Review

DOI: 10.5582/bst.2019.01326

The pro-tumor effect and the anti-tumor effect of neutrophils extracellular traps

Yufeng Liu, Lianxin Liu*

Department of General Surgery, the First Affiliated Hospital of Harbin Medical University, Harbin, China.

Summary **Significant advances in our understanding of neutrophil biology were made in the past several years. A newly discovered mechanism was discovered, the formation of neutrophils extracellular traps (NETs). The structure of NETs is composed of the DNA strand and neutrophil granule proteins. NETs were found to have an association with tumor progression. This review highlights the latest knowledge about the controversial effect on tumors of NETs. Pro-tumor and anti-tumor effects are described respectively. The probable mechanisms of the anti-tumor effect are related to its direct killing of cancer cells or stimulation of the immune system to fight against the tumor. The pro-tumor effect has a correlation with matrix metalloproteinase 9 (MMP-9), cathepsin G, and neutrophil elastase (NE). Moreover, the structure of the NETs makes it able to catch the circulating tumor cells, which could lead to metastasis. This review summarizes our knowledge about the proven roles of NETs in the progression of cancer with particular focus on the components of the NETs, and considers NETs as a potential target for cancer therapy.**

Keywords: Neutrophils extracellular traps; tumor; cancer metastasis

1. Introduction

For decades, neutrophils were considered to be a significant infection defender for both innate and acquired immune systems. It was understood that neutrophils act in two ways: either by releasing antimicrobial proteins through degranulation into extracellular space or by phagocytosis of pathogenic microbes. However, a series of recent findings suggest that neutrophils could play their roles in another way: neutrophils extracellular traps (NETs) (*1*).

NETs were first found in 2004 (*2*). When neutrophils are activated, they can form extracellular fibrous structures composed of DNA and some proteins from azurophilic, specific and tertiary granules derived from activated neutrophils. Among these components, histones comprise the highest proportion of NETs (*3*). The remainder of proteins that exist on the DNA scaffold include granular protein, cytoplasmic proteins, cytoskeletal proteins, and some other enzymes. Most of these molecules have been shown to participate in both direct and indirect pathogen-killing mechanisms. With such a number of proteins, as a result, NETs can influence the internal environment in different ways (*4*).

Although NETs play an important role in killing pathogenic microbes like bacteria or fungi, in some conditions when it is excessively generated, NETs can do harm to the human body (*5*). For instance, evidence showed that is can promote vasculitis and thrombosis. Moreover, NETs have been implicated in sterile inflammation diseases such as rheumatoid arthritis and systematic lupus erythematosus (*6*).

Recently, some studies have found that NETs are also involved with tumors. Nevertheless, these results go in two different directions: one is NETs can promote tumor proliferation, invasion, and metastasis, while the other suggests that NETs can inhibit cancer cell proliferation and invasion. Since this research was performed under different experimental conditions and the diseases being studied were different, there is not a clear conclusion on how NETs affect tumors or whether it is a pro-tumor factor or an anti-tumor factor.

Released online in J-STAGE as advance publication December 21, 2019.

**Address correspondence to:*
Dr. Lianxin Liu, Department of General Surgery, the First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, 150001, China.
E-mail: liulianxin@ems.hrbmu.edu.cn

Therefore, in this review, we aimed to elaborate on these findings and explore how NETs affect tumors and which mechanism could be a potential therapeutic target.

2. Formation of NETs in tumor progression

The formation of NETs also referred to as "NETosis" is a complicated process. It is related to the regulation of peptidyl arginine deiminase 4(PAD4) and reactive oxygen species (ROS). PAD4 could convert histone methylarginine residues to citrulline by a novel reaction termed demethylimination (*7*). The mouse with PAD4 knocked out can't generate NETs (*8*). However, NETs added with wild neutrophils can be generated again. Taken together, these findings suggest that PAD4 is an important factor that could regulate the formation of NETs. Another researcher found that when phorbol myristate acetate (PMA) adds to neutrophils, it could oxidize nicotinamide adenine dinucleotide phosphate (NADPH). NADPH-oxidase activation generates the superoxide anion (*9*). A series of down enzymes convert the superoxide to a series of other reactive oxygen species (ROS). After that, neutrophils release the DNA and relevant protein to form NETs.

