



Advances in the Mechanism of Action of Neutrophil Extracellular Traps in Gastrointestinal Tumors: A Review

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Introduction: Neutrophils are important immune cells in the body, extremely abundant, phagocytic and bactericidal, and usually involved in the defense against infectious diseases as immune become. However, a new reticulum structure has been discovered: neutrophil extracellular traps (NETs), which consists of various components such as DNA and proteins, etc. Current studies have found that NETs are closely associated with various diseases such as immune diseases, inflammation and tumors, and the study of the development and metastasis of gastrointestinal tumors has become a recent research hotspot. The clinical significance of NETs has been gradually highlighted, especially in the area of immunosuppression.

Methods: We reviewed a large amount of relevant literature, summarized the latest detection methods of NETs, explored the mechanism of NETs in gastrointestinal tumors and summarized the latest hotspot directions.

Results: NETs are involved in the development of gastrointestinal tumors, and are closely related to the proliferation and metastasis of gastrointestinal tumors. Higher levels of NETs are associated with poor prognosis of gastrointestinal tumors, promote local growth of tumors through various pathways, participate in tumor-related systemic injury, and promote tumor growth and metastasis by enhancing the mitochondrial function of tumor cells and awakening dormant tumor cells.

Discussion: NETs are highly expressed in tumors, and tumors and their microenvironment can promote the production of NETs, providing new ideas for the clinical diagnosis and treatment of gastrointestinal tumors. In this paper, we describe the basic information about NETs, explore the research mechanisms related to NETs in gastrointestinal tumors, and prospectively explore the clinical potential of hotspots and inhibitors related to NETs for gastrointestinal tumors, in order to provide new ideas and targets for the diagnosis and treatment of gastrointestinal tumors.

Keywords: neutrophil extracellular traps, gastrointestinal tumors, mechanism of action, research progress

Background

Gastrointestinal tumors are common clinical malignancies with extremely high morbidity and mortality rates, and recurrence and metastasis are the main causes of patient death.¹ The effect of comprehensive management is unsatisfactory, and there is an urgent need to investigate new anti-cancer molecular targets.²⁻⁵ Neutrophils are the leukocytes with the largest proportion in peripheral blood and usually constitute the first line of defense,⁶⁻⁹ but excessive activation leads to the release of large amounts of cytotoxic mediators such as reactive oxygen species and cytokines, which damage endothelial cells and lead to cell death. Therefore, NETs are a double-edged sword, which play a role in removing toxic substances while also causing imbalance in regulatory mechanisms and causing pathological damage to the organism, and at the same time can secrete in vivo NETs are an edged sword, which can affect the microenvironment of gastrointestinal tumors by scavenging toxic substances and causing pathological damage to the organism.¹⁰⁻¹³ The presence of NETs in gastrointestinal tumor tissues has been found in a number of studies, and they are involved in tumor biology through various pathways, promoting tumor cell cycle progression and metastatic potential, which has become the focus of clinical research in gastrointestinal surgery. In this paper, we summarize the basic characteristics of NETs,

related formation methods, detection methods and mechanisms of NETs in the development and progression of gastrointestinal tumors, and discuss the importance of NETs as a prognostic marker in the development of gastrointestinal tumors, which will further clarify the relationship between NETs and gastrointestinal tumors and will definitely provide new ideas for clinical prevention and treatment. This will provide new ideas for clinical prevention and treatment, and provide references for subsequent studies of related inhibitors.

NETs

Basic Characteristics of NETs

NETs are a special ultrastructure with a diameter of about 15–17 nm. It has two forms, the anchored net and the cell-free net, the cell-free net is considered to be divided; while the anchored net is described as linear, reticulated, without membrane structure envelope,^{12,14,15} when activated by tumor cells, it releases depolymerized DNA to the extracellular and uses this as a backbone to mosaic a variety of proteins, which together constitute NETs, which They usually include histones, elastase and peroxidase, etc.¹⁶ Participating in pathophysiological processes to achieve pathogen elimination, and mainly include two types, N1 and N2.¹⁷ The N1 phenotype is activated by reactive oxygen species or antibody-dependent cell-mediated cytotoxic effect on tumor cells and direct release of cytotoxicity to tumor cells. The N2 phenotype neutrophils promote tumor growth and disease progression through mechanisms such as reconstitution of the tumor extracellular matrix.

