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Research Progress of Extracellular Trapping Nets and Pancreatic Cancer

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ABSTRACT
Neutrophil extracellular trapping NETs (NETs) are a special mode of cell death. More and more studies have shown that NETs are involved in the pathophysiological processes of various disease formation, inflammation, tumors and other diseases. Pancreatic cancer is one of the most malignant tumors found at present, and due to its occult onset, it is difficult to make early diagnosis of pancreatic cancer patients, and its 5-year overall survival rate is only about 10%. At present, the incidence of pancreatic cancer is still increasing, and it has become one of the important factors threatening human life and health. Current studies have shown that NETs are an important factor involved in the proliferation, metastasis, chemoresistance of pancreatic cancer cells and the formation of venous thrombosis in pancreatic cancer patients. The author reviews this.

KEYWORDS
Neutrophil extracellular trap net; Pancreatic cancer; Research progress

1 NETs Overview

1.1 NETs Structure

NETs are a complex macromolecular complex formed after the death of special neutrophils. It mainly takes the DNA of neutrophils as the skeleton, and combines with a variety of intracellular protein particles to form a network structure. The core protein composition of NETs includes neutrophil elastase (NE), citrullinated histone H3 (CIT-H3) and myeloperoxidase (MPO) [1] In addition, NTEs also contain other non-core proteins such as histones and antibacterial proteins.

1.2 Formation Mechanisms of NETs

NETs can bind microorganisms and kill them. This process of killing microorganisms is named Neutrophil extracellular trap-net death (NETosis) [2]. The process of NETosis includes activation by induced mediators, burst of nicotinamide adenine dinudeotide phosphate (NADPH) and reactive oxygen species (ROS), transport of core protein to nucleus, NE modified histone, chromatin depolymerization, NET formation and release from cell membrane, etc. Many substances that promote inflammation will induce the formation of NET, and its mechanism may be more of a side effect of cytokine environment. At present, it is found that the mediators that can activate NETosis include microorganisms: bacteria (especially Staphylococcus aureus, Streptococcus), fungi, parasites, pathogen-related molecular patterns: such as lipopolysaccharide (LPS), and inflammatory mediators: interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), interferon, etc. At present, research shows that there are two main mechanisms of NETs: one is NADPH-dependent NETosis, and the other is NADPH-independent NETosis. NADPH-dependent NETosis requires the production of ROS by NADPH oxidase. ROS plays an important role in the production of NET [3], which can promote the disintegration of neutrophil granules, release enzymes such as MP0 and NE contained therein into the cytoplasm, and then enter the nucleus [4]. In the nucleus, serine protease and NE can shear histones to promote the decoagulation of chromatin [5], and then the nuclear membrane ruptures, and the decoagulated chromatin mixes with the proteins released by cytoplasmic granules to form NET [6-7]. Eventually, the cell membrane ruptures, releasing NET outside the cell. NADPH-independent NETosis requires extracellular calcium influx and is regulated by small conductance potassium channel 3 (SK3) and mitochondrial ROS [8]. Increased cytoplasmic calcium concentration activates peptidyl arginine deaminase 4 (peptidyl arginine deiminase4, PAD4) [9-10]. Activated PAD4 can modify histones, reduce their binding force to DNA, and depolymerize chromatin. After depolymerization, chromatin enters the cytoplasm, binds to MPO, NE and other granzymes and other proteins, and is finally released extracellularly to form NETs [11-12].

2 Association of NETs with Pancreatic Cancer

2.1 NETs Promote the Proliferation of Pancreatic Cancer

Current studies have found that the infiltration of a large number of immunosuppressive cells makes pancreatic cancer show a "cold tumor" tumor microenvironment immunologically. CD8 + T cells are lacking in the tumor, and T cell

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activation markers such as GZMB and IFNG The expression of genes is low, resulting in the dysfunction or loss of adaptive T cell immune function. NETs can further prevent the contact between immune cells and surrounding target cells, protecting pancreatic cancer cells from cytotoxic effects mediated by CD8 + T cells and natural killer (NK) cells [13]. The main feature of pancreatic cancer is the pro-connective tissue proliferative response that occurs with the progression of the disease, resulting in the deposition of dense extracellular matrix (ECM). One of the main components of ECM is collagen, which can promote the survival and invasion of cancer cells. Recent studies have shown that extracellular DNA in NETs can activate pancreatic stellate cells and form dense fibrous interstitium, thus promoting tumor proliferation [14]. Studies have shown that NE secreted by neutrophils can enter tumor cells and promote tumor cell proliferation by degrading insulin receptor substrate-1 (IRS-1), increasing the interaction between phosphatidylinositol 3-kinase (PI3K) and platelet-derived growth factor receptor (PDGFR) [23]. To sum up, NETs can promote the proliferation of pancreatic cancer, and inhibiting NETs can promote T cells and NK cells to strongly and effectively kill tumor cells, thus inhibiting the proliferation of pancreatic cancer.