Currently recognized stimuli for NETs formation and release include Nitric oxide (*10*), cytokines (*11*), microbes and their products (*12*), antimicrobial peptides (*13*) and some medicine like statins (14). The recently discovered anti-bacterial mechanism of NETs indicated their positive role in infections. There is an abundance of data suggesting that the processes between inflammation and neoplasia are kind of similar. The mediators and effector cells were found to be critical in the promotion and progression of the neoplastic process. Thus, people hypothesized NETs could be crucial in tumor development progress.

High expression of NETs is found in some malignant cancers. For example, researchers found that in the specimens of 8 patients with Ewing sarcoma there is a high expression of NETs and tumor-associated neutrophils. And in 2 of 8 patients, they observed NETs deposition in the tumor focus (*15*). Moreover, another study found that in a 9-day-old tumor of lewis lung carcinoma, a large number of neutrophils and extracellular chromatin were observed (*16*). Also, in other malignant tumors such as breast cancer and lymphoma, NETs are found with immunofluorescence or laser scanning confocal microscopy (*17,18*).

As NETs are observed in tumor tissues, people wonder whether cancer cells can stimulate the formation of NETs. Therefore, some researchers co-cultured cancer cells with vital neutrophils. As expected, when co-cultured with vital neutrophils, breast cancer cells (*19*), diffuse large B cell lymphoma and (*20*) non-small-cell lung cancer cells can stimulate neutrophils to form and release NETs. Cancer cells themselves can be an antigen to initiate NETosis and it can also release cytokines or make normal tissue damaged to generate nitric oxide to do the same. A study has proved that tumor-derived cytokine, IL-8 or murine homologue, can induce the formation of NETs. Besides, with the tumor's growth, it can lead to tissue damage and intravascular tumor thrombosis which can cause ischemic necrosis (*21*). Taken together, those factors can all stimulate the formation of NETs.

3. Controversial effect of NETs in tumor disease

Some researchers investigated whether NETs have correlations with tumor patients' prognosis. Interestingly, the prognosis depends on the tumor type. In breast cancer (*19*) and lung cancer (*22*), a larger amount of NETs are observed at an advanced stage than in the primary stage. And NETs levels could be an independent prognostic factor in pancreatic ductal adenocarcinoma (PDAC) (*23*). Tumor-infiltrating NETs predicted poor postsurgical survival of patients with PDAC. NETs were an independent prognostic factor in PDAC and incorporation of NETs along with the standard TNM stating system refined risk-stratification and predicted survival in PDAC with improved accuracy.

Those studies above all suggest that NETs indicate poor prognosis. Nevertheless, there are studies that suggest NETs deposition in tumor tissue has a cytotoxic effect. In malignant melanoma, NETs play an antineoplastic role (*24*). In the ulcerated area, the researchers detected more NETs, and NETs can come into contact with tumor cells. Then, surprisingly, they found that contacting NETs can inhibit melanoma cell migration and viability.

4. Anti-tumor effect of NETs

There is speculation that NETs play an anti-cancer role because of its direct killing of cancer cells or stimulation of the immune system to fight against the tumor.

Myeloperoxidase (MPO) is a component of NETs. MPO is present at 71.3 mg per gram of NET DNA, or a 1.01 molar amount (*25*). MPO could kill melanoma cells and inhibit their growth after implementation. The study found that patients with chronic granulomatous disease fail to make NETs because of the mutations that disrupt the ability of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to generate superoxide, which dismutases to hydrogen peroxide, the substrate of MPO. These patients are susceptible to infection and have a higher incidence of cancer than healthy people (*26*). Therefore, MPO is a representative component of NETs that makes NETs an anti-tumor factor.