Mode of NETs Formation

The formation of NETs is mainly associated with the regulation of peptidylarginine deiminase and reactive oxygen species and usually involves nuclear division and disintegration of the nuclear envelope with loss of cell polarization. After appropriate stimulation, the nucleus of neutrophils is deformed, chromatin is sparse, separation of the nuclear and granular membranes occurs, and NETs are released from the cell into the extracellular compartment due to cell membrane cleavage.^{16,18} In addition,¹⁹ it has been suggested that NETs can occur independently of cell death, a unique, rapid and reductase-II-independent process involving the secretion of nuclear chromatin, accompanied by the release of granule proteins through degranulation,¹⁹ which accumulate outside the cell and leave active nucleated cells to continuously ingest microorganisms, expel nuclear DNA through vesicular transport mechanisms, and participate in the formation of NETs (Figure 1).^{18,20,21} Nucleated neutrophils have intact cell membranes. The physiological properties, such as phagocytosis, are still retained. Among them, chromatin sparging is the most characteristic change in the composition of NETs.^{5,8,22} In addition, the release of large amounts of lipopolysaccharides in the presence of dysbiosis of the intestinal flora is an important prerequisite for stimulating formation.

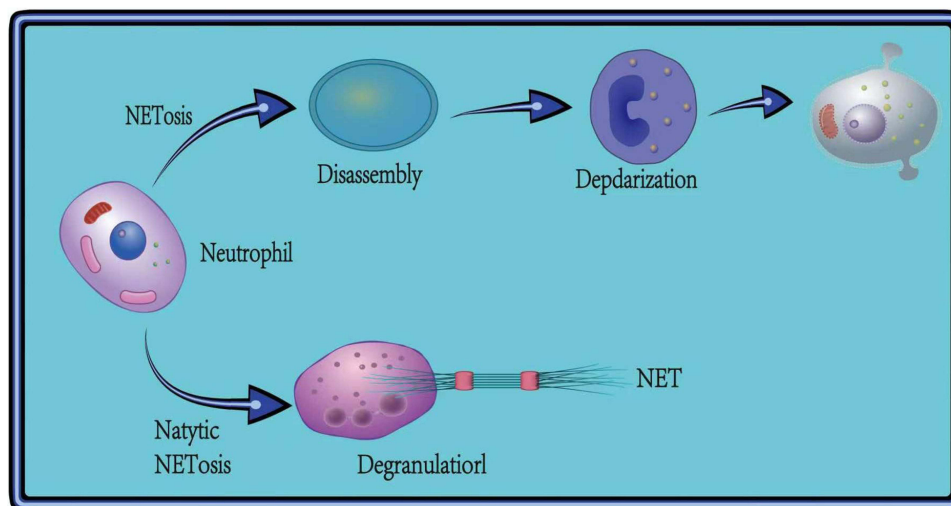


Figure 1 Formation of NETs.

Detection Methods and Progress of NETs

There is increasing evidence that NETs can promote the dissemination and metastasis of tumor cells, therefore, the detection of NETs in clinical treatment is more meaningful.^{23–26} Effective detection methods for NETs are beneficial for early screening of tumors, monitoring of recurrence and prognosis.^{13,27} In recent years, the detection of NETs is mostly based on simple fluorescent labeling staining method, which has a wide range of measurement and simple operation, but there is a considerable error in marker screening.²⁸ With the development of microscopy technology and digital analysis technology, the success rate of quantitative target NETs can be improved by the integrated use of sophisticated instruments (Table 1).

Fluorescent staining is a very common *in vitro* assay, and NETs are a kind of reticulation structure released to the extracellular surface after neutrophil activation by spiking, which establishes the importance of staining in the detection of NETs. The same commonly used method also includes enzyme-linked immunosorbent assay, which is poorly reproducible, but the method is sensitive, rapid and can be automated at low cost, making it the preferred option for detecting NETs. With the advancement of detection technology, in recent years, a number of methods such as confocal microscopy, *in vivo* microscopy, and *in vivo* imaging have emerged to analyze and study the tissue morphology and structural characteristics of objects using high resolution and high magnification, thus improving the detection rate and accuracy. Specific *in vivo* and *ex vivo* detection technology advances are shown in Table 1.

