and proliferation of circulating cancer cells (44). Zeng *et al* (45) reported that the upregulation of hydroxylase oxidase 1 in alveolar epithelial cell of metastatic breast cancer mice led to the accumulation of oxalic acid in the lungs. This accumulation promoted NET formation, thereby contributing to the establishment of premetastatic niches. Research has shown that before ovarian cancer metastasizes to the greater omentum, chemokines such as IL-8, monocyte chemoattractant protein-1, growth-regulated oncogenes and granulocyte colony-stimulating factor stimulate neutrophils to migrate and recruit to the greater omentum, where they form NETs and establish premetastatic niches. These NETs capture ovarian cancer cells and promote their colonization within premetastatic niches, thereby facilitating cancer cell metastasis (46). Additionally, research has shown that neutrophil infiltration and NET formation are first detected in distant target organs of tumors, followed by the development of metastatic lesions (47). This suggests that NETs secrete pro-inflammatory mediators such as matrix metalloproteinase-9 (MMP-9), which reshape the local microenvironment and extracellular matrix, promote the formation of premetastatic niches and facilitate the development of tumor metastases (48).

NETs awaken dormant cancer cells. Scattered dormant cancer cells can be reactivated leading to cancer cell proliferation. For example, Albrengues *et al* (49) demonstrated that lipase or tobacco exposure during inflammation can induce NET formation. The MMP-9 released by NETs sequentially cleaves laminin, and hydrolyzed laminin-111 provides an activation epitope for integrin $\alpha3\beta1$. This interaction awakens dormant cancer cells through the focal adhesion kinase/ERK signaling pathway, enabling cancer cells to re-enter the cell cycle and promote invasive lung metastasis. Another study found that β-amyloid protein secreted by cancer-associated fibroblasts can induce NETs production, whereas PAD4 inhibitors can prevent NET formation in melanoma mice, inhibiting tumor growth. Therefore, NETs serve a critical role in reactivating dormant cancer cells and promoting cancer cell proliferation (50).

NETs promote the invasion of cancer cells into blood vessels. As predominantly glycolytic cells, neutrophils derive minimal ATP from oxidative phosphorylation. They employ glycerol-3-phosphate signaling to directly modulate mitochondrial function through glycolytic pathways, a mechanism critical for maintaining mitochondrial polarization and ROS generation (51). Tumor-induced metabolic reprogramming in neutrophils shifts toward the glycolytic and pentose phosphate pathways, driving NET formation in an ROS-dependent manner. This process subsequently enhances HCC cell aggressiveness while downregulating tight junction proteins in adjacent endothelial cell, ultimately facilitating cancer intravasation and metastasis (52).

Cancer cells traverse the endothelial barrier of blood vessels through various mechanisms, entering the bloodstream before infiltrating distant tissues from outside blood vessels. This process ultimately leads to the dissemination and colonization of tumors in the target organs (53). Studies have shown that NETs can downregulate the expression of vascular endothelial cadherin, disrupt the integrity of endothelial cell connectivity, increase vascular permeability, and promote extravascular infiltration and migration of HCC cells (54,55). NETs can also upregulate the expression of tissue factor, phosphatidylserine and P-selectin while inducing platelet hypercoagulability, which facilitates cancer cell adhesion, retention and extravasation in blood vessels (56,57). Moreover, platelets can secrete heparin and proteases to degrade the vascular basement membrane, further promoting cancer cell invasion into the tissues (41). These findings highlight the role of NETs in regulating vascular-related proteins, enhancing cancer cell invasion into the blood vessels and facilitating distant metastasis.

5. NETs affect antitumor immunity

An immunosuppressive TME is closely associated with tumor immune escape (58). Under the influence of chronic inflammation, chemokines and metabolic factors, neutrophils migrate from the circulatory system to tumor tissue, where they release NETs that contribute to the formation of the premetastatic niche. This process promotes the infiltration of immunosuppressive cell and mediates tumor recurrence and metastasis, suggesting that NETs serve a crucial role in modulating the body's antitumor immune response (59), as shown in Fig. 2. The clinical application of immune checkpoint inhibitors (ICIs) such as anti-programmed cell death protein 1 (PD-1)/PD-L1 therapy has paved the way for tumor immunotherapy. Research indicates that inhibition of NET formation can markedly enhance the efficacy of anti-PD-1 therapy in CRC and liver, pancreatic and breast cancers (60).

NETs and immunosuppressive cells. NETs contribute to tumor progression by enhancing the function of various immunosuppressive cells present in the TME, such as MDSCs, regulatory T cells (Tregs), tumor-associated macrophages and Th17 cells (61). Yu *et al* (62) found that NETs can mediate macrophage polarization toward the M2 phenotype, promoting GC progression, and their synergy can also enhance the invasion and migration of A549 cells. NETs can regulate the expression of mitochondrial oxidative phosphorylation-related genes in naïve $CD4^+$ T cells via TLR4 receptors, inducing their differentiation into Tregs and facilitating the progression of non-alcoholic steatohepatitis into liver cancer (63). Furthermore, NETs promote Treg invasion, aiding in the local progression and metastasis of breast cancer (64). In acute lung injury (ALI) models, NETs have also been shown to induce $CD4^+$ T cell differentiation into Th17 cells, thereby contributing to the development of autoimmune pulmonary inflammation (65).

NETs and antitumor effector immune cells

NETs and dendritic cells (DCs). DCs are primary antigen-presenting cells in the body and serve a key role in inhibiting tumor progression by inducing antigen-specific adaptive immune responses (66). The effect of NETs on DC is multifaceted. NETs can activate DCs and present relevant antigens, thereby inducing specific immunity that inhibits the progression of nucleophosmin (NPM)-mutant myeloid leukemia. By contrast, NETs can induce DC apoptosis by causing mitochondrial damage (67).