2.2 NETs Can Facilitate PDAC Transfer

As one of the most remarkable biological characteristics of malignant tumors, tumor metastasis is a complex cascade process involving multiple steps and factors. In this process, tumor cells first need to break through the physical barrier of the primary tumor, obtain stronger migration ability through epithelial-mesenchymal transition, and then invade the surrounding extracellular matrix and basement membrane of blood vessels and lymphatic vessels to achieve vascular intravasation. After entering the circulatory system, these circulating tumor cells must withstand the shear force of blood flow and the attack of the immune system, stagnate in the microvessels of distant organs and complete vascular extravasation, and finally adapt, survive and proliferate in the unfamiliar microenvironment to form micrometastases, and then develop into clinically detectable metastatic tumors. The completion of this dynamic process not only depends on the intrinsic characteristics of tumor cells, but also is precisely regulated by the microenvironment of target organs and various molecular signaling pathways. At present, it is considered that the high mortality rate of pancreatic cancer is closely related to the susceptibility of tumor metastasis of pancreatic cancer. A growing number of studies have shown [15-17] that NETs can promote tumor progression and metastasis. As a special defense mechanism of neutrophils against pathogens, recent studies have revealed that neutrophil extracellular trap net plays an unexpected role in promoting cancer in tumor progression. This network structure, which is built by DNA skeleton, mosaic histones and granulins, reshapes the tumor microenvironment through various ways, creating favorable conditions for local invasion and distant dissemination of tumors. In terms of extracellular matrix remodeling, elastase and matrix metalloproteinase-9 carried by NETs have the ability to directly degrade extracellular matrix components. This proteolytic activity not only disintegrates the physical barrier, but also opens up migration channels for tumor cells. At the same time, growth factors originally anchored in the matrix are released, further stimulating the malignant behavior of tumor cells. At the same time, NETs act as a trapping network for circulating tumor cells by virtue of their viscous network structure. Through adhesion molecules such as β-integrin and intercellular adhesion molecules exposed on their surfaces, they interact with receptors on the surface of tumor cells, so that tumor cells that originally traveled rapidly in the blood flow can be retained and anchored to the surface of vascular endothelium, providing prerequisites for subsequent vascular extravasation and distant colonization. The destruction of blood vessel integrity by NETs further promotes the hematogenous dissemination of tumors. On the one hand, NETs components can directly damage the connection structure between vascular endothelial cells, resulting in increased vascular permeability; On the other hand, NETs induce microthrombosis by activating the coagulation cascade, which not only causes local blood flow stasis, but also creates a microenvironment conducive to tumor cell survival and immune escape. In terms of inducing malignant phenotypic transformation of tumor cells, NETs can activate the Toll-like receptor signaling pathway in tumor cells by releasing danger signal molecules such as high mobility group protein B1, and up-regulate the expression of various transcription factors, thereby driving the epithelial mesenchymal transition process, so that tumor cells can obtain stronger migration ability and stem cell-like characteristics. In addition, related studies have shown [18] that NETs can form a physical barrier between tumor cells and immune system cells, and inhibit the contact between immune cells and tumor cells, thereby promoting tumor metastasis. Studies have found [19] [20] [21] that cancer-associated fibroblasts (CAF) can promote tumor metastasis by secreting various growth factors, cytokines and chemokines. Related studies have shown [22] that NETs can promote the metastasis of pancreatic cancer cells by activating CAF to secrete vascular endothelial growth factor (VEGF). VEGF is a multifunctional vascular endothelial growth factor, which can accelerate vascular endothelial movement, improve vascular permeability and promote angiogenesis, and is an important factor involved in tumor metastasis. Studies have shown that NETs can induce the transformation of cancer cell epithelium into mesenchymal cells to promote their migration. The latest research shows [24] that in the mouse pancreatic cancer orthotopic transplanted tumor model, anti-CXCL1 treatment can reduce the invasion of neutrophils and the formation of NETs in pancreatic cancer, thus inhibiting the growth and metastasis of tumor cells. Taken together, NETs play a crucial role in the invasive and metastatic grade of pancreatic cancer.

