R E V I E W

Neutrophil Extracellular Traps in Tumors and Potential Use of Traditional Herbal Medicine Formulations for Its Regulation

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Abstract: Neutrophil extracellular traps (NETs) are extracellular fibers composed of deoxyribonucleic acid (DNA) and decorated proteins produced by neutrophils. Recently, NETs have been associated with the development of many diseases, including tumors. Herein, we reviewed the correlation between NETs and tumors. In addition, we detailed active compounds from traditional herbal medicine formulations that inhibit NETs, related nanodrug delivery systems, and antibodies that serve as "guiding moieties" to ensure targeted delivery to NETs. Furthermore, we discussed the strategies used by pathogenic microorganisms to evade NETs.

Keywords: neutrophil extracellular traps, cancer, traditional herbal medicine formulations, nano delivery systems

Introduction

Millions of people worldwide die from cancer each year. The number of new cases is expected to reach 20 million per year by 2025.[1] In recent years, various types of cancer have continued to pose a threat to human life, and cancer has increasingly become a major issue for society. In recent years, there has been an increasing interest in the immune system's role in cancer progression and how it influences treatment response.[2] The innate immune system is reported to play a critical role in the fight against cancer by modulating immune function.[3]

Neutrophils constitute the largest population of innate immune cells. Their functional mechanisms (eg, phagocytosis, degranulation) are important tools in the innate immune response.[4,5] Neutrophils are thought to play a major part in cancer progression, the angiogenesis of tumors, and regulation of the immune response in tumors.[6] In addition to macrophages, it has also been suggested that polarization occurs in murine neutrophils, where they can also undergo polarization toward a protumor (N2) or antitumor (N1) phenotype.[7] The natural antitumor effects of N1 neutrophils are attributed to their production of reactive oxygen species (ROS) and antibody-dependent cellular cytotoxicity.[8] In addition, N1 neutrophils can activate cluster of differentiation (CD)8$^+$ T cells as well as dendritic cells (DCs), and may even present tumor-specific antigens to them.[9] N2 neutrophils contribute to cancer development by assisting re-building of the extracellular matrix (ECM), accelerating lymphangiogenesis and angiogenesis, and affecting immune function by producing cytokines that promote tumor growth.[10] Even though neutrophils are believed to polarize towards N1 or N2 phenotypes in vivo, the specific conditions under which this process occurs are unclear, and the phenotypic differentiation between human neutrophils has not been clearly demonstrated.[11]

Neutrophil extracellular traps (NETs) are reticular structures with deoxyribonucleic acid (DNA) as a skeleton and various proteins as surface "decorations".[12] NETs were first described by Brinkmann et al in 2004.[13] At first, they were found to capture and kill pathogens and bacteria, so were called "NETs".[14] NETs play a crucial role in infectious as well as non-infectious conditions and are also essential to the defense of the host. Although NETs have been described as beneficial in combating pathogens, their detrimental effects are only now becoming evident. NETs are involved in various pathological conditions, such as diabetes, wound healing, atherosclerosis, coagulation disorders, and periodontitis.[15,16] Excessive numbers of NETs also have a significant impact on cancer development (Please see the Graphic Abstract).[17]

NETs formation in human tissues was first demonstrated by histopathological analysis of Ewing sarcoma biopsy samples. Six of the eight tissue samples contained tumor-associated neutrophils, and two had NETs. It was found that the formation of NETs was closely associated with relapses and metastatic complications despite treatment with chemotherapy.[18,19] Several subsequent studies have shown the presence of NETs in peripheral blood and tumor samples collected from patients and animals with both primary and metastatic tumor. Neutrophils from mice suffering from lung cancer have been found to release NETs more frequently than those from healthy mice. Overproduction of NETs occurred in conjunction with intravascular coagulation induction and microvascular thrombosis in these animals.[20] NETs also are able to wrap and shield tumor cells from interactions with cytotoxic immune cells, resulting in impaired activity of $CD8^+$ T cells and NK cells.[21] Of course, NETs do not always play a bad role. The unexpected fact is that NETs themselves have the function of killing tumors. Because of the heterogeneity of tumor, the location of NETs formation matters. In the primary tumor niche, NETs are found to be pro-tumor, like their effects in more aggressive disease states.[15,22] Therefore, NETs-targeted therapies become more and more potential for therapeutic implications of cancer treatment.[23,24]

Right now, most cancers are treated with surgery, chemotherapy, radiotherapy, and immunotherapy. Among them, the use of chemotherapeutic drugs can be effective, however, it may result in adverse effects on normal cells, multidrug resistance, insufficient efficacy, and metastasis.[25] The limitations of conventional drugs have prompted researchers to explore alternatives. Accordingly, traditional herbal medicines (THMs) offer numerous advantages over synthetic drugs because of their diverse chemical composition, minimal toxicity to normal cells, low side effects, and accessibility, giving them the advantage of being both an effective and affordable alternative.[26] Furthermore, compared with chemical drugs, THMs may work through various mechanisms, including regulating cancer-related signaling pathways, inhibiting cell proliferation, causing cell apoptosis and autophagy. Same trend is also found on active ingredients from THMs, like baicalein and ginsenoside Rg3, not only inducing apoptosis and triggering autophagy of tumor cells, but also reducing the resistance of tumor cells to the chemotherapeutic drugs.[27,28] Because of the distinguish characterization of THMs, it was reported that 32% of all small molecule drugs approved between January 1981 and September 2019 were natural products or derivatives of natural products. Furthermore, 51% of all the 1211 small molecule drugs that were approved globally between 1981 and 2014 were derivatives of natural products.[29] A report published in 2016 revealed that the US Food and Drug Administration (FDA) approved 547 natural products and their derivatives for use as medications from 1827 to 2013. They are prescribed for treating various diseases, primarily different types of cancers, infections, and hypertension. Similarly, the study found that 68% of all 136 small-molecule drugs approved for cancer treatment between 1940 and 2014 were derived from natural products.[30]

However, the therapeutic potential of THMs restricted in most cases by their poor solubility in bodily fluids, low permeability, poor stability, and bioavailability. To achieve the maximized advantages of natural compounds, a variety of functional nanocarriers, such as liposomes, dendrimers, micelles, and nanoparticles have been developed,[31,32] aiming to improve half-life, especially for volatile compound, and achieve targeting drug delivery.[33–35]

Taking the NETs contribution to cancer progression into consideration, this review summarizes the relationship and specific molecular mechanisms between NETs and tumor-related events, aiming to provide information about recent advances in the application of THM-original compounds-based nanodelivery systems to target the NETs against tumor progress, invasion, and migration.

What are NETs?

Originally, it was believed that NETs formation occurred due to the death of neutrophil cells, referred to as NETosis. Later, researchers discovered that living neutrophils may also release NETs. Typically, this occurs after neutrophils are activated, then they become flat within a few minutes, their nuclear membranes disintegrate within an hour, chromatin decondensed, and the outer and inner nuclear membranes separate. The nucleoplasm and cytoplasm merge into homogenous clumps after the nuclear membrane separates into individual vesicles. Condensation and rounding of cells cause the cytoplasmic membrane to rupture, forming fibrous bundles called NETs.[36]

Structure of NETs

NETs have a reticular form which takes the depolymerized chromatin as the basic framework (Figure 1). NETs are strongly negative-charged web-like structures, embedded with various active proteins: histones, myeloperoxidase (MPO), neutrophil elastase (NE), cathepsin G, calreticulin, and proteinase 3 (PR3) (Table 1).[37–40]

Biomarkers of NETs

Studies have shown that NETs may exist in primary tumor tissue, metastatic tumor tissue, and/or the circulatory system of tumors. Perhaps this is because extracellular DNA enzymes degrade NETs into soluble products that are released into the peripheral bloodstream. Several studies have shown a correlation between infiltrated NETs in tumor tissue and NETs in peripheral blood.[41] Many markers are used in studies to visualize and assess NETs production, such as NE, MPO, cell-free DNA (cfDNA), MPO-DNA, high-mobility group protein B1 (HMGB1), nucleosomes, and H3Cit.[42–47] However, cfDNA is not NETs-specific. Apoptosis or necrosis can occur in any type of cell. Apoptosis causes extracellular DNA to be released in the form of apoptotic bodies (collapsed cell debris or large fragments), membrane-bound particles, or free DNA.[48] Notably, citrullination denotes the conversion of arginine residues in protein–peptide chains into citrulline residues under the action of PAD, which is an important process of protein post-translational modification.[49] For example, PAD4 catalyzes the H3Cit arginine residues (Arg 2, 8, 17), which is thought to be a special way to trigger neutrophils to respond to infection (ie, NETs formation). Therefore, H3Cit is the most specific biomarker for NETs.[45,46,50–52]

Where Does NETs Come from?