The interplay between NETs and DCs promotes DC activation and maturation, enabling the presentation of NET-associated antigens (68). Unlike other forms of cell death

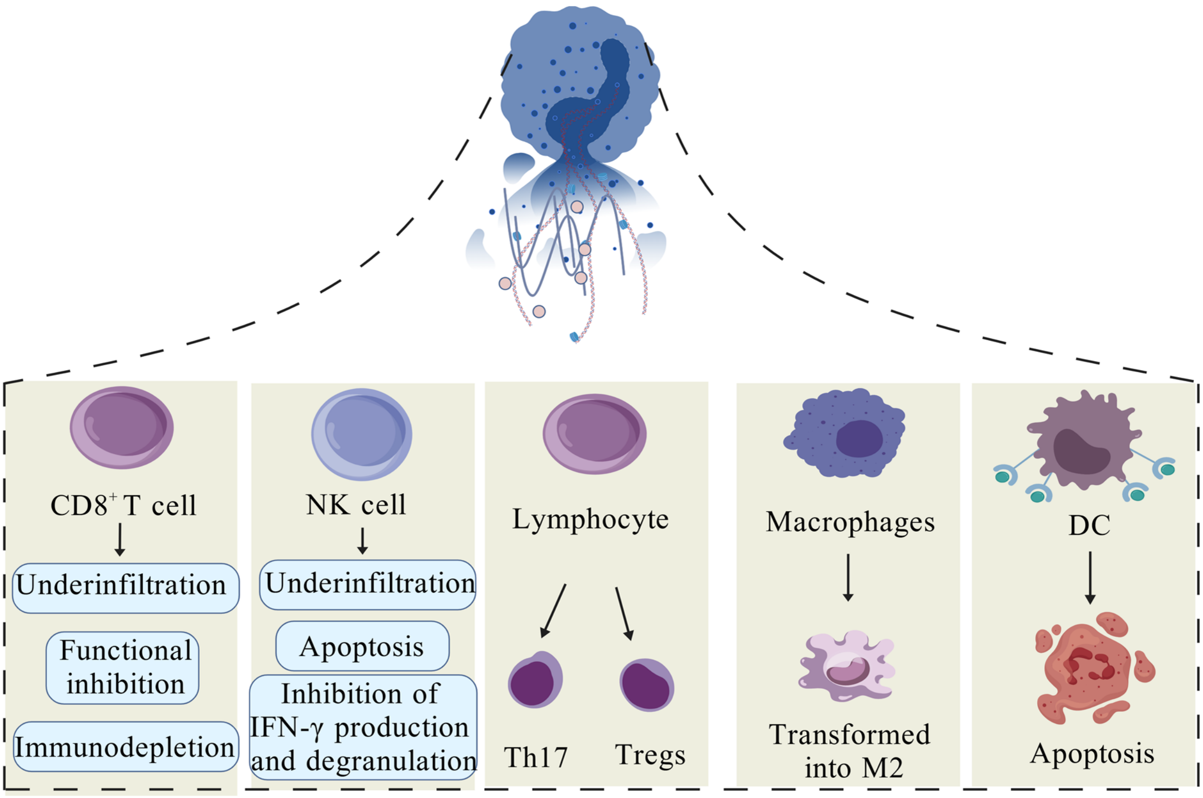

Figure 2. Immunosuppressive effects of NETs on various immune cells in the tumor microenvironment. NETs inhibit antitumor immunity by multiple mechanisms: They reduce infiltration of CD8$^+$ T cells and NK cell; induce apoptosis of CD8$^+$ T cells and lymphocytes; impair NK cell degranulation and IFN-γ production; promote transformation of Th17 cells and Tregs; and drive macrophage polarization toward the M2 phenotype. These coordinated effects lead to immune cell dysfunction and depletion, contributing to tumor immune evasion. Figure made using BioGDP.com. IFN-γ, interferon-γ; NETs, neutrophil extracellular traps; NK, natural killer; Treg, regulatory T cell; DC, dendritic cell; Th17, T helper type 1.

that induce protein denaturation, NETs preserve the native conformation and enzymatic activity of DNA-associated proteins, thereby retaining their immunogenic epitopes. In addition to neutrophils, leukemia cells also generate extracellular traps that display tumor-specific antigens, including mutant NPM. This oncogenic variant, characterized by nucleolar-to-cytoplasmic translocation due to the mutation, is spatially repositioned on NETs-like structures during leukemia cell trap formation (69).

NETs and CD8$^+$ T cells. Dysfunction of CD8$^+$ T cells is closely associated with tumor initiation, progression and immune evasion. NETs act as a physical barrier around cancer cells, promoting tumor progression by restricting CD8$^+$ T cell infiltration (38). In HCC, NETs exert immunosuppressive effects through a recently elucidated molecular mechanism. Song *et al* (70) demonstrated that NETs-DNA directly binds to transmembrane and coiled-coil domains 6 (TMCO6), a transmembrane protein on CD8$^+$ T cells. This binding inhibits T cell receptor signaling and blocks NF-κB p65 nuclear translocation, leading to impaired T cell proliferation, functional exhaustion and apoptosis. Notably, this interaction creates a positive feedback loop: NETs-DNA/TMCO6 binding promotes TGFβ1 secretion from exhausted T cells, which further stimulates NET formation. The clinical relevance of this pathway is supported by evidence that PAD4 inhibitors suppress tumor growth in wild-type but not TMCO6-knockout mice, confirming TMCO6 as a critical mediator (70). Additionally, TMCO6-expressing CD8$^+$ T cells in clinical samples exhibit pronounced exhaustion phenotypes, and combining TMCO6 deficiency with anti-PD-1 therapy abrogates NETs-driven HCC progression (70). A Study on breast cancer demonstrated that targeting NETs can inhibit T cell infiltration and significantly improve the efficacy of anti-PD-1 therapy (71).