The components that comprise the highest proportion of neutrophil extracellular trap proteins are histones. Especially, H2A, H2B, and H3 are present

in amounts of 379, 299 and 199 mg per gram of NET DNA. Histones, another important element of the NETs, are able to do damage to epithelial cells and consequently do damage to the blood vessels feeding the tumor. Also, it represents a potential attachment site for pathogens and carries antibacterial activity. Studies have shown that integrin mediates cancer cell adhesion by binding to fibronectin, which co-localize with histone H3 and the web-like structure of NETs (*25*).

Not only histones provide a site for the pathogen to bind, but the DNA structure also plays an important role in capturing cancer cells. The study we mentioned above (*24*) found that when melanoma cell line A375 was co-cultured with NETs, their ability to metastasize and proliferate declined. In vivo experiment showed the same result. However, when co-cultured with neutrophils' DNA *in vitro* or with DNase treatment *in vivo*, cancer cells' ability to proliferate and metastasize can't be inhibited. These findings suggest that it is the web-like structure but not the DNA that causes this anti-effect. The web-like structure promotes the adhesion of melanoma cells similar to the mechanism for capturing microbes (*2*).

5. Pro-tumor effect of NETs

As we all know, NETs could improve immune capacity to eliminate microbes or cancer cells. However, the special structure and proteases may degrade extracellular matrix and promote metastasis of neoplastic cells. What's more, the tumor's microenvironment can predispose neutrophils to release NETs. Some studies hypothesized that the scaffold structure of NETs can stimulate platelet adhesion and contribute to formation of blood clots in progression of cancer (*27,28*).

These findings suggest that NETs could promote tumor progression and metastasis within the tumor. However, there is no study to explain clearly the mechanism. Some published literature reports NETs-derived components anti-tumor effects. As previously said, neutrophils extracellular traps component include neutrophil-derived chromatin, enzymes, antimicrobial proteins, and peptides. Some of these components have been proved to promote cancer proliferation and metastasis. These include matrix metalloproteinase 9(MMP-9), cathepsin G, and neutrophil elastase (NE). It is known that NETs could adhere to cancer cells. As a result, the adhesion may provide a microenvironment for tumor cells and those functional molecules to contact with each other where the biologically active proteins have a high local concentration. These events can act to promote proliferation, inhibit apoptosis and induce metastasis.

Neutrophil elastase is a kind of serine protease that is stored in azurophilic granules of neutrophils. During the process of "NETosis", activated neutrophils can release the neutrophil elastase into the extracellular matrix. The main physiological function of NE is to clear pathogens during infection. However, NE can also degrade extracellular matrix, which leads to tissue damage. Both *in vivo* and *in vitro*, neutrophil elastase has demonstrated a number of pro-tumorigenic roles. In some global NE deletion mouse models, tumor burden is significantly reduced, which proves that NE plays an important role in tumor development.

Using the model of lung adenocarcinoma, mice lacking NE showed a longer survival time than the control group mice with NE. In vitro, NE could also enhance proliferation and migration of the tumor cells. When the A549 cell is co-cultured with polymorphonuclear leukocytes, cell proliferation is increased. Interestingly, it is inhibited while A549 is co-cultured with NE-/- polymorphonuclear leukocytes. What's more, adding NE inhibitors into culture media, proliferation of the tumor cell is attenuated (*29*). The pro-tumor effects of NE were proved to be mediated by the phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) pathway. Moreover, this NE-mediated proliferation of A549 cells is attenuated by specific PI3K inhibitors. Therefore, researchers wonder what is activated downstream. To that end, it was found that the degradation of insulin receptor substrate-1 leads to PI3k activation and increases proliferation (*30*). Other studies proved that NE could also increase the concentration of transforming growth factor α, vascular endothelial growth factor(VEGF) and platelet-derived growth factor (PDGF) in the tumor cell culture in the media (*31*). These findings suggest that NE may promote release of these pro-tumor factors into the extracellular environment, promoting interactions with their cognate receptors (*32*). Taken together, these mechanisms implicate the direct and indirect roles for NE in promoting tumor progression.