The production pathway of NETs is quite unique and different from that of autophagy, apoptosis, and necrosis.[53] Neutrophils can be stimulated to release NETs by various means, such as lipopolysaccharide (LPS), phorbol myristate acetate (PMA), complement 5a, complement 3a, interleukin (IL)-6, and IL8 (Table 2).[11] A type of cell death known as "NETosis" results in the release of NETs, and the stimulation method determines how NETs are produced.[54,55] In addition, recent studies have shown that autophagy is also involved in regulating NETs production.[56] The phagocyte receptor separates NE from the nucleus by promoting phagosome formation, so NE drives chromatin depolymerization by processing histones.[57] In general, there are two ways of generating NETs.
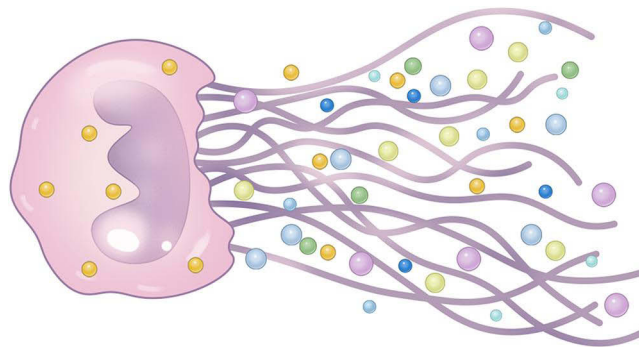

Figure 1 Structure of NETs.

Table 1 Structure of NETs

Protein Type	Composition
Primary particle	Myeloperoxidase, neutrophil elastase, cathepsin G, defensins
Secondary particles	Alkaline phosphatase, lactoferrin, lysozyme, Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), collagenase
Tertiary particles	Cathepsin, matrix metalloproteinases
Histone	H1/H2A/H2B/H3/H4

Table 2 Stimulators of NETs Formation

Inductive Agent	NETosis	Receptor	Ref.
LPS	Vital NETosis	TLR4	[58,59]
PMA	Suicide NETosis	PKC	[11,60–62]
C5a	Vital NETosis	C5aR	[58,59,63–65]
C3a	Vital NETosis	C3aR	[66]
IL-6	Suicide NETosis	CXCR1/2	[67]
IL-8	Suicide NETosis	CXCR1/2	[60,64,68]
GM-CSF	Vital NETosis	GM-CSFR	[63]
G-CSF	Vital NETosis	C-SFR	[20]
TNF-α	Suicide NETosis	TNF-αR	[67,69]
HMGB1	Suicide NETosis	TLR4/RAGE	[70,71]
Calcium ionophore A23187	Vital NETosis	Ca^{2+}	[72]
Glucose	Suicide NETosis	–	[73]
Pathogenic microorganisms	Suicide NETosis/vital NETosis	–	[54,74]

Abbreviations: C3aR, complement 3a receptor; C5aR, complement 5a receptor; CFSR, colony-stimulating factor; CXCR, CXC chemokine receptor; GM-CSF, granulocyte-macrophage colony stimulating factor; GM-CSFR, granulocyte-macrophage colony stimulating factor receptor; G-CSF, granulocyte colony-stimulating factor; HMGB1, high-mobility group protein B1; RAGE, receptor for advanced glycation end products; TNF-α, transforming growth factor α; TNF-αR, transforming growth factor α receptor.

One way is through suicidal NETosis, in which the rupture of nuclear and plasma membranes and chromatin depolymerization are involved.[75] This pathway dependents upon nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), which produces ROS.[76,77] Initial stimulation of neutrophils activates NOX and Raf/mitogen-activated ERK kinase/extracellular signal-regulated kinases (Raf/MEK/ERK) signaling via protein kinase C (PKC) signaling, which subsequently stimulates ROS production. After that, citrullinated histone-3 (H3Cit) is induced by the calcium-dependent enzyme peptidyl arginine deiminase (PAD)4 present in the nucleus of neutrophils,[78] resulting in chromatin depolymerization.[39,79,80] Neutrophils release NETs within 2–4 h of being activated, and then they usher in their own death.[72,81,82]

PMA is a classic suicidal NETosis stimulus that activates PKC and Raf/MEK/ERK pathways. During this time, many ROS are generated rapidly in the cell, which rupture and dissolve granule and neutrophil membranes. Rupture of the granule membrane releases many protein components into the cytoplasm, such as NE, MPO, matrix metallopeptidase 9 (MMP9), and cathepsin G. The released NE and MPO contribute to permeabilization of the nuclear membrane and unfolding of intracellular chromatin.[83] Some reports have indicated that cyclin-dependent kinase-4 and −6 are also involved in the dissolution and rupture of nuclear and cell membranes.[84] Receptor-interacting protein kinase 3 (RIPK3), mixed lineage kinase domain-like protein (MLKL), and necroptotic factors are also thought to mediate NETs formation.[85] ROS-dependent neutrophil necroptosis drives NETs generation through the formation of surface MLKL.[86] After stimulation, RIPK3 is "turned on" in the nucleus and mediates MLKL activation, rupturing the nuclear envelope and allowing DNA to leak into the cytoplasm.[87] RIPK1 and caspase-8 have been shown to have negative regulatory roles in producing RIPK3/MLKL-mediated NETs.[86,87] PKC activation also contributes to the activation of peptidyl arginine deiminase 4 (PAD4) and then H3Cit,[88] which is considered to be the molecular feature of NETs formation.[89] Eventually,

this process leads to chromosome depolymerization and the generation of a many DNA fibers. Afterwards, under the combined action of ROS and various proteases, the neutrophil membrane ruptures and releases the chromatin-fiber network attached to various proteins, such as NE and MPO.

The other pathway for generating NETs is called vital NETosis.[60] Vital NETosis is a mild method to generate NETs. In this pathway, NETs production is activated by microbe-specific molecular patterns of pathogen-associated molecular patterns or endogenous damage-associated molecular patterns recognized by host recognition receptors.[90] When complement proteins or toll-like receptors (TLRs) in neutrophils "sense" the presence of pathogens, they stimulate NETs production.[66] This process involves chromatin transportation by vesicles and the "resealing" of the cell membrane,[91] which signifies that NETs production does not kill neutrophils.[83] Bryan et al[92] observed that NETs contain intracellular vesicles and cell-membrane ingredients of human neutrophils upon infection by Gram-positive bacteria, which showed that chromatin was delivered to the extracellular space without cell-membrane rupture.

Vital NETosis is distinct from suicidal NETosis dependent upon the production of NADPH-mediated ROS.[93] Pilsczek et al[94] reported that neutrophils rapidly release vesicles containing nuclear DNA in vitro. Speziale described the pathway of NETs production by *Staphylococcus aureus* infection through complement receptors and TLR-2 ligands, which are independent of activation of NADPH enzymes.[95] In addition to releasing nuclear DNA, neutrophils release mitochondrial DNA (mtDNA) to generate NETs.[90,96] Mitochondria also play an important part in this NADPH-independent pathway[97] because they can release mitochondrial-derived ROS,[72] which then mediate the extracellular release of mtDNA to generate NETs without cell rupture.[63,98] Vital NETosis occurs much faster than suicidal NETosis: vital NETosis takes only 5–60 min to release NETs. Interestingly, after generating NETs by neutrophils through vital NETosis, they continue to survive and retain relevant immune activity.[92,99]

NETs in Cancer

The relationship between NETs and cancer is incompletely understood. Many NETs were first detected in the tumor tissue of patients with Ewing's sarcoma and found to be strongly associated with a poor prognosis.[19] Pastor et al[100] used MPO, NE, and circulating DNA as markers for NETs. They found that the levels of these markers were positively correlated with the probability of a diagnosis of metastatic colorectal cancer, suggesting that NETs are important players in the occurrence and development of cancer. In one study, enzyme-linked immunosorbent assays of MPO-double-stranded DNA were used to visualize NETs. These NETs were found to be related to the disease stage of patients with lung or upper-gastrointestinal (esophagogastric) adenocarcinoma. The level of NETs in patients with advanced (stage III and IV for lung adenocarcinoma, and stage II and III for upper-gastrointestinal adenocarcinoma) was significantly higher than that in patients with local tumors (stage I and II).[22] One study observed more NETs in the peripheral blood of patients with ventricular aneurysm.[101] Simultaneously, multiple signaling pathways (eg, Smad, mitogen-activated protein kinase (MAPK), Ras homolog gene family, member A (RhoA)) were activated, and the development of cardiac fibrosis was observed, which was considered to denote aggravation of ventricular aneurysms.[101]

In addition, certain factors in the tumor microenvironment associated with NETs, such as IL-8, IL-17, granulocyte colony-stimulating factor (G-CSF), and CXC chemokine receptor (CXCR), are also directly or indirectly involved in cancer progression.[102–104] For example, IL-8 stimulates the aggregation and apoptosis of neutrophils but also participates in NETs generation, resulting in deterioration of the disease.[104,105] Nie et al[106] found that IL-8 secreted by diffuse large B cell lymphoma (DLBCL) stimulated NETs production through Src, p38, and ERK pathways, and NETs, in turn, upregulated TLR9 expression from DLBCL, which finally activated nuclear factor-kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), and p38 pathways to promote tumor progression.