In addition to inhibiting infiltration, NETs can notably induce CD8$^+$ T cell dysfunction in various tumor models. For instance, NETs reduce the secretion of effector cytokines such as IFN-γ and IL-2 in CD8$^+$ T cells, thereby promoting lung cancer progression (72). The PD-L1 protein component in cancer cells can inhibit their function by binding to PD-1 on the T cell surface (73). Therefore, targeting NETs holds promise for improving CD8$^+$ T cell function and enhancing the efficacy of immunotherapy.

NETs and NK cells. NETs can inhibit the antitumor activity of NK cells through multiple mechanisms. First, the physical barrier formed by NETs prevents NK cells from effectively contacting the cancer cells. Second, NETs can degrade pro-inflammatory cytokines such as IL-2 and IL-15, which are essential for the activation, proliferation and function of NK cells (74). NETs may indirectly suppress NK cell antitumor functions by isolating and degrading these cytokines. Additionally, NETs can directly induce NK cell apoptosis through angiopoietin-2 and inflammatory cytokines that trigger apoptotic signaling (75).

Additionally, cathepsin G, an important component of NETs, can inhibit NK cell activation, IFN-γ release and degranulation by downregulating expression of the activation

Table II. Summary of therapeutic strategies targeting NETs in cancer.

Agent/strategy	Mechanism of action	Study type/stage	Key findings
DNase I	Degrades NETs DNA backbone, disrupting NETs structure	Preclinical	Reduces liver metastasis in CRC mouse models; improves anti-CTLA-4 therapy efficacy in MSS/pMMR CRC, leading to complete tumor regression; inhibits NETs, prevents tumor cell entrapment and suppresses metastasis; restores local immune responses by increasing CD8$^+$ T cell infiltration
AAV-mediated DNase I gene transfer	Liver-specific DNase I expression for sustained NET degradation	Preclinical	Single intravenous injection achieves sustained DNase I expression in liver; suppresses liver metastasis development; inhibits neutrophil infiltration and NET formation in tumor tissues; restores CD8$^+$ T cell response
DNase I-AuNPs	Radiosensitization via AuNPs + NET degradation via DNase I release	Preclinical	Enhances radiotherapy efficacy through AuNP-mediated radiosensitization; sustained DNase I release degrades ROS-induced NETs; prevents interaction between free tumor cell and vasculature; reduces metastasis risk
PAD4 inhibitors	Inhibits PAD4-mediated histone citrullination, blocking NETs	Preclinical	Blocks NET formation at the initiation stage; reduces tumor growth and metastasis in preclinical models
Combination: DNase I + anti-CTLA-4	NET degradation + immune checkpoint blockade	Preclinical	Complete response rate of >50% in primary tumors; complete responders were immune to tumor rechallenge; suggests DNase I may enhance both response rate and duration of ICIs
Combination: DNase I + galunisertib in NPs	NET degradation + TAN suppression + immune modulation	Preclinical	Tumor microenvironment-responsive NPs co-deliver DNase I and galunisertib; degrades NETs and suppresses TANs; reshapes immunosuppressive TME; reactivates CD8$^+$/CD4$^+$ T cells and dendritic cells

NETs, neutrophil extracellular traps; CRC, colorectal cancer; CTLA-4, cytotoxic T lymphocyte associated protein 4; MSS, microsatellite stable; pMMR, proficient mismatch repair; AuNPs, gold nanoparticles; ROS, reactive oxygen species; PAD4, peptidyl arginine deiminase 4; ICI, immune checkpoint inhibitors; TANs, tumor-associated neutrophils; TME, tumor microenvironment.

receptor NKp46 (76). Furthermore, MMP-9, which is abundant in NETs, can contribute to NK cell dysfunction and promote tumor immune evasion (77). NETs can interact with other factors in the TME to regulate NK cell functions. In GC models, NETs have been shown to increase the expression of angiopoietin-2 in the TME, which is closely associated with the induction of a resting state in NK cells (78). This suggests that NETs may indirectly affect NK cell antitumor activity by modulating the composition of the TME. Cheng *et al* (79) found that inhibiting NETs markedly enhanced NK cell function and inhibited liver cancer progression, indicating that further research on the interactions between NETs and NK cells could provide new insights for optimizing NK cell-based therapies.

Strategies for targeting NETs. As NETs can affect antitumor immunity in various ways, exploring strategies to target NETs holds promise for enhancing the efficacy of immunotherapy in tumors. Under normal conditions, NETs are rapidly degraded through the synergistic action of extracellular free DNA enzymes and macrophages (80). Additionally, DCs can promote NET degradation via phagocytosis (80). However, further research is required to determine whether other immune cells contribute to NET degradation. External factors such as environmental temperature also regulate NET degradation in the body (81). Although there is currently limited research on the regulatory mechanisms of NET degradation pathways, it is speculated that these phenomena may be associated with DNA enzyme activity. Currently, several therapeutic strategies targeting NETs have been investigated, as summarized in Table II. These approaches primarily focus on two key mechanisms: Degrading existing NETs and inhibiting NET formation.