Matrix metalloproteinase 9(MMP-9) is another crucial component of NETs that could promote tumor metastasis *via* the degradation of the extracellular matrix. In a study, a researcher found a decreased frequency of invasive tumors in a mice group with MMP-9 inhibitor. Furthermore, this finding was supported by immunohistochemical analysis of squamous cell carcinoma, which proved that MMP-9 existed mostly in invading granulocytes. Thus, the authors indicated that MMP-9 is associated with increased proliferation of neoplastic cells. Besides, MMP-9 could also facilitate tumor cells by inhibiting apoptosis in tumor cells (*33*). In the study above, the authors demonstrated an 81% increase in metastatic foci after tail vein injection of LLC cells compared to MMP-$9^{-/-}$ mice. Moreover, it proved that MMP-9 in metastatic tumor foci is derived from infiltrated neutrophils and NETs (*34*). NETs-derived MMP-9 could also improve tumor angiogenesis, which could maintain both primary and metastatic tumor growth.

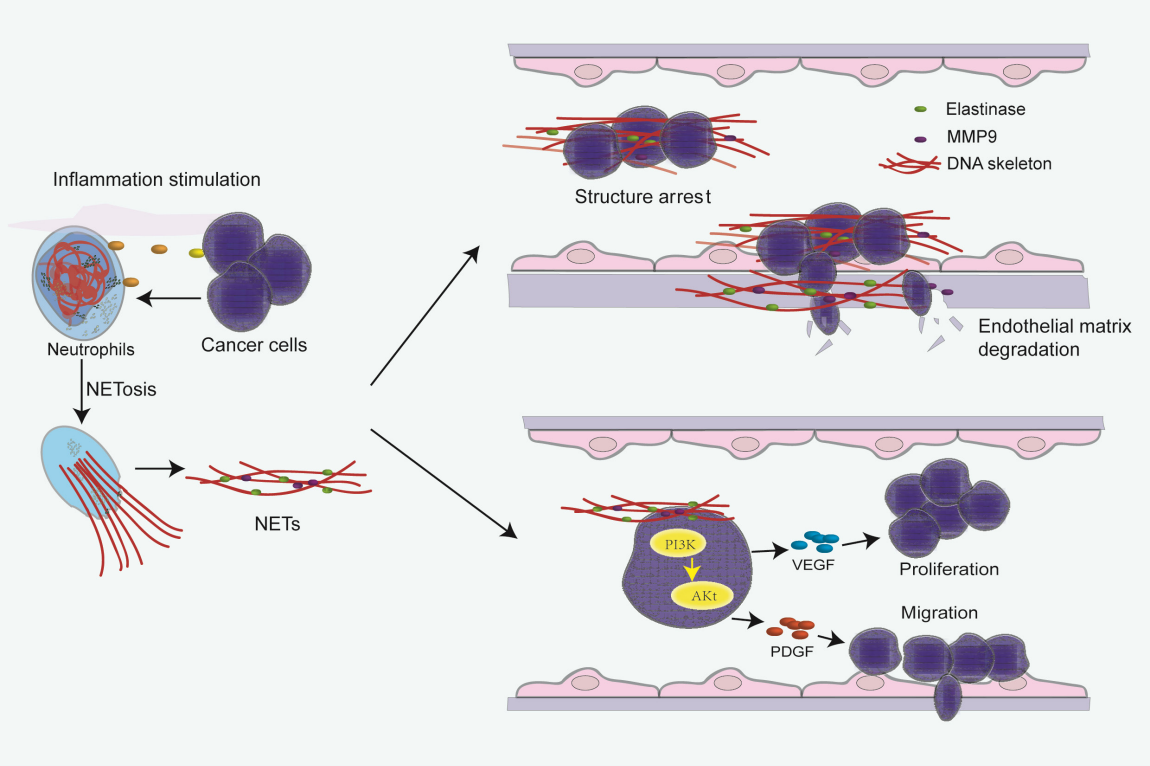

Figure 1. The pro-tumor mechanism of NETs. Cancer cells could promote neutrophils to release NETs. The net structure of NETs makes it able to catch the circulating tumor cell and carry them to other organs. The MMP-9 could degrade the extracellular matrix. NE and elastase on the NETs could promote tumor proliferation and migration via VEGF and PDGF signal pathways.

Some studies hypothesized MMP-9 takes part in liberating VEGF from the ECM. Inhibition of MMP-9 with the small molecule inhibitor R94138 can cause a decrease of angiogenic and an 80% reduction in the number of tumors (*35*). Taken together, these findings suggest that NETs-derived MMP-9 is a mediator of angiogenesis in this animal model. Moreover, this effect can be inhibited by anti-VEGF antibodies. With the addition of heparanase, this effect can be replicated. These all indicate that MMP-9 promotes tumor progression by liberating VEGF from the extracellular matrix *via* degradation activity.

Besides NE and MMP-9, there is another representative peptidase in NETs that promote tumor progression, cathepsin G. It is a peptidase inside the azurophilic granules (*35,36*). Cathepsin G could degrade bacteria during phagocytosis and remodel ECM (*37*). It has been proved that cathepsin G could also facilitate angiogenesis and tumor cell migration. People know that tumor cells could aggregate in the vasculature and form tumor emboli at a distant location. Cathepsin G showed the ability to facilitate formation of tumor aggregates in a mouse model of breast cancer (*38*). This aggregation was mediated by intracellular adhesion *via* E-cadherin. It also proved that inhibition of cathepsin G reduced aggregation of tumor cells. Tumor cells aggregation progress is mediated by binding of cathepsin G to their cell surface, benefited by its enzymatic activity.