Some proteins of NETs are also thought to be involved in tumor progression. NE and MMP9 have been shown to be actively involved in cancer progression.[62,107,108] NE is a serine protease. It is an important part of NETs but also involved in NETs formation.[109] NE is actively involved in the genesis and growth of tumors.[110] The NE-specific small-molecule inhibitor sivelestat has been shown to reduce the growth of prostate-cancer xenografts in athymic mice.[111]

NETs have been suggested as potential therapeutic targets in several tumor types (Table 3). Accordingly, various approaches have emerged to fight against NETosis or eliminate NETs, such as degrading the DNA skeleton, inhibiting expression of PAD4 or NE, targeting cathepsin C, blocking or interfering with signaling pathways.[112–121] However recent

Table 3 Effect of NETs on the Occurrence and Development of Tumors

Tumor Type	Effects of NETs	Mechanism	Ref.
Breast cancer	Promote tumor metastasis	Activate NF-κB signaling and promote human breast cancer	[68]
		Promote migration of breast cancer cells through AKT and STAT signaling pathways	[103]
	Awaken dormant tumor cells	Awakening of dormant tumor cells and cell arousal are associated with NETs-led ECM remodeling	[125]
Colorectal cancer (CRC)	Promote tumor metastasis	Stimulate IL-8 production in CRC cells, while overproduced IL-8, in turn, activates NETs formation and NETs-mediated tumor metastasis	[105]
	Induce cancer-associated thrombosis	Platelet-induced NETs from patients with gastric cancer contribute to procoagulant activity in patients with CRC	[126]
Oral squamous cell carcinoma (OSCC)	Promote tumor metastasis	Accelerate the transformation from oral lichen planus to OSCC	[59]
Pancreatic cancer	Promote tumor metastasis	Mediate NETs to induce EMT	[61]
		Promote EMT, migration and invasion through the IL-1β/EGFR/ERK pathway	[127]
Nonalcoholic steatohepatitis-related hepatocellular carcinoma (NASH-HCC)	Promote tumor growth	Regulate Tregs through metabolic reprogramming, and create an immunosuppressive hepatic microenvironment that favors the development and growth of tumor cells in the liver of patients with NASH	[128]
Cholangiocarcinoma	Suggests adverse prognosis	A high level of NETs can be used as a marker of a poor prognosis for cholangiocarcinoma	[129]
Gastric cancer (GC)	Promote tumor metastasis	Regulate TGF-β signaling to activate EMT and promote GC metastasis	[130,131]
Epithelial ovarian cancer (EOC)	Promote tumor metastasis	Bind to ovarian tumor cells and promote metastasis	[132]
Small-cell lung cancer (SCLC).	Induce cancer-associated thrombosis	cfDNA as a marker of NETs has been linked to SCLS-associated venous thromboembolism	[133]
Hepatocellular carcinoma (HCC)	Suggests adverse prognosis	A high level of NETs can be a marker of a poor prognosis for hepatocellular carcinoma	[129]
	Promote tumor metastasis	Capture HCC cells and trigger tumor metastasis by activating TLR4/9-COX2 signaling	[134]
Bladder cancer (BC)	Induce immune killing	Bacillus Calmette–Guerin vaccine-stimulated NETs contribute to the recruitment of T cells and macrophages to inhibit tumor growth	[135]
Abdominal aortic aneurysm (AAA)	Promote tumor growth	Early AAA promotes IL-1β release by neutrophils and induces NETs production, which accelerates AAA progression	[136]
Glioma	Promote tumor growth	Mediate glioma progression through the HMGB1/RAGE/IL-8 axis	[137]
Anaplastic thyroid cancer (ATC)	Promote tumor growth	ATC-induced neutrophils directly maintain the viability of tumor cells in a NETs-dependent manner	[138]
Ulcerative melanoma	Kill tumor directly	Adhere to tumors via integrins and inhibit the migration and invasion of tumor cells	[124]
Gallbladder cancer (GBC)	Induce cancer-associated thrombosis	Accelerate the formation of tumor-associated blood clots and aggravate the extent of tumor-induced harm	[139]

Abbreviations: AKT, protein kinase B; COX, cyclooxygenase; cfDNA, cell-free DNA; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ERK, extracellular regulated protein kinases; HMGB1, high-mobility group protein B1; IL, interleukin; NETs, neutrophil extracellular traps; NF-κB, nuclear transcription factor-κB; RAGE, receptor of advanced glycation end products; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TGF-β, transforming growth factor β.

studies have also revealed another side of NETs: blockade of tumor progression. NETs have been reported to induce the killing of immune cells. NETs induced by a cigarette-smoke extract in vitro can drive the maturation and activation of plasmacytoid DCs (eg, CD40, CD86) and initiate a T cell-mediated immune response.[122] It was reported that the injection of NETs-DNA and NETs protein into subcutaneous tumors resulted in greater recruitment of T cells, which

led to cancer-cell death and a reduced tumor volume.[123] NETs may also be directly involved in the killing of tumor cells.[124] Few reports have focused on the active anti-tumor effect of NETs, but it cannot be ignored because it suggests a "third way" to treat NETs-related tumors (ie, reliance on NETs) in addition to inhibiting NETosis and eliminating existing NETs.

Relationship with Tumor Metastasis

NETs are actively involved in tumor metastasis, which is the main factor of tumor-associated death (Figure 2).[22,140–142] Studies have suggested that tumor metastasis is related to epithelial–mesenchymal transition (EMT). Specifically, epithelial tumor cells lose their ability to adhere and gain the ability to migrate to mesenchymal cells through EMT.[143] NETs have been reported to enhance the migration of and invasion by tumor cells by inducing EMT,[144,145] but thrombomodulin can degrade HMGB1 and NETs components to inhibit EMT, thereby reducing the risk of tumor-cell migration.[61] NETs also promote tumor metastasis by degrading thrombospondin-1 (TSP-1), which is an important inhibitor of metastasis.[114] In addition, due to their special structure, NETs have an outstanding ability to capture tumor cells and promote adhesion between tumor cells and endothelial cells (ECs).[11,112] Then, NE derived from NETs increases the permeability of ECs through vascular endothelial-cadherin proteolysis,[145] and causes damage to ECs,[146] making it easier for tumor cells to break through the vascular wall and exudate to distant organs.[11,112] Capture of tumor cells by NETs could also enable them to avoid immune surveillance by CD4$^+$ T cells, further preventing them from being destroyed by CD8$^+$ T cells and natural killer (NK) cells.[128,147]

Furthermore, NETs, tumor cells, NETs-related cells, factors, signaling pathways, and genes, may form an optimized positive loop that promotes tumor metastasis. In a mouse model of spontaneous breast cancer, researchers found that NETs mediated NF-κB activation and upregulated expression of downstream genes related to the growth and metastasis of tumor cells, such as cyclin D1, zinc finger protein SNAI1 (SNAIL), IL-8, and IL-1β. Expression of these factors activated neutrophils to release NETs.[68]