Notably, inhibiting PAD4 activity or promoting NET degradation through DNase I can effectively inhibit tumor progression, demonstrating the therapeutic potential of antagonizing NETs (38). Sivelestat, an NE inhibitor clinically used for ALI and acute respiratory distress syndrome, suppresses NET formation by inhibiting NE activity. Sivelestat-mediated NET inhibition effectively reduces tumor metastasis by specifically inhibiting NE, which is essential for NET formation. NE released during NETs activates ERK signaling to promote cancer cell migration, and blocking NE with sivelestat suppresses both NETs and cancer cell motility (82,83).

Myeloid cells suppress antitumor immunity through metabolic reprogramming, notably via l-arginine depletion mediated by ARG1 (84). In pancreatic cancer, NET released by spontaneously activated neutrophils establish specialized microdomains where cathepsin S (CTSS) proteolytically processes human ARG1 into truncated isoforms with enhanced enzymatic efficiency under physiological pH conditions (85). This NET-associated ARG1 variant potently inhibits T lymphocyte proliferation, an immunosuppressive effect that is reversible through either human ARG1-specific monoclonal antibodies or CTSS cleavage inhibition, although conventional small-molecule inhibitors remain ineffective. Crucially, combinatorial targeting of ARG1 with ICB rescues CD8$^+$ T cell functionality in pancreatic tumors. Therapeutically, human ARG1-neutralizing antibodies not only amplify intratumoral infiltration of adoptively transferred tumor-specific CD8$^+$ T cells, but also synergistically enhance the therapeutic efficacy of ICB.

IL-17 orchestrates neutrophil recruitment and induces NET formation while excluding cytotoxic CD8$^+$ T cells from the pancreatic TME. Therapeutic neutralization of IL-17 notably restores tumor sensitivity to dual ICB targeting of the PD-1 and cytotoxic T-lymphocyte associated protein 4 checkpoints. Notably, pharmacological suppression of neutrophil infiltration or genetic inhibition of PAD4-dependent NETs recapitulates the therapeutic benefits observed with IL-17 blockade, confirming the central role of this cytokine in NET-mediated immunosuppression (12). OX40 receptor activation enhances the functional capacity of effector T cells while concurrently suppressing regulatory Treg activity through the dual mechanisms of population reduction and functional deactivation. A study has demonstrated that anti-OX40 agonistic antibodies potentiate antitumor immunity through two strategic approaches: Monotherapy by direct T cell modulation and combination therapy through synergistic enhancement of checkpoint inhibitor efficacy. This bimodal therapeutic strategy achieves superior immune activation compared with single-pathway interventions (86).

In summary, while multiple mechanisms exist for NET degradation in the body, research on the cells involved in this process and the regulatory factors governing it are still underdeveloped. Deeper exploration of NET degradation mechanisms, identification of potential influencing factors and understanding how these mechanisms vary under different pathological conditions could uncover new regulatory pathways or targets for NETs-targeted strategies in tumor therapy.

6. Conclusions and future perspectives

NETs have emerged as critical targets in the study of tumor metastasis and recurrence. A growing body of research has uncovered various mechanisms by which NETs promote tumor metastasis. These include inducing EMT in cancer cells, capturing and protecting circulating cancer cells, facilitating extravascular infiltration of cancer cells, stimulating inflammatory responses, promoting the formation of pre-metastatic niches and reactivating dormant cancer cells at metastatic sites. NETs interact with inflammation, immune responses and the TME to collectively regulate tumor cell proliferation and migration, exhibiting dynamic changes in spatial dimensions. The mechanisms by which NETs facilitate metastasis across primary lesions, the circulatory system and metastatic sites are highly complex, with interactions between NETs and TME varying across different tumor types.

Existing research suggests that targeting NETs can notably enhance the efficacy of ICIs and NK cell-based therapies. Additionally, NETs have shown promise as materials for developing DC tumor vaccines because of their unique biological characteristics such as high immunogenicity and viscosity (87). However, despite gaining some insight into the role of NETs in tumor biology and antitumor immunity, current research remains insufficient. NETs not only interact with immune cell but also with non-immune components in the TME, such as the extracellular matrix in liver cirrhosis tissue and intestinal bacteria (52,77), which collectively influence antitumor immunity. Therefore, it may be crucial to explore the impact of NETs on antitumor immunity from a holistic perspective of the TME. Additionally, the role of NETs in tumor immunity may vary among individuals owing to factors such as tumor type, patient age and genetic background (28). However, individual differences in NETs have not been explored extensively. Therefore, further research is needed to identify the targets related to NETs function, assess the roles of NETs in different patients by combining various targets and identify suitable tumor patients for the clinical application of targeted NETs strategies. Future research should focus on the detailed biological characteristics of NETs, their dynamic changes in the TME and their interactions with immune cell within the TME. A deeper understanding of the complex functions of NETs in the TME, along with targeted NETs therapies, could lead to notable breakthroughs in tumor immunotherapy and provide more effective and safer treatment strategies for patients with cancer.

The limitations of this review include the predominance of preclinical studies, limited clinical evidence, uncharacterized NETs heterogeneity across tumors, insufficient understanding of NETs-immune interactions in the TME, and the need to evaluate off-target effects of NETs-targeting strategies before clinical translation.

The present review is tailored for a multidisciplinary audience in oncology and immunology, including basic researchers investigating NETs mechanisms in cancer progression, clinical oncologists seeking to leverage NETs as biomarkers or therapeutic targets in immunotherapy. By integrating preclinical models, clinical data and emerging therapeutic strategies, the present review bridges mechanistic understanding with actionable innovations, fostering collaboration across cancer biology, immunology and drug development.