Except for the components of NETs, the special structure also contributes to its tumorigenicity. First, it is well known that circulating tumor cells (CTC) contribute a lot to cancer metastasis. Due to the web-like structure and the stickiness of it, NETs are able to arrest intravascular bacteria. Thus, it may be able to capture CTC and cause adhesion to it in the same way. There is a researcher who tested whether NETs could augment tumor metastasis. In a septic mouse model, which has a larger amount of NETs, after injection with LLC cells, enhanced tumor cells arrested by NETs could be directly visualized compared to healthy controls (*39*). Moreover, deletion of neutrophils abrogates this effect and decreases the amount of CTC adhesion within the liver. When neutrophils with DNase contact tumor cells, the effect was abrogated. This proved that it is NETs but not neutrophils that promotes tumors (*40*). Furthermore, another researcher used PMA to induce a mouse model to induce NETs. In the PMA treatment group, the adhesion ability of lung carcinoma cells was increased fivefold when compared to the control group. This phenomenon was abrogated when using DNase or NETs formation inhibitor (*18*). This mechanism may be correlated by the β1-integrin that expressed both NETs and a cancer cell surface. Taken together, as NETs could carry tumor cells just like they carry other pathogenic microbes to an adjacent area where there are more antibacterial proteins or peptides, this function of

it could promote tumor cell metastasis. Besides, NETs may promote a more malignant phenotype in cancer cells because of the interaction of NETs and tumor cells (Figure 1).

6. NETs as potential therapeutic targets

Some studies measured extracellular DNA and extracellular DNase levels in some patients' samples. It showed that mean DNase I levels were lower than the healthy control samples. Moreover, extracellular DNA levels were higher than the healthy control samples. For many years, researchers believed that cell-free DNA comes from mostly tumor cells. By now, it is known that NETs play a part in extracellular DNA and contribute to tumor progression and metastasis.