Some proteins expressed by tumors interact with NETs and promote metastasis. The transmembrane protein coiled-coil domain containing 25 (CCDC25) on tumor cells was thought to be attracted by NETs and cause distal metastasis of tumor cells because NETs-mediated metastasis of tumor cells did not occur after knockout of CCDC25 expression.[148] Circulating tumor cells (CTCs) derived from primary tumors have also been considered to be important factors in promoting metastasis.[62,149] Specifically, tumor-derived CTCs activate expression of the serine PR3 on neutrophil membranes, which leads to IL-1β activation in lung neutrophils and stimulates ROS generation through the p38/MAPK signaling pathway to induce NETs.[114] In addition, CTCs in blood undergo mesenchymal–epithelial transition
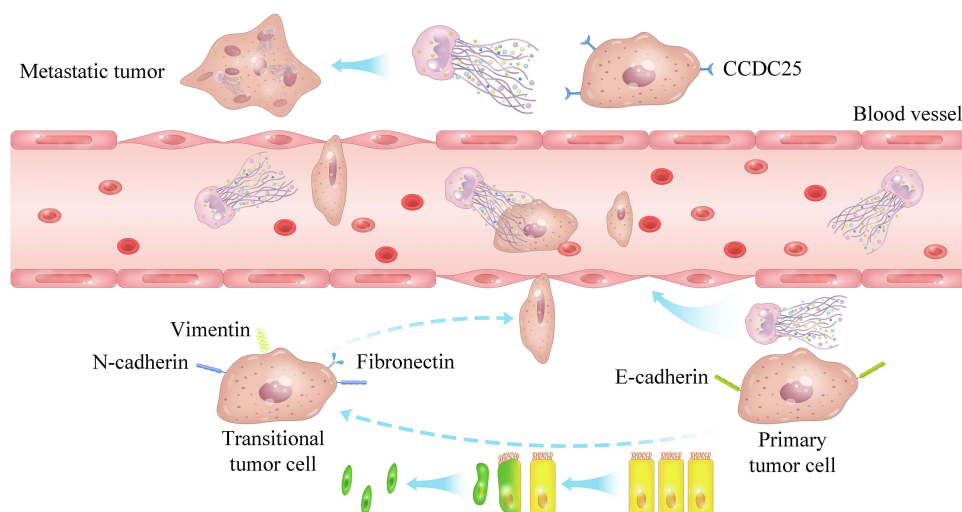

Figure 2 How NETs promote tumor metastasis.

after reaching other body parts through the blood circulation, regain the adhesion ability of epithelial tumor cells, and form metastases.[150]

Relationship with Dormant Tumor Cells

Tumor cells from a primary tumor tend to spread to other locations. During this process, T cells and NK cells destroy them if they start to proliferate and may lead them into a dormant state. However, tumor cells in the dormant state cannot be detected clinically.[151] NETs have been shown to "awaken" dormant tumor cells to cause tumor recurrence and promote their malignant growth and spread.[18,146,152] Albrengues et al[144] found that inflammation induced by tobacco smoke and LPS promoted the invasive lung metastasis of dormant tumor cells. NE and MMP9 from NETs induced the hydrolysis of laminin (important component of the ECM). This process involves activating the signal transduction of integrin $\alpha_3\beta_1$ and awakening dormant tumor cells to proliferate through the focal adhesion kinase/ extracellular regulated protein kinases/mixed lineage kinase domain-like protein/yes-associated protein 1 (FAK/ERK/MLCK/YAP) signaling pathway.[144] In addition, they also found that NE and MMP9 hydrolyzed TSP-1, which inhibits tumor awakening and metastasis.[144] Treatment with PAD4 inhibitors or DNA enzymes inhibited NETs formation and prevented the activation and metastasis of dormant tumor cells (Figure 3).[144] Unfortunately, data on NETs awakening and promoting the development of dormant tumor cells are scarce, and the research in this area must be deepened.

Relationship with Cancer-Associated Thrombosis (CAT)

CAT is a major indicator of patient mortality, and NETs are active participants in CAT (Figure 4).[153–156] Thrombosis depends upon the adhesion, activation, and aggregation of platelets. Fibrin and von Willebrand factor (vWF) provide a "scaffold" for platelet adhesion.[157,158] The stability of a thrombus in CAT events is also dependent upon the support
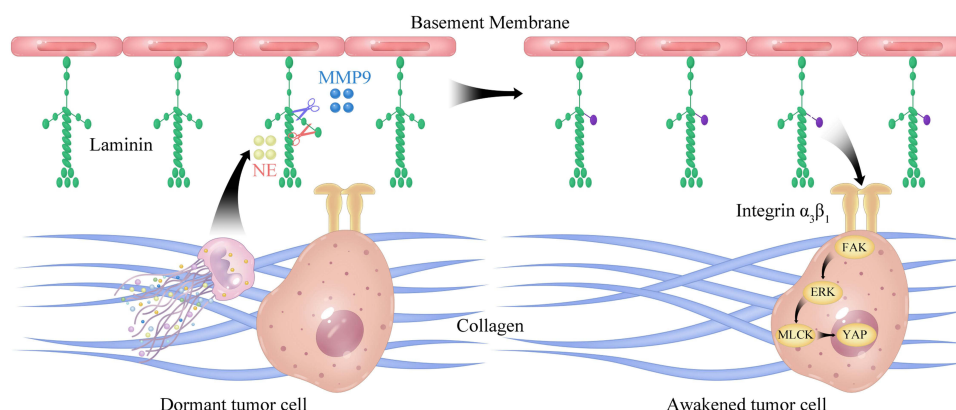

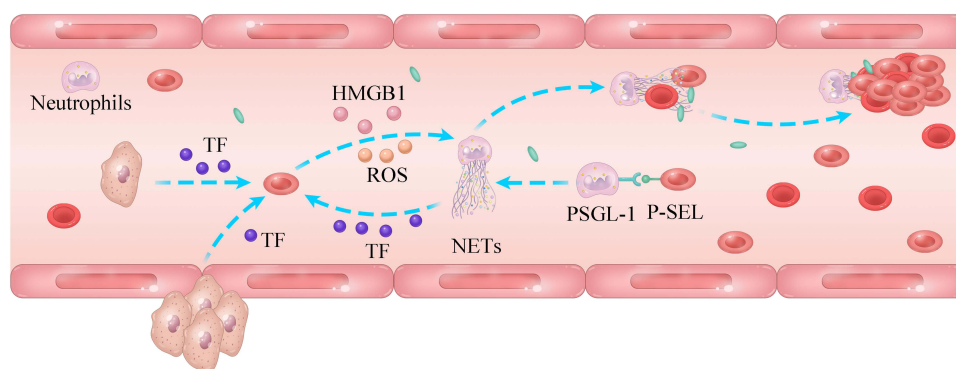**Figure 3** NETs awakening tumor dormant cells.

Figure 4 Role of NETs in cancer-related thrombosis.

provided by NETs. NETs can damage ECs,[15] which release vWF.[159] Adhesion of many NETs onto the vascular system (vWF-mediated) can cause thrombosis by providing "stents" for the adhesion and activation of platelets and thrombin production.[112] In addition, NETs bind to plasma proteins such as fibrinogen, vWF, and fibronectin; they also contribute to the development of arterial thrombosis.[160,161] Fuchs et al initially reported the relationship between NETs and thrombosis. For the first time, they disclosed that NETs provide stents for thrombus formation.[162] Evidence for the prothrombotic capacity of NETs was based on the finding that NETs entangle tumor cells and allow them to spread.[153] The G-CSF level was found to be increased significantly (NETosis) in mice bearing human pancreatic BxPc-3 tumors.[163] Moreover, an increased thrombin density and denser fibrin were observed in tumor-bearing mice; unsurprisingly, DNase I administration reduced the thrombus weight significantly.[163]

Furthermore, tissue factors (TFs) are important participants in CAT. TFs trigger thrombosis by activating the coagulation cascade.[162,164] Tumor cells are thought to have high expression of TFs,[165–167] which are the key to platelet activation.[168] Activated platelets can promote NETs production through an interaction between P-selectin (P-SEL) expressed on the surface on cells and P-selectin glycoprotein ligand-1 expressed by neutrophils,[169] but also release stored HMGB1, which can combine with TLR4 expressed by neutrophils to stimulate NETs production.[71] Surprisingly, NETs also mediate the release of TFs to accelerate thrombosis.[170,171] The specific mechanism may be that NE and cathepsin G derived from NETs inhibit the signaling pathway that inhibits TFs.[172] In receptor for advanced glycation end products (RAGE)-knockout mice bearing pancreatic cancer, the serum levels of TFs were reduced, and NET-induced platelet accumulation was decreased significantly.[173] Therefore, NETs are expected to become new targets for treating cancer-related thrombosis, hopefully improving the prognosis and survival chances of patients suffering from cancer.