Acknowledgements

Not applicable.

Funding

The present work was funded by the National Natural Science Foundation of China (grant no. 82505411) and the Social Development Project of Yixing Science and Technology Bureau (grant no. 2024SF12).

Availability of data and materials

Not applicable.

Authors' contributions

MG and HW wrote the original draft. JZ and ZH contributed to the literature search, data analysis, interpretation of findings, and critical selection of relevant studies. NW generated the figures, ensured visual data accuracy, and contributed to drafting the manuscript. AX and TD reviewed and edited the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

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. Amulic B, Cazalet C, Hayes GL, Metzler KD and Zychlinsky A: Neutrophil function: From mechanisms to disease. Annu Rev Immunol 30: 459-489, 2012.
2. Mantovani A, Cassatella MA, Costantini C and Jaillon S: Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11: 519-531, 2011.
3. Johnson SS, Liu D, Ewald JT, Robles-Planells C, Pulliam C, Christensen KA, Bayanbold K, Wels BR, Solst SR, O'Dorisio MS, *et al*: Auranofin inhibition of thioredoxin reductase sensitizes lung neuroendocrine tumor cells (NETs) and small cell lung cancer (SCLC) cells to sorafenib as well as inhibiting SCLC xenograft growth. Cancer Biol Ther 25: 2382524, 2024.
4. Shahzad MH, Feng L, Su X, Brassard A, Dhoparee-Doomah I, Ferri LE, Spicer JD and Cools-Lartigue JJ: Neutrophil extracellular traps in cancer therapy resistance. Cancers (Basel) 14: 1359, 2022.
5. Fang Q, Stehr AM, Naschberger E, Knopf J, Herrmann M and Stürzl M: No NETs no TIME: Crosstalk between neutrophil extracellular traps and the tumor immune microenvironment Front Immunol 13: 1075260, 2022.
6. 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.
7. Takei H, Araki A, Watanabe H, Ichinose A and Sendo F: Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis. J Leukoc Biol 59: 229-240, 1996.
8. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y and Zychlinsky A: Neutrophil extracellular traps kill bacteria. Science 303: 1532-1535, 2004.
9. Neubert E, Meyer D, Rocca F, Günay G, Kwaczala-Tessmann A, Grandke J, Senger-Sander S, Geisler C, Egner A, Schön MP, *et al*: Chromatin swelling drives neutrophil extracellular trap release. Nat Commun 9: 3767, 2018.
10. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V and Zychlinsky A: Novel cell death program leads to neutrophil extracellular traps. J Cell Biol 176: 231-241, 2007.
11. Ravindran M, Khan MA and Palaniyar N: Neutrophil extracellular trap formation: Physiology, pathology, and pharmacology. Biomolecules 9: 365, 2019.
12. Marin-Esteban V, Turbica I, Dufour G, Semiramoth N, Gleizes A, Gorges R, Beau I, Servin AL, Lievin-Le Moal V, Sandré C and Chollet-Martin S: Afa/Dr diffusely adhering Escherichia coli strain C1845 induces neutrophil extracellular traps that kill bacteria and damage human enterocyte-like cells. Infect Immun 80: 1891-1899, 2012.
13. Lewis HD, Liddle J, Coote JE, Atkinson SJ, Barker MD, Bax BD, Bicker KL, Bingham RP, Campbell M, Chen YH, *et al*: Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. Nat Chem Biol 11: 189-191, 2015.
14. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, *et al*: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 13: 463-469, 2007.
15. 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.
16. Cumming J, Maneshi P, Dongre M, Alsaed T, Dehghan-Nayeri MJ, Ling A, Pietras K, Patthey C and Öhlund D: Dissecting FAP+ cell diversity in pancreatic cancer uncovers an interferon-response subtype of cancer-associated fibroblasts with tumor-restraining properties. Cancer Res 85: 2388-2411, 2025.
17. Hedrick CC and Malanchi I: Neutrophils in cancer: Heterogeneous and multifaceted. Nat Rev Immunol 22: 173-187, 2022.
18. Al Qutami F, AlHalabi W, Vijayakumar A, Rawat SS, Mossa AH, Jayakumar MN, Samreen B and Hachim MY: Characterizing the inflammatory profile of Neutrophil-Rich Triple-negative breast cancer. Cancers (Basel) 16: 747, 2024.
19. Hsu BE, Tabariès S, Johnson RM, Andrzejewski S, Senecal J, Lehuédé C, Annis MG, Ma EH, Völs S, Ramsay L, *et al*: Immature Low-density neutrophils exhibit metabolic flexibility that facilitates breast cancer liver metastasis. Cell Rep 27: 3902-3915.e6, 2019.
20. Rice CM, Davies LC, Subleski JJ, Maio N, Gonzalez-Cotto M, Andrews C, Patel NL, Palmieri EM, Weiss JM, Lee JM, *et al*: Tumour-elicited neutrophils engage mitochondrial metabolism to circumvent nutrient limitations and maintain immune suppression. Nat Commun 9: 5099, 2018.
21. Partouche S, Goldberg I, Halperin E, Atamna B, Shacham-Abulafia A, Shapira S, Samara A, Gover-Proaktor A, Leader A, Spectre G, *et al*: Interferon-α inhibits NET formation in neutrophils derived from patients with myeloproliferative neoplasms in a neutrophil Sub-Population-specific manner. Int J Mol Sci 25: 13473, 2024.
22. Teijeira A, Garasa S, Ochoa MC, Villalba M, Olivera I, Cirella A, Eguren-Santamaria I, Berraondo P, Schalper KA, de Andrea CE, *et al*: IL8, neutrophils, and NETs in a collusion against cancer immunity and immunotherapy. Clin Cancer Res 27: 2383-2393, 2021.
23. Shang A, Gu C, Zhou C, Yang Y, Chen C, Zeng B, Wu J, Lu W, Wang W, Sun Z and Li D: Exosomal KRAS mutation promotes the formation of tumor-associated neutrophil extracellular traps and causes deterioration of colorectal cancer by inducing IL-8 expression. Cell Commun Signal 18: 52, 2020.
24. Nie M, Yang L, Bi X, Wang Y, Sun P, Yang H, Liu P, Li Z, Xia Y and Jiang W: Neutrophil Extracellular traps induced by IL8 promote diffuse large B-cell lymphoma progression via the TLR9 signaling. Clin Cancer Res 25: 1867-1879, 2019.
25. Carmona-Rivera C, Carlucci PM, Goel RR, James E, Brooks SR, Rims C, Hoffmann V, Fox DA, Buckner JH and Kaplan MJ: Neutrophil extracellular traps mediate articular cartilage damage and enhance cartilage component immunogenicity in rheumatoid arthritis. JCI Insight 5: e139388, 2020.
26. Wang WW, Wu L, Lu W, Chen W, Yan W, Qi C, Xuan S and Shang A: Lipopolysaccharides increase the risk of colorectal cancer recurrence and metastasis due to the induction of neutrophil extracellular traps after curative resection. J Cancer Res Clin Oncol 147: 2609-2619, 2021.