Therefore, people hypothesized that using DNase I to degrade the cell-free DNA could inhibit tumor progression. Salganik *et al*. used spontaneous lymphatic leukemia in the mouse as an animal model to detect the effect of injection of DNase I. The study found that the injection results in a decrease in lymph node size and prolonged survival time by 12 weeks (*41*). Other studies found that pre-treatment of DNase I led to an inhibition of cancer cell metastasis (*42*).

In many tumor models, we have already seen that DNase I treatment can reverse some pro-tumor effects of some factors. Thus, DNase I treatment may be a treatment target for cancer. Administration of human DNase I on adults and children with other diseases did not lead to severe adverse effects (*43*). With that, using DNase I alone or combining DNase I with other means seems available to treat some cancers. Recently, a number of clinical trials using DNase I treatment are in progress. However, there is no such treatment that can significantly reduce tumor size or metastasis in humans.

As the above said, peptidyl arginine deiminase 4 is a critical protein to regulate the formation of NETs. Using the PAD4 inhibitor can lead to a similar result inhibiting NETs formation. Nowadays, by using the mouse model with the PAD4 gene knocked out, researchers could explore various kinds of factors in a NETs-free microenvironment (*44*).

Lastly, NETs could capture microbes threatening people's life. Patients with such treatment are susceptible to other diseases such as sepsis, and other life-threatening conditions. Although the treatments above cannot inhibit all formation of NETs, it is still a challenge for people to overcome.

7. Concluding remarks

Despite that there are a number of studies about NETs, it is difficult to prove whether NETs have a pro-tumor effect or an anti-tumor effect. However, in the data that present the anti-tumor effect of NETs, like in Fiona Schedel's study (*24*), the researcher added NETs other than with neutrophils into the cell culture media. It may be the components of NETs that play an anti-tumor role. These factors may have some binding sites with specific tumor cell surfaces. But the formation process of NETs and the migration of NETs can do harm to the peripheral tissue. Prolonged damage may lead to gene mutation or cause the normal cell to acquire tumorigenicity.

Whatever the mechanisms of NETs are, they show important value in clinical diagnosis and treatment. The circulating NETs may be a diagnostic marker or a prognostic indicator. DNase I is a potential treatment, as well as other NETs inhibitors. Moreover, a single component of NETs could be a therapeutic target for a kind of disease. Taken together, the recent development and safe utilization of NETs are promising and NETs are warranted for further investigation in the cancer field.

References

1. Branzk N, Papayannopoulos V. Molecular mechanisms regulating NETosis in infection and disease. Semin Immunopathol. 2013; 35:513-530.
2. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532-1535.
3. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, Garlanda C, Romani L, Gascan H, Bellocchio S, Bozza S, Cassatella MA, Jeannin P, Mantovani A. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. J Exp Med. 2007; 204:793-804.
4. Jorch SK, Kubes P. An emerging role for neutrophil extracellular traps in noninfectious disease. Nat Med. 2017; 23:279-287.
5. Gregory AD, Houghton AM. Tumor-associated neutrophils: new targets for cancer therapy. Cancer Res. 2011; 71:2411-2416.
6. Erpenbeck L, Schon MP. Neutrophil extracellular traps: protagonists of cancer progression? Oncogene. 2017; 36:2483-2490.
7. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, Hayama R, Leonelli L, Han H, Grigoryev SA, Allis CD, Coonrod SA. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol. 2009; 184:205-213.
8. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med. 2010; 207:1853-1862.
9. Karlsson A, Dahlgren C. Assembly and activation of the neutrophil NADPH oxidase in granule membranes. Antioxid Redox Signal. 2002; 4:49-60.
10. Keshari RS, Jyoti A, Kumar S, Dubey M, Verma A, Srinag BS, Krishnamurthy H, Barthwal MK, Dikshit M. Neutrophil extracellular traps contain mitochondrial as well as nuclear DNA and exhibit inflammatory potential. Cytometry A. 2012; 81:238-247.
11. Gonzalez-Aparicio M, Alfaro C. Influence of Interleukin-8 and Neutrophil Extracellular Trap (NET)