Relationship with Cancer Drug Resistance

In addition to actively participating in tumor development, metastasis, and awakening dormant tumors, NETs also mediate drug resistance during tumor chemotherapy, radiotherapy, and immunotherapy (Figure 5). Dying cancer cells induce neutrophil recruitment and release IL-1β, which immediately stimulates neutrophils to release NETs. NETs-related integrin-$\alpha_v\beta_1$ and MMP9 lead to the emergence of subsequent EMT and drug resistance through TGF-β activation.[174] The clinical use of DNase (Pulmozyme®) targeting extracellular DNA restored the sensitivity of myeloma cells to doxorubicin, thus prolonging the survival time of tumor-bearing animals.[175] Besides of chemo drug-related resistance, it was found that radiation induced NETs production in a TLR4-dependent manner by stimulating the release of HMGB1 from bladder tumor cells, while NETs may prevent the infiltration of CD8$^+$ T cells in tumor cells to promote radiotherapy resistance, which could be attenuated by administration of NE inhibitors (NEi) and DNase I.[176] IL-17 was uncovered to be the main cause of drug resistance in pancreatic cancer treatment, which is responsible for recruiting neutrophils and triggering NETs. IL-17 promotes the inactivation of CD8$^+$ T cells by stimulating NETs production, thus reducing the sensitivity of checkpoint blockade, that is, drug resistance.[105]

Targeting NETs
THMs Targeting NETs

Several studies have reported that THMs can be used to treat immune system-related diseases.[177–179] The anti-NETs active substances from THMs have been structurally characterized (Table 4). These active ingredients are categorized into three groups based on their reactions.

The first group of active ingredients inhibits the proteins of NETs (histone, NE and MPO) or proteins that affect the production of NETs.[194] Using PAD4 (mediating histone citrullination), NE or MPO inhibitors have been observed conclusive evidence of the reduction of NETs.[195–199] An example is andrographolide, the active component of *Andrographis paniculate*. Andrographolide can inhibit PMA-induced upregulation of PAD4 expression, reverse citrullination of histone-3, and inhibit autophagy of neutrophils and NETs production. The second group is to inhibit NETs generation by blocking ROS from NADPH or mitochondria, which is an important mediator in NETs generation. Flavonoids were found to inhibit ROS formation and block the formation pathway of NOX-dependent NETosis. The third group is signaling-pathway inhibitors in NETs generation. The signaling pathways involved in the generation of
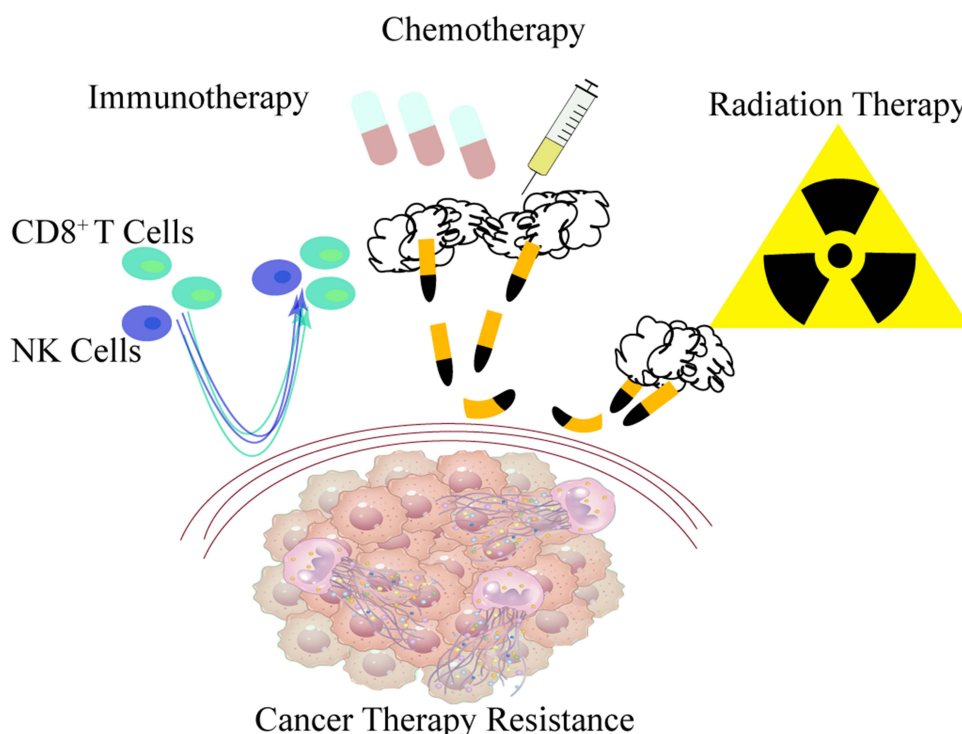
Figure 5 Role of NETs in cancer drug resistance.

NETs are relatively complex. Typical examples are safflor yellow A, salvianolic acid B, and anhydrosafflor yellow B, which are the main three active ingredients of Xuebijing injection. They inhibit NETs generation by blocking the Raf/MEK/EPK signaling pathway.

The active ingredients of THMs are worth investigating. However, combining these active ingredients of THMs to develop new preparations with higher drug loading, higher bioavailability, stronger targeting, and more convenient drug delivery is a major challenge.

Nanomedicine Preparations for Targeting NETs

An excessive number of NETs is closely related to the occurrence and development of tumors and various autoimmune diseases. Hence, inhibiting NETosis or eliminating existing NETs could be a strategy against NETs. Since NETs were found to be linked to tumors, multiple approaches have been employed to inhibit NETs to curb tumor metastasis. These include DNase I (degrades the skeleton of existing NETs) or inhibitors of PAD4, NE, MPO, or H3cit (inhibit NETs production). However, the use of most of these inhibitors has ceased in the clinical stage due to low oral bioavailability and poor targeting. Therefore, improving the inhibitory effect and targeting ability of these inhibitors is a considerable challenge.

However, the rapid development of nanotechnology has overcome most of the shortcomings of non-specific chemotherapeutic drugs. The use of nanoparticles in cancer treatment is advantageous because it can increase the half-life of drugs in circulation and delivery to tumor cells in a precise and controlled manner while sparing healthy cells from toxicity.[200–202] Nanoparticles (size > 5 nm) can accumulate passively at the tumor site due to weakened barrier integrity, a condition referred to as enhanced permeability and retention (EPR).[203,204] Furthermore, nanoparticles conjugated with active targeting ligands dramatically increase the concertation of formulation in the targeting sites.[205]

In recent years, functional nanoparticles have become a better choice for anti-tumor therapies.[206] For example, DNase I degrades the structure of NETs and is hoped to resist NETs-mediated tumor progression. However, its short half-life in plasma and poor stability hampers the ability of DNase I to block NETs-mediated tumor progression.[197,207] To address

Table 4 Traditional Herbal Medicine Targeting NETs

Target	Active Ingredients	Source	Route of Application	Model	Dose	Mechanism	Disease	Ref.
Protein	Andrographolide	*Andrographis paniculata*	i.p.	C57BL/6 mice	25, 50 mg/kg	Inhibited PMA-induced upregulation of PAD4 expression, reversed histone-3 citrullination, and inhibited autophagy of neutrophils and NETs production.	Rheumatoid arthritis	[180]
	HMEI-A (self-naming)	*Hirudinaria manillensis*	Co-incubation	PMNs	10 μg/mL	Inhibit NETs formation.	Inflammation	[181]
	Chikusetsusaponin V	*Panax japonicus*	p.o.	C57BL/6 mice	50, 100, 200 mg/kg	Inhibit NETs formation by reducing HMGB1 expression.	Acetaminophen-damaged liver	[182]
	Polysaccharide (KSWP)	*Kochia scoparia*	i.g.	ICR mice	300 μL (5 g/kg)	Selective inhibition of human neutrophil elastase and significant inhibition of NETs formation in a dose-dependent manner.	Acute lung injury	[183]
	-	SiWuTang	p.o.	C57BL/6 J mice	2.6, 5.2, 10.4 g/kg	Inhibits NETs production by reducing cathepsin, elastase, and MPO levels.	Bile duct ligation-induced liver fibrosis	[184]
ROS	Polydatin	*Polygonum cuspidatum*	i.p.	DBA/1 mice	45 mg/kg	Inhibit NETs production by blunting ROS production.	Rheumatoid arthritis; collagen-induced arthritis	[185]
	Sal B and DHT I	Danshen	i.p.	BALB/c athymic nude mice	780 mg/kg	Block the activity of MPO and NADPH to disrupt NETs formation.	Tumor	[186]
	Gingerol	Ginger	Co-incubation	PMNs	1–10 μM	Inhibits NETosis by blunting ROS production.	Lupus and antiphospholipid syndrome-related NETs	[187]
	TTC	*Celastrus orbiculatus*	Co-incubation	PMNs	5–80 μg/mL	Inhibits ROS and blocks NETs generation in a NOX-dependent pathway.	Inflammation	[188]
	Epicatechin, catechin hydrate, rutin trihydrate	–	Co-incubation	PMNs	4–100 μM; 4–100 μM; 0.1–150 μM	Inhibit the formation of ROS-dependent NETs.	NETs- related diseases	[189]
	Quercetin	–	i.p.	C57BL/6 mice	30 mg/kg	Inhibits neutrophil infiltration and ROS production through autophagy-dependent pathways.	Rheumatoid arthritis	[190]

(*Continued*)

Table 4 (Continued).