27. Guan X, Lu Y, Zhu H, Yu S, Zhao W, Chi X, Xie C and Yin Z: The crosstalk between cancer cells and neutrophils enhances hepatocellular carcinoma metastasis via neutrophil extracellular Traps-associated Cathepsin G component: A potential therapeutic target. J Hepatocell Carcinoma 8: 451-465, 2021.
28. 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.
29. Kaltenmeier CT, Yazdani H, van der Windt D, Molinari M, Geller D, Tsung A and Tohme S: Neutrophil extracellular traps as a novel biomarker to predict recurrence-free and overall survival in patients with primary hepatic malignancies. HPB (Oxford) 23: 309-320, 2021.
30. 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.
31. Zhu T, Zou X, Yang C, Li L, Wang B, Li R, Li H, Xu Z, Huang D and Wu Q: Neutrophil extracellular traps promote gastric cancer metastasis by inducing epithelial-mesenchymal transition. Int J Mol Med 48: 127, 2021.
32. Xia X, Zhang Z, Zhu C, Ni B, Wang S, Yang S, Yu F, Zhao E, Li Q and Zhao G: Neutrophil extracellular traps promote metastasis in gastric cancer patients with postoperative abdominal infectious complications. Nat Commun 13: 1017, 2022.
33. Yang J, Jin L, Kim HS, Tian F, Yi Z, Bedi K, Ljungman M, Pasca di Magliano M, Crawford H and Shi J: KDM6A loss recruits Tumor-associated neutrophils and promotes neutrophil extracellular trap formation in pancreatic cancer. Cancer Res 82: 4247-4260, 2022.
34. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, Huang D, Li J, Li H, Chen F, *et al*: DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature 583: 133-138, 2020.
35. Dongre A and Weinberg RA: New insights into the mechanisms of Epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol 20: 69-84, 2019.
36. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, Campbell K, Cano A, Casanova J, Christofori G, *et al*: Guidelines and definitions for research on epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 21: 341-352, 2020.
37. Najmeh S, Cools-Lartigue J, Rayes RF, Gowing S, Vourtzoumis P, Bourdeau F, Giannias B, Berube J, Rousseau S, Ferri LE and Spicer JD: Neutrophil extracellular traps sequester circulating tumor cells via β1-integrin mediated interactions. Int J Cancer 140: 2321-2330, 2017.
38. Teijeira Á, Garasa S, Gato M, Alfaro C, Migueliz I, Cirella A, de Andrea C, Ochoa MC, Otano I, Etxeberria I, *et al*: CXCR1 and CXCR2 chemokine receptor agonists produced by tumors induce neutrophil extracellular traps that interfere with immune cytotoxicity. Immunity 52: 856-871.e8, 2020.
39. Alfaro C, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, Alignani D, Labiano S, Azpilikueta A, Rodriguez-Paulete A, *et al*: Tumor-produced Interleukin-8 attracts human Myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). Clin Cancer Res 22: 3924-3936, 2016.
40. Zhang Y, Chandra V, Riquelme Sanchez E, Dutta P, Quesada PR, Rakoski A, Zoltan M, Arora N, Baydogan S, Horne W, *et al*: Interleukin-17-induced neutrophil extracellular traps mediate resistance to checkpoint blockade in pancreatic cancer. J Exp Med 217: e20190354, 2020.
41. 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, 2021.
42. Zha C, Meng X, Li L, Mi S, Qian D, Li Z, Wu P, Hu S, Zhao S, Cai J and Liu Y: Neutrophil extracellular traps mediate the crosstalk between glioma progression and the tumor microenvironment via the HMGB1/RAGE/IL-8 axis. Cancer Biol Med 17: 154-168, 2020.
43. Deng J, Kang Y, Cheng CC, Li X, Dai B, Katz MH, Men T, Kim MP, Koay EA, Huang H, *et al*: DDR1-induced neutrophil extracellular traps drive pancreatic cancer metastasis. JCI Insight 6: e146133, 2021.
44. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, *et al*: VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438: 820-827, 2005.
45. Zeng Z, Xu S, Wang F, Peng X, Zhang W, Zhan Y, Liu Z and Liang L: HAO1-mediated oxalate metabolism promotes lung pre-metastatic niche formation by inducing neutrophil extracellular traps. Oncogene 41: 3719-3731, 2022.