2.3 NETs Promote Thrombosis in Pancreatic Cancer

Thrombosis is a process in which blood components abnormally accumulate and coagulate in blood vessels under various inducements, forming blood clots. Its occurrence is based on three major factors: vascular endothelial injury, blood flow changes and abnormal blood components: endothelial injury exposes collagen, activates platelets and coagulation reactions; Slow or disordered blood flow causes local accumulation of coagulation factors; Hypercoagulable blood increases the risk. Thrombosis is one of the common complications of tumor patients, and blood hypercoagulability is one of its causing factors. Even if there is no clear cause, cancer patients usually have hypercoagulability, so the risk of venous thromboembolism (VTE) in cancer patients is significantly higher than that in normal people [25]. Some Korean scholars [26] evaluated the circulating levels of NETs markers and hypercoagulable markers in 62 patients with pancreatic biliary malignant tumors and 30 healthy controls, and found that the formation of NETs can enhance the hypercoagulability of blood, and indicated that NETs inhibitors may be potential therapeutic agents to reduce hypercoagulability. Some researchers [27-29] have confirmed that NETs are not only a component of venous thrombosis, but also participate in the formation and maintenance of thrombosis. NETs can cause platelet adhesion, activation and aggregation, thereby stimulating thrombus formation and providing a mesh scaffold for thrombus formation. Gould et al. induced neutrophils to release intact NETs through PMA, and found that it has procoagulant activity and can significantly enhance plasma thrombin production. Related studies have shown that compared with the control group, pancreatic cancer tumor-bearing mice can reduce the thrombus size of tumor-bearing mice by neutrophil depletion or DNaseI administration [30]. Taken together, NETs are a key bridge connecting inflammation, tumor and coagulation, and have been proved to be an important driver of pancreatic cancer thrombosis. In the inflammatory microenvironment of pancreatic cancer, tumor and stromal cells secrete cytokines and continuously induce neutrophils to release NETs; NETs activate platelets, enrich coagulation factors and damage endothelium through their DNA backbone, transforming local inflammation into a systemic hypercoagulable state, significantly increasing the risk of thrombosis.

2.4 NETs Induce Chemoresistance in Pancreatic Cancer

For patients with inoperable tumors, chemotherapy is the core means to control tumors and prolong survival. However, chemotherapy resistance severely restricts its long-term efficacy. Whether it is primary or secondary drug resistance, it can reduce the sensitivity of tumor cells, make chemotherapy ineffective, and manifest as disease progression and shortened survival time. The mechanism of drug resistance is complex, involving drug efflux, target change, enhanced DNA repair, apoptosis inactivation and microenvironment protection. Mousset [31] found that chemotherapy will reshape the immune microenvironment while killing tumors: a large number of neutrophils are recruited and penetrate into the tumor site, releasing NETs under the stimulation of chemotherapy stress signals. These NETs weaken the effect of chemotherapy through multiple mechanisms such as physically isolating drugs, activating tumor cell survival pathways, and remodeling the inflammatory microenvironment. Recent studies have shown that this process begins with the direct action of chemotherapeutic drugs: when cytotoxic drugs enter the body, they will up-regulate the expression of chemokines such as CXCL1 and CXCL5 in the tumor microenvironment. These chemokines act as recruitment signals, attracting a large number of neutrophils to gather to the tumor site. At the same time, in the process of chemotherapy drugs killing tumor cells, apoptotic or necrotic tumor cells will release a large amount of ATP. These extracellular ATPs act as danger signals and bind to P2RX7 purinergic receptors adjacent to the surface of surviving tumor cells to activate the intracellular NLRP3 inflammasome complex. Activation of the NLRP3 inflammasome in turn activates caspase-1, which cleaves and processes the inactive precursor protein pro-IL-1β into biologically active IL-1β. The release of IL-1β constitutes an induction signal for the formation of NETs. Under its action, neutrophil, which was previously recruited into the tumor microenvironment, is activated and releases NETs. The formation of NETs is not the end point, but plays a "capture" function through its network structure to enrich TGF-β continuously secreted by tumor cells. More importantly, the matrix metalloproteinase 9 carried by NETs is capable of proteolytic processing of TGF-β, converting it from an inactive latent form to a mature form with signaling activity. Activated TGF-β eventually binds to TGF-β receptor 1 on the surface of tumor cells, initiating intracellular signal transduction, and inducing tumor cells to enter chemoresistance. The elucidation of this pathway provides a clear intervention strategy for overcoming chemoresistance. Experimental evidence shows that adding PAD4 enzyme inhibitors to block the release process of NETs, or using DNase I to degrade the formed NETs, can effectively restore the sensitivity of tumor cells to chemotherapeutic drugs and improve the chemotherapeutic response rate of metastatic tumors. To sum up, NETs play a key mediating role in the occurrence and development of chemoresistance in pancreatic cancer, and targeting the formation or function of NETs is expected to become an important adjuvant treatment for sensitizing chemotherapy and improving the prognosis of pancreatic cancer patients.

3 Outlook

NETs can not only form inflammatory reaction to promote the occurrence of pancreatic cancer, but also play an important role in the proliferation and metastasis of pancreatic cancer. Therefore, can further study the role of NETs and their inhibitors in the treatment of pancreatic cancer become a new idea in clinical work? This still needs a lot of experiments to study and discuss.

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