Target	Active Ingredients	Source	Route of Application	Model	Dose	Mechanism	Disease	Ref.
Signaling pathway	–	ReDuNing injection (approval number Z20050217)	i.p.	C57BL/6 Mice	5, 10 mL/kg	Inhibits NETs generation by blunting the MAPK pathway.	Acute lung injury	[191]
	SYA, HSYA, AHSYB	Xuebijing injection (approval number Z20040033)	i.p.	C57BL/6J mice	5×10^{-5} mol/kg	Inhibits the production of inflammatory factors, infiltration of inflammatory cells, and NETs production by inhibiting activation of TLR signaling.	Acute lung injury	[192]
	–	Ephedra–almond herbal pair	Co-incubation	PMNs	0.5–8 mM	Inhibits NETs formation by blunting expression of the HMGB1/TLR4 pathway.	Asthma	[193]

Abbreviations: AHSYB, anhydrosafflor yellow B; DHT I, 5,16-dihydrotanshinone; HSYA, hydroxysafflor yellow A; HMGB1, high-mobility group protein B1; i.g., intragastric; i.p., intraperitoneal injection; KSWP, polysaccharide of *Kochia scoparia*; MPO, myeloperoxidase; MAPK, mitogen-activated protein kinase; NADPH, nicotinamide adenine dinucleotide phosphate; NETs, neutrophil extracellular traps; NOX, NADPH oxidase; PAD, peptidyl arginine deiminase; PMA, phorbol Myristate Acetate; PMNs, polymorphonuclear cells; p.o., peros; ROS, reactive oxygen species; Sal B, salvianolic acid B; SYA, safflor yellow A; TLR, toll-like receptor; TTC, total terpenoids of *Celastrus orbiculatus*.

this issue, researchers encapsulated DNase on the surface of nanoparticles. They achieved a more stable and longer plasma half-life than those in the free-DNase group. Under identical conditions, compared with the free-DNase group, the nanoparticle group had a higher drug concentration in the blood and lead to tumor inhibition.[141] The research status of nanomedicine preparations targeting NETs is summarized in Table 5. Three main strategies for combating NETs have been reported: elimination of existing NETs, inhibition of NETosis and inhibition of neutrophils (the cells that produce NETs).

Besides, multidrug-combination nanomedicine preparations against NETs-related tumors are also an option. DNase–chemotherapy agent multifunction nanomedicine preparations could achieve synergistic antitumor effects. Recently, preparations with paclitaxel (as the core) and a polymorphic sequence of cell-penetrating DNase I-substrate peptides (as the shell) have achieved good results in inhibiting the growth and metastasis of malignant tumors.[209]

However, most anti-NETs nanomedicine preparations are aimed at degrading the DNA structure of NETs. In contrast, research on nanomedicine preparations for some proteins, TFs, and signaling pathways involved in NETosis and multidrug combination therapy is scarce. The study of preparations targeting NETs specifically is even rarer. Hence, some examples of antibodies targeting NETs are provided below. We hope that these antibodies can be used for studying specifically targeted NETs preparations in the future.

Progress in Antibody Research for Targeting NETs

Antibodies that target NETs specifically for application in against NETs-involved diseases are summarized in Table 6. These antibodies can be divided into three groups. One group targets histone, such as antibody 7C10, which targets H3cit. The latter is a specific marker to produce NETs. Mouse-derived antibody 7C10 can bind specifically to H3cit at positions 2, 8, and 17. More importantly, 7C10 does not react with mono- or bis-H3cit. The second group targets proteins on NETs, such as PAD4. The participation of PAD4 is essential for generating NETs. PAD4 is involved in catalyzing the arginine residues of H3cit. The rabbit polyclonal antibody ab50247 can bind specifically to the 74-kDa PAD4 protein and does not cross-react with other subtypes. The third group binds specifically to the intact nucleosome (the main component of NETs). Monoclonal antibody 2C5 can elicit nucleosome-restricted specificity. Conjugating antibodies to nanoparticles can lead to a better effect in degrading NETs. 2C5-modified DNase I micelles can target and eliminate NETs; this is a good example of employing antibodies as targeting moieties for nanomedicines against NETs.[229]

Taking Advantage of NETs Anti-Tumor Properties

In tumor-related diseases, neutrophils can exert positive and negative effects depending on the neutrophil subtype (N1 or N2). N1 shows anti-tumor activity, while N2 shows tumor-promoting properties. TGF-β and interferon-β have a serious impact on the expression of neutrophil subtypes. TGF-β leads to transformation from the N1 phenotype to the N2 phenotype. Interferon-β induces the change in the opposite direction. Moreover, NETs are the most important way for N2 neutrophils to exert negative effects.[75] Previously, it has been reported that NETs promote tumor development, and high levels of NETs have been associated with a low chance of survival and poor prognosis in patients with cancer.[244,245] Hence, must NETs be synonymous with bad outcomes? Some components of NETs, such as NE and MPO,[246,247] have been shown to have antitumor activity. Therefore, developing therapies that employ NETs to fight against tumors may be a feasible strategy.[248]

A recent study by Schedel et al found that melanoma cells adhered to NETs via integrins, and that NETs inhibited melanoma metastasis because co-cultured NETs had a cytotoxic effect on melanomas.[124] Another study showed that NETs induced by bacillus Calmette–Guerin (BCG) inhibited tumors by attacking tumor cells and increasing the infiltration of immune cells.[135] NETs may be cytotoxic to capture hepatocellular carcinoma cells and may limit their metastasis.[134,249] Arelaki et al conducted in vitro experiments to study the effect of NETs on the progression of solid tumors when they are co-cultured with a colon tumor cell line (Caco-2).[250] NETs were induced by PMA and septic serum-induced apoptosis in a concentration-dependent manner, and DNase I or heparin could attenuate this effect.

The "ideal" drug carrier should have good biocompatibility, be non-toxic, biodegradable, and avoid rapid clearance in vivo. Employing living cells as drug carriers have recently attracted attention. Natural cells have incomparable biocompatibility and targeting ability. Using the functional properties of neutrophils to release NETs in a targeted

Table 5 Nanomedicine Preparations Used to Target NETs

Target	Nanomedicine Preparation	Preparation Type	Route of Application	Model	Dose	Curative Effect	Disease	Ref.
DNA	ALG-SNase	NPs	p.o.	C57BL/6 mice	25 or 75 mg/kg	Degrade the DNA skeleton of NETs to reduce the amounts of NETs.	Ulcerative colitis	[208]
DNA	mP-NPs-DNase/PTX	NPs	i.v.	BALB/c nude; ICR mice	10 mg/kg	Release DNase I in response to MMP9 to degrade NETs structure.	Non-small cell lung cancer and breast cancer	[209]
DNA	GODM-gel	Hydrogels containing nanoparticles	ext.	BALB/c mice SD rats New Zealand white rabbits	0.5 mL	Neutralize the acidity of tumors and lyse NETs to enhance the killing function of NK cells.	HCC	[210]
DNA	PLGA-PD-DNase I	NPs	i.p.	BALB/c mice P47 phox$^{-/-}$ mice ACTB-ECFP mice C3-Tag mice Lysm-EGFP mice	75 U/mouse	Degrade NETs structure to inhibit tumor metastasis.	Lung metastasis of breast cancer	[141]
DNA	PLGA-PD-PEG-DNase1-NPs	NPs	i.v.	C57BL/6 mice	100 U/mouse	Reduce the cfDNA level, inhibit NETs production, NF-κB activation and cytokine secretion.	SARS-CoV-2 sepsis	[211]
DNA	PAAP/DNase I	NPs	i.v.	BALB/c mice	70 mg/kg	Induce tumor-cell apoptosis and decompose NETs-DNA to prevent NETs-induced tumor metastasis to the liver.	Liver metastasis in CT26 colon cancer and 4T1 breast cancer	[212]
DNA	DNase I MG	Hydrogels containing nanoparticles	Co-incubation	PMNs	0.6 mg/mL	Degrade the DNA structure of NETs.	NETs-mediated inflammation and micro-thrombosis	[213]
DNA	AuPB@mPDA@DNase I	NPs	i.v.	C57BL/6 mice	150 μg/mL, 100 μL	Degrade NETs structure, break the shielding of NETs for contact between immune cytotoxic cells and tumors, and eliminate the capture of NETs for circulating tumor cells.	Colorectal cancer	[214]
DNA	DNase-I pMNSs	NPs	i.v.	C57BL/6 mice	100 U/mouse	Dissolve NETs structure, inhibit NETs production, and reduce the level of proinflammatory cytokines.	SARS-CoV-2 sepsis	[215]
DNA	PDA NPs	NPs	Articular injection	SD rats	2.5 mg/kg	Clear cfDNA and inhibit expression of inflammatory factors.	Rheumatoid arthritis	[216]
DNA	HDNaselipo	NPs	i.v.	C57BL/6 mice	128 IU	Prolong the plasma half-life and increase the efficacy of DNase in degrading the NETs structure.	NETs-related diseases	[217]
DNA	PLGA-b-PDMA cNPs	NPs	i.v.	CIA rats	12.5, 25 mg/kg	Clear cfDNA and inhibit nucleic acid mediated TLR activation, downregulate inflammatory cytokines to inhibit inflammatory progression.	Rheumatoid arthritis	[218]