NETs Regulatory Mechanism

NETs Degrade the Extracellular Matrix

The extracellular matrix reticulum acts as a barrier for tumor cells to invade and enter the circulatory system, therefore, the process of degrading the extracellular matrix reticulum can effectively promote the metastasis of GI tumors. It has been shown that protein-degrading enzymes present in NETs can enhance tumor invasion and metastasis by dissolving the extracellular matrix meshwork. Matrix metalloproteinase 9 (MMP-9) is an important component of NETs, and several studies^{29–31} have indicated that MMP-9 plays an important role in cell proliferation and apoptosis, and also degrades extracellular matrix components to prepare tumor cells for migration and accelerate GI tumor metastasis. Studies have shown^{5,22} that neutrophil elastase in NETs has synergistic effects with metalloproteinases and is able to degrade protein inhibitors. Most scholars believe that neutrophil elastase has a role in degrading extracellular matrix,^{23,24,32} but little has been reported on the specific mechanism of NETs neutrophil elastase in tumor metastasis, and more experimental studies are still needed to gain insight (Figure 2).

Table 1 Advances in *in vitro* and *in vivo* Testing Technology digestive Tract Tumors

	Methods	Advantages	Disadvantages
In situ and in vivo assays	Western blotting	Sensitive and specific	Many variables, time-consuming
	In Vivo Microscopy Technology	Objective, more efficient and accurate	Expensive and difficult to select suitable specimens
In vitro assays	In Vivo Imadigestive tract tumorsng Technology	Real-time monitoring	Complex operation with many influencing factors
	Cell immunofluorescence staining	Wide range	Difficult to screen
	Enzyme-linked immunosorbent assay	Low cost	Poor repeatability
	Fluorescence spectrometry	High throughput, rapid detection	Low specificity
	Microplate color development	Small amount	High numerical impact when samples are small
	Flow Cytometry	High accuracy, automatic, quantitative and rapid analysis	Limitations of detection
	Confocal microscopy	Clear and easy to screen	Cumbersome steps

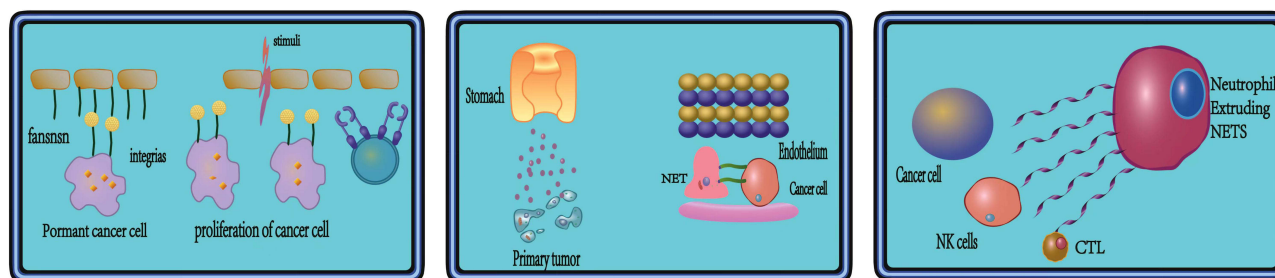


Figure 2 NETs regulatory mechanism.

NETs is Involved in Immunoregulation of Gastrointestinal Tumors

Immunomodulation is a fundamental step in the development of gastrointestinal tumors and an important step affecting the development of gastrointestinal tumors, which can be blocked or promoted by the immune system.^{17,33–36} NETs in gastrointestinal tumors tissues expresses programmed cell death protein 1 significantly higher than surrounding normal tissues,³⁷ inhibiting the proliferation of CD8⁺ T cells and disrupting their anti-immune function, thus promoting gastrointestinal tumors progression.^{8,9,38–43} PD-1 overexpression in NETs has also been shown to be associated with tumor progression and a reduced natural killer cell response.^{5,9,44} Also, NETs can assist tumor cells in evading tumor cells by releasing the pro-inflammatory cytokine TNF- α activates nuclear factors that assist tumor cells to evade cytotoxic T cell killing.^{45–49} NETs inhibits CD4⁺T cell proliferation and prevents T cell attack on tumor cells, it's also secretes growth factors to nourish gastrointestinal tumors cells. IL-10 is a potent immunosuppressive cytokine associated with cancer, and secretion of TGF- α and IL-10 by M2-type NETs induces expression of programmed death protein ligands as a way to inhibit the killing of gastrointestinal tumors by T cells.^{27,50,51} Immunomodulation plays a major role in the development of gastrointestinal tumors, activating activating factors, exerting programmed control, and influencing immune tolerance, and further understanding of immunomodulatory mechanisms, uncovering immune verification sites, and blocking immunosuppression are essential for the clinical treatment of gastrointestinal tumors.