Formation in the Tumor Microenvironment: Is There a Pathogenic Role? J Immunol Res. 2019; 2019:6252138.
12. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007; 176:231-241.
13. Kraemer BF, Campbell RA, Schwertz H, Cody MJ, Franks Z, Tolley ND, Kahr WH, Lindemann S, Seizer P, Yost CC, Zimmerman GA, Weyrich AS. Novel anti-bacterial activities of beta-defensin 1 in human platelets: suppression of pathogen growth and signaling of neutrophil extracellular trap formation. PLoS Pathog. 2011; 7:e1002355.
14. Chow OA, von Kockritz-Blickwede M, Bright AT, Hensler ME, Zinkernagel AS, Cogen AL, Gallo RL, Monestier M, Wang Y, Glass CK, Nizet V. Statins enhance formation of phagocyte extracellular traps. Cell Host Microbe. 2010; 8:445-454.
15. Berger-Achituv S, Brinkmann V, Abed UA, Kuhn LI, Ben-Ezra J, Elhasid R, Zychlinsky A. A proposed role for neutrophil extracellular traps in cancer immunoediting. Front Immunol. 2013; 4:48.
16. Ho-Tin-Noe B, Carbo C, Demers M, Cifuni SM, Goerge T, Wagner DD. Innate immune cells induce hemorrhage in tumors during thrombocytopenia. Am J Pathol. 2009; 175:1699-1708.
17. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, Scadden DT, Wagner DD. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc Natl Acad Sci U S A. 2012; 109:13076-13081.
18. Najmeh S, Cools-Lartigue J, Rayes RF, Gowing S, Vourtzoumis P, Bourdeau F, Giannias B, Berube J, Rousseau S, Ferri LE, Spicer JD. Neutrophil extracellular traps sequester circulating tumor cells *via* beta1-integrin mediated interactions. Int J Cancer. 2017; 140:2321-2330.
19. Park J, Wysocki RW, Amoozgar Z, *et al*. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci Transl Med. 2016; 8:361ra138.
20. An Z, Li J, Yu J, Wang X, Gao H, Zhang W, Wei Z, Zhang J, Zhang Y, Zhao J, Liang X. Neutrophil extracellular traps induced by IL-8 aggravate atherosclerosis *via* activation of NF-kappaB signaling in macrophages. Cell Cycle. 2019; 18:2928-2938.
21. Yang C, Sun W, Cui W, Li X, Yao J, Jia X, Li C, Wu H, Hu Z, Zou X. Procoagulant role of neutrophil extracellular traps in patients with gastric cancer. Int J Clin Exp Pathol. 2015; 8:14075-14086.
22. Rayes RF, Mouhanna JG, Nicolau I, Bourdeau F, Giannias B, Rousseau S, Quail D, Walsh L, Sangwan V, Bertos N, Cools-Lartigue J, Ferri LE, Spicer JD. Primary tumors induce neutrophil extracellular traps with targetable metastasis promoting effects. JCI Insight. 2019; 5.
23. Jin W, Xu HX, Zhang SR, Li H, Wang WQ, Gao HL, Wu CT, Xu JZ, Qi ZH, Li S, Ni QX, Liu L, Yu XJ. Tumor-Infiltrating NETs Predict Postsurgical Survival in Patients with Pancreatic Ductal Adenocarcinoma. Ann Surg Oncol. 2019; 26:635-643.
24. Schedel F, Mayer-Hain S, Pappelbaum KI, Metze D, Stock M, Goerge T, Loser K, Sunderkotter C, Luger TA, Weishaupt C. Evidence and impact of neutrophil extracellular traps in malignant melanoma. Pigment cell & melanoma research. 2019.
25. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, Brinkmann V, Jungblut PR, Zychlinsky A. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog. 2009; 5:e1000639.
26. Metzler KD, Fuchs TA, Nauseef WM, Reumaux D, Roesler J, Schulze I, Wahn V, Papayannopoulos V, Zychlinsky A. Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity. Blood. 2011; 117:953-959.
27. Demers M, Wagner DD. Neutrophil extracellular traps: A new link to cancer-associated thrombosis and potential implications for tumor progression. Oncoimmunology. 2013; 2:e22946.
28. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD, Jr., Wrobleski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci U S A. 2010; 107:15880-15885.
29. Houghton AM, Rzymkiewicz DM, Ji H, *et al*. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med. 2010; 16:219-223.
30. Gregory AD, Hale P, Perlmutter DH, Houghton AM. Clathrin pit-mediated endocytosis of neutrophil elastase and cathepsin G by cancer cells. J Biol Chem. 2012; 287:35341-35350.
31. Wada Y, Yoshida K, Tsutani Y, Shigematsu H, Oeda M, Sanada Y, Suzuki T, Mizuiri H, Hamai Y, Tanabe K, Ukon K, Hihara J. Neutrophil elastase induces cell proliferation and migration by the release of TGF-alpha, PDGF and VEGF in esophageal cell lines. Oncol Rep. 2007; 17:161-167.
32. Wada Y, Yoshida K, Hihara J, Konishi K, Tanabe K, Ukon K, Taomoto J, Suzuki T, Mizuiri H. Sivelestat, a specific neutrophil elastase inhibitor, suppresses the growth of gastric carcinoma cells by preventing the release of transforming growth factor-alpha. Cancer Sci. 2006; 97:1037-1043.
33. Acuff HB, Carter KJ, Fingleton B, Gorden DL, Matrisian LM. Matrix metalloproteinase-9 from bone marrow-derived cells contributes to survival but not growth of tumor cells in the lung microenvironment. Cancer Res. 2006; 66:259-266.
34. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell. 2000; 103:481-490.
35. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci U S A. 2006; 103:12493-12498.
36. Segal AW. How neutrophils kill microbes. Annu Rev Immunol. 2005; 23:197-223.
37. Campanelli D, Detmers PA, Nathan CF, Gabay JE. Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties. J Clin Invest. 1990; 85:904-915.
38. Gabay JE, Almeida RP. Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. Curr Opin Immunol. 1993; 5:97-102.
39. McDonald B, Spicer J, Giannais B, Fallavollita L, Brodt P, Ferri LE. Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated

mechanisms. Int J Cancer. 2009; 125:1298-1305.
40. Spicer JD, McDonald B, Cools-Lartigue JJ, Chow SC, Giannias B, Kubes P, Ferri LE. Neutrophils promote liver metastasis *via* Mac-1-mediated interactions with circulating tumor cells. Cancer Res. 2012; 72:3919-3927.
41. Salganik RI, Martynova RP, Matienko NA, Ronichevskaya GM. Effect of deoxyribonuclease on the course of lymphatic leukaemia in AKR mice. Nature. 1967; 214:100-102.
42. Hawes MC, Wen F, Elquza E. Extracellular DNA: A Bridge to Cancer. Cancer Res. 2015; 75:4260-4264.
43. Guichard MJ, Patil HP, Koussoroplis SJ, Wattiez R, Leal T, Vanbever R. Production and characterization of a PEGylated derivative of recombinant human deoxyribonuclease I for cystic fibrosis therapy. Int J Pharm. 2017; 524:159-167.
44. van der Windt DJ, Sud V, Zhang H, Varley PR, Goswami J, Yazdani HO, Tohme S, Loughran P, O'Doherty RM, Minervini MI, Huang H, Simmons RL, Tsung A. Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. Hepatology. 2018; 68:1347-1360.

(Received December 4, 2019; Revised December 12, 2019; Accepted December 15, 2019)