NETosis	ZD-E-1	Nanoflowers	i.v.	BALB/c mice ICR mice BALB/c mice	2,5,10 µmol/kg	Inhibit histone-3 citrullination and NETs formation to reduce the growth and metastasis of tumors.	Tumor development	[219]
NETosis	α2,8-Sialylated NPs	NPs	Co-incubation	PMNs	10 µg/mL	Inhibit ROS production and rupture of neutrophil membranes to prevent the formation and release of NETs.	–	[220]
NETosis	DOX-HYD-BSA NPs	NPs	i.v.	CD1 mice C57BL/6 mice	0.2 mg/kg	Inhibit the activation and induce the apoptosis of neutrophils.	Sepsis	[221]
NETosis	TA-Zn-Gen NPs	NPs	i.p.	C57BL/6 mice	10 mg/kg	Scavenge ROS and reduce ROS-induced DNA release/ damage to improve inflammation.	Sepsis	[222]
NETosis	hUC-MSC-EV	NPs	i.v.	C57BL/6 mice	100 µg/ 100 µL	Reduce levels of proinflammatory cytokines and inhibit NETs production.	Liver IRI	[223]
NETosis	PLGA-LPV@M	NPs	i.v.	BALB/c mice	100 µg/ mL, 200 µL	Inhibit neutrophil activation and NETs production.	COVID-19 and inflammation	[224]
NETosis	LA/DOX NPs	NPs	i.v.	BALB/c mice	90 mg/kg	Block the NF-κB signaling pathway and P-SEL to inhibit NETs generation.	Breast cancer and metastasis.	[225]
NETosis	M@M-Ag-Sil-MA	Hydrogels containing nanoparticles	ext.	C57BL/6 mice	0.2 mL	Inhibit NETs formation and regulate the immune microenvironment to accelerate wound healing.	Diabetic wound	[226]
NETosis	ICMV-Sive	NPs	i.p.	BALB/c mice	50 mg/kg	Inhibit the NETs production mediated by NE and reduce expression of proinflammatory cytokines.	Lung damage	[115]
Neutrophil	MDPNs	NPs	i.v.	C57BL/6 mice	10 µL/ mouse	Compete with receptors on the neutrophil surface for complement and prevent complement-mediated neutrophil activation.	–	[227]
Neutrophil	PD@RPPT/sV NCs	NPs	i.v.	SD rats	400 µg siRNA/kg and 2.5 mg DXM/kg	Downregulate expression of proinflammatory cytokines to inhibit the recruitment and infiltration of neutrophils and improve cardiac function.	MIRI	[228]

Abbreviations: DOX, doxorubicin; DXM, dexamethasone; ext., external use; HCC, hepatocellular carcinoma; i.p., intraperitoneal injection; i.v., intravenous injection; IRI, ischaemia–reperfusion injury; MIRI, myocardial ischemia-reperfusion injury; MMP9, matrix metalloproteinase 9; MSC, mesenchymal stem cell; NETs, neutrophil extracellular traps; NF-κB, nuclear transcription factor-κB; NK, natural killer cell; P-SEL, P-selectin; PLGA, poly lactic-co-glycolic acid; p.o., peros; ROS, reactive oxygen species; TLR, toll-like receptor; NPs, nanoparticles.

Table 6 Potential Antibody Targets of NETs

Ligand	Target	Mechanism of Action	Ref.
7RHistone	pAb #07-596	Detects histone H4 citrullination on arginine 3	[230]
	mAb ab80256	Reacts specifically with human histone H3 citrulline at positions 2, 8, and 17	
	mAb ab176843	Binds to citrulline at the second position of histone H3	
	mAb 2C5	Recognizes the complete chromatin containing histone 2/3 in the NETs structure	[231]
	mAb tACPA	Recognizes and blocks N-terminal citrulline epitopes in histones 2A and 4	[232,233]
	RA RmAb	Recognizes NETs from peripheral blood and/or neutrophils in RA joints	[234]
Citrullinated histones	ACPA	Reacts with citrullinated histone 4 of NETs	[235]
	mAb 11D3	Reacts specifically with human histone H3 citrulline at positions 2, 8, and 17	[236]
	mAb 7C10	Reacts specifically with human histone H3 citrulline at positions 2, 8, and 17	[230]
	pAb ab5103	Binds strongly to 2, 8, and 17 peptides on histone H3 citrulline	[230,237]
	mAb ab176843	Binds to citrulline at two positions of histone H3	[89]
	mAb 4Cit	CitH3 mAb (4 Cit) recognizes four citrulline sites: H3 citrullinated R2, R8, R17, and R26	[238]
PAD4	mAb ab214810	/	[97]
	mAb ab128086	/	[239]
	pAb ab50247	Reacts specifically with the 74-kDa PADI4/PAD4 protein	[240]
Ly6G	mAb 1A8	Binds to Ly6G and consumes neutrophils to inhibit their production of NETs	[241,242]
MPO, LF, NE	P-ANCA	Targets antigens in NETs, such as MPO, LF, and NE	[243]

Abbreviations: ACPA, anti-citrullinated peptides antibodies; LF, lactoferrin; Ly6G, lymphocyte antigen 6 complex locus G6D; MPO, myeloperoxidas; mAb, monoclonal antibody; NE, neutrophil Elastase; NETs, neutrophil extracellular trap; PAD, peptidyl arginine deiminase; RA, rheumatoid Arthritis; /, not available.

manner to deliver and release endocytosed drugs to the target site (hijacking NETosis) is a novel and efficacious therapeutic approach.[251–253] Cheng et al[254] prepared a liposome fused by a mycoplasma membrane to inhibit tumor development. This liposome preparation retained the characteristics of the mycoplasma membrane so that it would be recognized and "swallowed" by neutrophils. When neutrophils recognize inflammatory signals, NETs are released. With the release of NETs, the drugs were also excreted to tumor tissue. Wu et al[255] prepared $_T$LiplT nanoparticles by encapsulating IR780 molecules and tyrosinase-related protein 2 (TRP-2) peptides (both promoted T-cell activation and induced a potent immune response) in cell-penetrating peptide-TAT functionalized liposomes. Then, the preparation was endocytosed by neutrophils (NEs) to form $_T$LiplT/NEs. When neutrophils migrated actively to inflammatory tumor sites, IR780 molecules and TRP-2 peptide were liberated through the release of NETs and exerted a curative effect. In addition, in recent years, some "biological micromotors" have been reported to enhance the natural targeting ability of cells and shorten the time of drug delivery to target sites.

Escape of Microorganisms from NETs

Certain bacteria, parasites, and fungi have developed strategies to resist NETs or elimination. Additionally, some pathogens have even been shown to be capable of modifying NETs in order to utilize them for nutrition or to avoid being recognized by other immune cells.[55] Most microorganisms employ three main strategies to avoid NETs: degradation of NETs, modification of their surface structures to increase resistance, and suppression or inhibition of formation of NETs (Table 7).[55,256] This concept provides great inspiration, and we may be able to learn from this experience and combine it with preparation technology to avoid or counteract the adverse effects of NETs on tumors.