46. Lee W, Ko SY, Mohamed MS, Kenny HA, Lengyel E and Naora H: Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum. J Exp Med 216: 176-194, 2019.
47. Zhou AF, Tan Y and Zhang HJ: Colon cancer cell-derived exosomes activate neutrophil through HMGB1 to promote the proliferation, invasion and migration of colon cancer cell. Chin J Oncol Prev Treat 14: 20-25, 2022.
48. Norouzi F, Eini P and Tahmasebi S: The role of NETosis in breast cancer: Mechanistic insights and biomarker potential. Breast Cancer Res 27: 180, 2025.
49. Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, Upadhyay P, Uyeminami DL, Pommier A, Küttner V, *et al*: Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science 361: eaao4227, 2018.
50. Munir H, Jones JO, Janowitz T, Hoffmann M, Euler M, Martins CP, Welsh SJ and Shields JD: Stromal-driven and Amyloid β-dependent induction of neutrophil extracellular traps modulates tumor growth. Nat Commun 12: 683, 2021.
51. Fresneda Alarcon M, Abdullah GA, Nolan A, Linford C, Meschis MM, Cross AL, Sellin A, Phelan MM and Wright HL: The small molecule inhibitor 3PO is a modulator of neutrophil metabolism, ROS production and NET release. Clin Exp Immunol 219: uxaf012, 2025.
52. Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER and Slingerland JM: Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. CA Cancer J Clin 67: 378-397, 2017.
53. Alshahrani A, Alsubait A, Asiri Z, Alghamdi S, Bin Saqyah S, Alqahtani T, Fitaihi R, Altuwaijri N and Jamous YF: Deciphering molecular pathways driving cancer invasion and metastasis: Advances and therapeutic prospects. Front Oncol 15: 1684896, 2025.
54. Vestweber D: VE-cadherin: The major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. Arterioscler Thromb Vasc Biol 28: 223-232, 2008.
55. Jiang ZZ, Peng ZP, Liu XC, Guo HF, Zhou MM, Jiang D, Ning WR, Huang YF, Zheng L and Wu Y: Neutrophil extracellular traps induce tumor metastasis through dual effects on cancer and endothelial cells. Oncoimmunology 11: 2052418, 2022.
56. Coupland LA and Parish CR: Platelets, selectins, and the control of tumor metastasis. Semin Oncol 41: 422-434, 2014.
57. Li JC, Zou XM, Yang SF, Jin JQ, Zhu L, Li CJ, Yang H, Zhang AG, Zhao TQ and Chen CY: Neutrophil extracellular traps participate in the development of cancer-associated thrombosis in patients with gastric cancer. World J Gastroenterol 28: 3132-3149, 2022.
58. Chen C, Wang Z, Ding Y and Qin Y: Tumor microenvironment-mediated immune evasion in hepatocellular carcinoma. Front Immunol 14: 1133308, 2023.
59. Shi Y, Wu D, Wang Y, Shao Y, Zeng F, Zhou D, Zhang H and Miao C: Treg and neutrophil extracellular trap interaction contributes to the development of immunosuppression in sepsis. JCI Insight 9: e180132, 2024.
60. Shen XT, Xie SZ, Zheng X, Zou TT, Hu BY, Xu J, Liu L, Xu YF, Wang XF, Wang H, *et al*: Cirrhotic-extracellular matrix attenuates aPD-1 treatment response by initiating immunosuppressive neutrophil extracellular traps formation in hepatocellular carcinoma. Exp Hematol Oncol 13: 20, 2024.
61. Jin K, Li T, van Dam H, Zhou F and Zhang L: Molecular insights into tumour metastasis: Tracing the dominant events. J Pathol 241: 567-577, 2017.
62. Yu C, Zhou G, Shi Z, Yu L and Zhou X: TREM1 facilitates the development of gastric cancer through regulating neutrophil extracellular traps-mediated macrophage polarization. Dig Liver Dis 56: 1237-1247, 2024.
63. Wang H, Zhang H, Wang Y, Brown ZJ, Xia Y, Huang Z, Shen C, Hu Z, Beane J, Ansa-Addo EA, *et al*: Regulatory T-cell and neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. J Hepatol 75: 1271-1283, 2021.
64. Li H, Li J, Bai Z, Yan S and Li J: Collagen-induced DDR1 upregulates CXCL5 to promote neutrophil extracellular traps formation and Treg infiltration in breast cancer. Int Immunopharmacol 120: 110235, 2023.