NETs Promote Proliferation of Gastrointestinal Tumor Cells

Several studies have shown that NETs can promote tumor cell proliferation.^{8,52} It's found that NETs-derived DNA in mouse pancreatic cancer cells promoted the growth of pancreatic cancer; deficiency of neutrophil elastase from NETs led to a decrease in tumor cell proliferation and an increase in apoptosis.^{32,53} In addition, the inflammatory microenvironment is one of the important features of tumor proliferation. NETs are closely associated with the inflammatory microenvironment of tumors, creating a microenvironment that promotes inflammation and thus further recruiting neutrophil aggregation.^{23,54} It's found that blocking NETs improved the liver tumor microenvironment and slowed the progression to hepatocellular carcinoma by reducing mononuclear macrophage infiltration and their production of inflammatory cytokines. Lipopolysaccharide stimulates the upregulation of neutrophil complement receptor expression and activates the complement cascade reaction causing the formation of NETs, which promotes the conversion of coagulation and tumor-associated neutrophils to a pro-tumor phenotype (N2 type), leading to the development of small bowel cancer.

NETs Affect Tumor Vascular Stability

The maintenance and stability of vascular integrity mainly depends on the tight interconnection of endothelial cells of blood vessels, and an intact vascular wall plays a barrier role for tumor metastasis. NETs-related proteases promote tumor growth and metastasis by causing down-regulation of endothelial calcium mucin expression, which leads to disruption of the integrity of cell-cell junctions and vascular leakage. It has also been found that neutrophil elastase, a component of NETs, can stimulate the expression of vascular endothelial growth factor and promote tumor growth and invasion.

Hot Spot Exploration

Studies have shown that the formation and degradation of NETs is a complex process influenced by multiple factors. The current hot research on NETs in tumors is mainly in the NETs-related inhibitors.

The overexpression of protein arginine deiminase 4 (PAD4) in neutrophils has been found in many malignant tumors in humans^{9,45,55} and it has been found that overexpression of PAD4 in cancer cells can induce NETs reticulation⁴⁶; and PAD4 inhibitors can promote gene expression, inhibit tumor cell proliferation, and thus induce apoptosis.^{47,56} However, whether PAD4 inhibitors affect tumor cell proliferation and distant metastasis by inhibiting the release of NETs from neutrophils still needs to be further confirmed. Whether NETs inhibitors have synergistic effects with chemotherapeutic agents, radiotherapy, targeted therapy and immunotherapy also needs to be further investigated. Suppression of T cell function is the focus of current research on tumor immunosuppressive mechanisms,⁵² however However, neutrophils can overexpress and secrete nitric oxide synthase, leading to T cell suppression.^{53,57–59} Anti-PD-L1 immunotherapy is a popular GI treatment, and studies have confirmed that anti-PD-1 immunotherapy can act on malignant tumor cells through mechanisms other than T lymphocyte involvement, while NETs can suppress T cells, further delaying the progression of malignant GI tumors and the disruption of the immune microenvironment. Therefore, using neutrophil mechanism as the target of anti-PD-L1 immunotherapy is a new breakthrough point for tumor immunotherapy targeting PD-L1/PD-1 signaling pathway⁵⁴ and is expected to be applied in the clinical treatment process.

Conclusion

NETs, as an emerging research hotspot with wide clinical value, have opened up a whole new research field and provided new explanations for studying the biological behavior of gastrointestinal tumors. Not only can they kill pathogens, but also are closely related to tumor development and metastatic recurrence, and are important prognostic factors for tumor patients, participating in tumor progression through multiple pathways. Although the mechanism of NETs in gastrointestinal tumors has not been fully elucidated yet, and relevant experimental and research reports are very limited and only superficial mechanisms have been explored, this is an area worthy of further study and is expected to be further applied in the clinical setting, and assessing the effect of targeted NETs on tumor development will have great prospects for application. At present, NETs inhibitors have become a relatively new tumor treatment strategy in clinical research, which has attracted much attention. Due to the specificity of tumors and the lack of a large number of clinical studies and differences in animal models, the real application of NETs inhibitors in clinical treatment is still open to question, and a large number of experiments are still needed to study and explore, and further improvement of detection methods is needed to achieve early screening as much as possible. There is great potential for future development of related inhibitors, and targeted drugs for NETs formation mechanism can also provide more options to control the development of gastrointestinal tumors. Overall, these findings strongly suggest the use of NETs as a potential target.

Ethics Standards

This article does not contain or involve any human participants and/or animals.

Informed Consent

For this type of study, formal consent was not required.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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