Typical examples of pathogenic microorganisms evading NETs are summarized in Table 7. There are three ways that microorganisms evade NETs. The first one is excreting DNase to degrade the DNA skeleton of NETs. Group-A streptococci contain two genes that encode DNA enzymes. In vivo studies have found that DNase can resist bactericidal effects by degrading the DNA skeleton of NETs. The second way is inhibiting NETosis. A typical example is hepatitis B virus (HBV), which reduces ROS production and NETosis by inhibiting ERK, p38, and MAPK signaling pathways with HBV-C and HBV-E proteins. The third way is utilizing the surface structure and adjusting the surface charge. *Streptococcus pneumoniae* can acylate the lipoteichoic acid on the surface through the surface DLT gene locus to make

Table 7 Escape of Microorganisms from Killing by NETs

Mechanism	Microorganisms	Important structure/Ingredient/ Signaling Pathway/Gene Locus	Ref.
DNase degrades the structure of NETs	Group-A streptococci	DNase	[257]
	Mycoplasma bovis	MnuA	[258]
	Mycoplasma pneumoniae	Mpn491	[259]
	African trypanosome parasites	TatD DNase	[260]
	Streptococcus pneumoniae	EndA	[261]
	Staphylococcus aureus	Nuclease	[262]
	Candida albicans	3'NT/NU	[263]
	Leishmania species	3'NT/NU	[264]
Inhibition of NETosis	Human immunodeficiency virus	GP120-CD209	[265,266]
	Chlamydia trachomatis	CPAF	[267]
	Trichinella spiralis	Calreticulin	[268]
	Hepatitis B virus	ERK, p38 and MAPK	[269]
	Group-A streptococci	Streptolysin O	[270]
	Staphylococcus aureus	EaP	[95]
	Pseudomonas aeruginosa	Sialic acid	[271]
Surface structure/ charge	*Aspergillus fumigatus*	RodA	[272]
	Streptococcus pneumoniae	DLT operon	[273]
	Streptococcus pneumoniae	PspA	[274]
	Staphylococcus aureus	FnBPB	[95]
	Staphylococcus aureus	LuKAB	[275]
	Neisseria gonorrhoeae	OPa	[276]

Abbreviations: EaP, extracellular adherence protein; ERK, extracellular regulated protein kinases; MAPK, mitogen-activated protein kinase; MnuA, a kind of nuclease on the membrane of *Mycoplasma bovis*II; Mpn491, a secreted magnesium-dependent nuclease of *Mycoplasma pneumoniae*; TatD DNase, metal-dependent nuclease from parasites; EndA, nuclease expressed on the surface of *Streptococcus pneumoniae*; 3'NT/NU, 3'-nucleotidase/nuclease; GP120-CD209, the membrane protein-GP120 of *HIV* binds to the type II transmembrane protein-CD209 expressed in DC cells to promote the production of LI-10 which can inhibit NETosis; CPAF, chlamydial protease-like activating factor; RodA, the main protein component on the surface of *Aspergillus fumigatus*; DLT, a gene locus on the bacterial surface; PspA, the surface protein A of *Streptococcus pneumoniaei*; FnBPB, *Staphylococcus aureus* cell wall anchoring protein; LuKAB, leukocidin released by biofilms; OPa, outer membrane protein of *Neisseria gonorrhoeae*.

its surface carry the same charge as that of the cationic peptide in NETs to repel its capture. In addition, the N-terminal region of the surface protein A of *S. pneumoniae* can show a negative charge to repel the negatively charged NETs-DNA to escape its killing.

Targeting Microorganisms Using NETs Mimicking Strategy

Excretion of NETs is a special type of neutrophil immune response. NETs adhere to the surface of bacteria to prevent infection. Inhibition of infection through the bionic pathway of NETs is a relatively new research direction. Recently, Huang et al[277] developed a supramolecular assembly system, BQH-GGFF, to mimic NETs. This supramolecular assembly system was used to capture Methicillin-resistant Staphylococcus epidermidis and prevent its transmission in vivo. The supramolecular assembly system they prepared had good antibacterial activity. Chen et al[278] developed a nano-gel using zinc oxide nanoparticles and DNA to mimic the structure of NETs. Compared with the control group, this NETs-like structure alleviated the clinical symptoms of mice stimulated by LPS. They investigated the anti-inflammatory properties of this NETs-like structure in vivo. Compared with the ZnO group, this preparation inhibited the proliferation of more bacteria, inhibited the entry of Escherichia coli into the circulation effectively, and greatly prolonged the survival time of mice infected with *E. coli*.

Conclusions and Future Prospectives

In recent years, increased attention has been given to NETs and their role in the poor prognosis of tumors. The structural components targeting NETs can effectively inhibit the growth and metastasis of tumor cells. This review elaborates the

relationship between NETs and tumors and explores targeting strategies to reduce the adverse effects of NETs. The active components of THMs and nanomedicine-preparation technology were combined to be able to combat the adverse effects of NETs. However, further studies are needed to improve the anti-NETs efficiency.

Firstly, NETs inhibition-related studies are limited to animal or cell experiments, there is a paucity of formal clinical trials against NETs and related tumors, including NCT00536952 and NCT02462265 against NETs-DNA, NCT03400332 to improve sensitivity to immune checkpoint blockade, NCT03161431, NCT03473925 that blocks CXCR1 and CXCR2 to impede neutrophil recruitment to generate NETs, and NCT02370238, which combines the traditional herbal extract paclitaxel with a molecular drug (Reparixin) targeting CXCR1/2 for the treatment of metastatic triple-negative breast cancer. Accelerating the development of proteins and pathway inhibitors in the process of NETosis will be an effective way to block tumor development.

Secondly, the mechanism of the relationship between NETs and tumor is very complex and incompletely understood. Often, tumor-cell migration accelerates the death of a patient. The reticular nature of "camouflage" NETs enables them to capture tumor cells and makes the immune surveillance of the "sheriff" $CD4^+$ T cells ineffective, making them unable to transmit the killing signal to the "executioner" NK cells and $CD8^+$ T cells. NETs act on a tumor through the intermediate bridge (upstream and downstream cells, signaling pathways, and proteins) to accelerate its metastasis which, in turn, activates neutrophils to release NETs. Such layer-by-layer optimization necessitates vigilance, and finding ways to block such optimization may become the focus of treating NETs-related tumors. NETs can also awaken dormant tumor cells and make them proliferate. Two NETs-derived proteins, NE and MMP9, sequentially cut and remodel laminin in the ECM and degrade TSP-1, which may be the culprits of this awakening mechanism. Cancer-associated thrombosis has also attracted attention. NETs provide platelets and coagulation factors for thrombus formation-stents. Unfortunately, research on NETs and tumor-associated thrombi is relatively scarce.

Thirdly, exploration of the relationship between the N1 subtype neutrophils and NETs that exert anti-tumor activity is an important research area. Two subtypes of neutrophils, N1 and N2, have important roles in tumor development. $CD16^{high}CD26L^{bim}$ with anti-tumor activity may be mediated by NETs and could be an excellent candidate for N1 neutrophils. Research on the relationship between N1 neutrophils and anti-tumor NETs is still insufficient. Most of literatures are focused on the anti-tumor activity of certain proteins of NETs. Moreover, only a few of studies have examined the interaction between NPs and neutrophils. Due to the complexity of this interaction, further research is needed to gain a more comprehensive understanding of the mechanisms underlying the potential toxicity of NPs toward neutrophils, as well as emphasizing neutrophils and NET roles in cancer biology. Signaling pathways and chemokines play various roles in the regulation process, but many of their functions are still poorly understood. Researchers are still investigating the underlying regulatory mechanisms of neutrophil functions, and new properties are being discovered as research into neutrophils continues.

Finally, future research efforts should be made to discover effective herbal compounds and elucidate the mechanisms of action as well as the safety and efficacy of them. Although several studies reported the improved safety of THMs, the intrinsic toxicity associated with certain herbs should still be given due consideration. For the nanocarriers, to improve drug-targeting ability, antibodies could be employed as accurate targeting moieties. A nanomedicine preparation delivering active compounds modified with NETs-specific antibodies could help to reduce the adverse effects of NETs in tumors. Moreover, the NETs structure can be simulated to prepare drug-delivery systems and to hijack NETosis to deliver antineoplastic drugs. Hence, if faced with NETs-related cancer, an alternative method to fight NETs, that is, relying on NETs, will be available.

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