65. Wilson AS, Randall KL, Pettitt JA, Ellyard JI, Blumenthal A, Enders A, Quah BJ, Bopp T, Parish CR and Brüstle A: Neutrophil extracellular traps and their histones promote Th17 cell differentiation directly via TLR2. Nat Commun 13: 528, 2022.
66. Chen W, Chen H, Yang ZT, Mao EQ, Chen Y and Chen EZ: Free fatty acids-induced neutrophil extracellular traps lead to dendritic cells activation and T cell differentiation in acute lung injury. Aging (Albany NY) 13: 26148-26160, 2021.
67. Tripodo C, Bassani B, Jachetti E, Cancila V, Chiodoni C, Portararo P, Botti L, Valenti C, Perrone M, Ponzoni M, *et al*: Neutrophil extracellular traps arm DC vaccination against NPM-mutant myeloproliferation. Elife 11: e69257, 2022.
68. Donis-Maturano L, Sánchez-Torres LE, Cerbulo-Vázquez A, Chacón-Salinas R, García-Romo GS, Orozco-Uribe MC, Yam-Puc JC, González-Jiménez MA, Paredes-Vivas YL, Calderón-Amador J, *et al*: Prolonged exposure to neutrophil extracellular traps can induce mitochondrial damage in macrophages and dendritic cells. Springerplus 4: 161, 2015.
69. Kyrysyuk O and Wucherpfennig KW: Designing cancer immunotherapies that Engage T cells and NK cells. Annu Rev Immunol 41: 17-38, 2023.
70. Song M, Zhang C, Cheng S, Ouyang D, Ping Y, Yang J, Zhang Y, Tang Y, Chen H, Wang QJ, *et al*: DNA of Neutrophil extracellular traps binds TMCO6 to impair CD8+ T-cell immunity in hepatocellular carcinoma. Cancer Res 84: 1613-1629, 2024.
71. Taifour T, Attalla SS, Zuo D, Gu Y, Sanguin-Gendreau V, Proud H, Solymoss E, Bui T, Kuasne H, Papavasiliou V, *et al*: The tumor-derived cytokine Chi3l1 induces neutrophil extracellular traps that promote T cell exclusion in triple-negative breast cancer. Immunity 56: 2755-2772.e8, 2023.
72. Kaltenmeier C, Yazdani HO, Morder K, Geller DA, Simmons RL and Tohme S: Neutrophil extracellular traps promote T cell exhaustion in the tumor microenvironment. Front Immunol 12: 785222, 2021.
73. Schauer C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, Lell M, Manger B, Rech J, Naschberger E, *et al*: Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med 20: 511-517, 2014.
74. Abel AM, Yang C, Thakar MS and Malarkannan S: Natural killer cells: Development, maturation, and clinical utilization. Front Immunol 9: 1869, 2018.
75. Zhang Y, Wang Z, Lu Y, Sanchez DJ, Li J, Wang L, Meng X, Chen J, Kien TT, Zhong M, *et al*: Region-Specific CD16+ neutrophils promote colorectal cancer progression by inhibiting natural killer cells. Adv Sci (Weinh) 11: e2403414, 2024.
76. Valayer A, Brea D, Lajoie L, Avezard L, Combes-Soia L, Labas V, Korkmaz B, Thibault G, Baranek T and Si-Tahar M: Neutrophils can disarm NK cell response through cleavage of NKp46. J Leukoc Biol 101: 253-259, 2017.
77. Peng YP, Zhang JJ, Liang WB, Tu M, Lu ZP, Wei JS, Jiang KR, Gao WT, Wu JL, Xu ZK, *et al*: Elevation of MMP-9 and IDO induced by pancreatic cancer cells mediates natural killer cell dysfunction. BMC Cancer 14: 738, 2014.
78. Yang S, Zou X, Li J, Yang H, Zhang A, Zhu Y, Zhu L and Zhang L: Immunoregulation and clinical significance of neutrophils/NETs-ANGPT2 in tumor microenvironment of gastric cancer. Front Immunol 13: 1010434, 2022.
79. Cheng Y, Gong Y, Chen X, Zhang Q, Zhang X, He Y, Pan L, Ni B, Yang F, Xu Y, *et al*: Injectable adhesive hemostatic gel with tumor acidity neutralizer and neutrophil extracellular traps lyase for enhancing adoptive NK cell therapy prevents post-resection recurrence of hepatocellular carcinoma. Biomaterials 284: 121506, 2022.
80. Demkow U: Molecular mechanisms of neutrophil extracellular trap (NETs) degradation. Int J Mol Sci 24: 4896, 2023.
81. Lazzaretto B and Fadeel B: Intra-and extracellular degradation of neutrophil extracellular traps by macrophages and dendritic cells. J Immunol 203: 2276-2290, 2019.
82. Jia W, Mao Y, Luo Q, Wu J and Guan Q: Targeting neutrophil elastase is a promising direction for future cancer treatment. Discov Oncol 15: 167, 2024.
83. Wang CL, Wang Y, Jiang QL, Zeng Y, Yao QP, Liu X, Li T and Jiang J: DNase I and Sivelestat ameliorate experimental hindlimb Ischemia-reperfusion injury by eliminating neutrophil extracellular traps. J Inflamm Res 16: 707-721, 2023.
84. Katzenelenbogen Y, Sheban F, Yalin A, Yofe I, Svetlichnyy D, Jaitin DA, Bornstein C, Moshe A, Keren-Shaul H, Cohen M, *et al*: Coupled scRNA-Seq and intracellular protein activity reveal an immunosuppressive role of TREM2 in cancer. Cell 182: 872-885.e19, 2020.
85. Canè S, Barouni RM, Fabbi M, Cuozzo J, Fracasso G, Adamo A, Ugel S, Trovato R, De Sanctis F, Giacca M, *et al*: Neutralization of NET-associated human ARG1 enhances cancer immunotherapy. Sci Transl Med 15: eabq6221, 2023.
86. Fu Y, Lin Q, Zhang Z and Zhang L: Therapeutic strategies for the costimulatory molecule OX40 in T-cell-mediated immunity. Acta Pharm Sin B 10: 414-433, 2020.
87. Gao F, Peng H, Gou R, Zhou Y, Ren S and Li F: Exploring neutrophil extracellular traps: Mechanisms of immune regulation and future therapeutic potential. Exp Hematol Oncol 14: 80, 2025.