Review Article

Influence of Interleukin-8 and Neutrophil Extracellular Trap (NET) Formation in the Tumor Microenvironment: Is There a Pathogenic Role?

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In this review, we will highlight several studies that revolve around interleukin-8 (IL-8) and show the multiple facets that could take in the tumor microenvironment. Chemokines that attract neutrophils (to a large extent, IL-8) can have a bimodal behavior inducing the migration of them in the first place and later favoring the formation of NETs in the place of emission focus of the chemokine. Also, this mechanism occurs when neutrophils migrate to tumor cells and where the extrusion of NETs in the tumor is observed. A possible participation of NETs in cancer progression was considered; however, until now, it is difficult to decide if NETosis plays a pro- or antitumor role, although it is necessary to emphasize that there is more experimentation focused on the protumorigenic aspect of the NETs. The formation of NETs has a relevant role in the inhibition of the immune response against the tumor generated by neutrophils and in turn favoring the processes involved in the development of tumor metastasis. It is striking that we do not have more complete information about the effects of circulating chemokines on neutrophils in cancer patients and hence the suitability of this review. No one has observed to date the impact that it could have on other cell populations to inhibit the arrival of neutrophils and the formation/elimination of NETs. However, the extent to which NETs affect the function of other cells of the immune system in the tumor context has not been directly demonstrated. It is necessary to identify possible combinations of immunotherapy that involve the modulation of neutrophil activity with other strategies (immunomodulatory antibodies or adoptive cell therapy). Therefore, knowing the mechanisms by which tumors take advantage of this ability of neutrophils to form NETs is very important in the search for antitumor therapies and thus be able to take advantage of the possible immunotherapeutic combinations that we currently have in clinical practice.

1. Characteristics and Effects of IL-8

Interleukin-8 (IL-8), also known as CXCL8, is a proinflammatory chemokine [1] of CXC type that is processed to give rise to a functionally competent protein of 77 amino acids in the case of IL-8 produced by parenchymal cells and 72 amino acids in the case of the one produced by monocytes and macrophages. The production of IL-8 is mainly regulated by NF-κB transcription factors and in minor media by NF-IL6 [2]. IL-8 is a fundamental chemokine to promote tissue infiltration by polymorphonuclear leukocytes [3, 4]. This chemokine is not conserved between species since there is no homolog in the mouse genome that is difficult to study including their functions in murine models genetically transformed.

The biological effects of IL-8 are exerted through two surface receptors called CXCR1 and CXCR2 [5, 6]. These receptors share a remarkable similarity and homology in their sequence that suggests that they are the product of a gene duplication. The signals from these receptors are

transmitted through the plasma membrane through conformational changes that expose regions in the intracellular loops of the receptor. These conformational changes allow G proteins to bind (mainly $G\alpha i$, although possibly other G proteins insensitive to pertussis toxin are also involved) [7]. Activation of G proteins determines the activation of PI3Kinase, phospholipase C, and members of the RAS family [8]. These events in turn determine the activation of the AKT-mTOR pathway, the activation of PKC, and the entry of ionic calcium into the cytosol [9]. The reorganization of the cytoskeleton is mainly mediated by Rho GTPases and the FAK kinase [10] that reorganizes via ARP 2/3 the actin cytoskeleton [9]. These signaling pathways can have effects on multiple leukocyte functions in addition to migration [11].

The chemoattraction of neutrophils to the inflammatory focus is mediated by different substances, among which a family of chemokines stand out those that act on the CXCR1 and CXCR2 receptors. The signals from these receptors are transmitted through the plasma membrane to G proteins (mainly $G\alpha i$, although other G proteins insensitive to pertussis toxin are also possibly involved). Both CXCR1 and CXCR2 receptors do not share the same ligands. CXCR1 is activated only in response to CXCL1, CXCL6, and CXCL8, while CXCR2 is activated by several CXC chemokines, in addition to the aforementioned, such as $GRO\alpha$, $GRO\beta$, the neutrophil-activating peptide GPC-2, NAP-2, and ENA-78. The exposure of these receptors to their ligands determines the intracellular internalization and therefore the desensitization of the cell to the chemokine [12]. In addition, the functions of CXCR1 and CXCR2 do not overlap, since the first in addition to chemotaxis seems to play an important role in the activation of the microbicidal capacity of polymorphonuclear leukocytes [13].

The expression of receptors for IL-8 in cancer cells, endothelial cells, and tumor-associated macrophages suggests that the secretion of IL-8 by cancer cells should have an intense effect on the tumor microenvironment [9, 14]. IL-8 determines in endothelial cells proangiogenic effects that include the proliferation, survival, and migration of vascular endothelial cells [15]. It is also thought that IL-8 has beneficial autocrine and paracrine effects for the tumor cells themselves [9, 14].

The effects of IL-8 in leukocyte populations of cancer patients are not well known. It is possible that they regulate the entry into the malignant tissue of myeloid populations. We have shown that IL-8 attracts and retains dendritic cells specialized in inducing T lymphocyte responses [16]. We have also seen that IL-8 produced by xenografted human tumors in mice determines the disorientation of the migration pattern of human dendritic cells without affecting their immunogenic capacity [17]. It is striking that we do not have information about the effects of circulating IL-8 on neutrophils in cancer patients and hence the suitability of this review.

The development of anti-IL8 humanized monoclonal antibodies, such as ABX-IL-8, has allowed at studying the effect of suppressing IL-8 signaling in tumor progression [18]. Thus, it has been seen that the administration of ABX-IL-8 to mice carrying xenografts of bladder cancer decreases their tendency to metastasize and progress [18], as also happens in similar models of melanoma and prostate cancer [19]. Recently, it has been documented that IL-8, through its proangiogenic effects, is implicated in resistance to VEGF inhibitor drugs such as sunitinib or bevacizumab [20].

It is important to keep in mind not only the effects of IL-8 in the tumor microenvironment, since we must not forget the chemotactic effects on the innate response mediated by circulating leukocytes against infections. It is well known that cancer patients have a higher incidence of infections by pyogenic and fungal bacteria [21]. Some cases can be explained by neutropenia secondary to myelosuppression by different chemotherapeutic agents [22]. However, in cases with normal leukocyte levels in the peripheral blood, there is also a marked tendency to infectious processes that are frequently serious. It is possible that elevated levels of IL-8 disorient the migration of polymorphonuclear leukocytes and make it difficult for them to follow the gradient of IL-8 to migrate to sites of acute infection [23]. If this is correct, it can be explained that the plasma concentration of IL-8 will determine, at least, a certain propensity to develop acute infections and/or to increase its severity and duration.

2. IL-8 Action on Polymorphonuclear Cells

The interaction of cells expressing specific receptors for a specific chemokine with chemokine agonist determines two molecular consequences: (1) polarization and cell migration towards the chemokine concentration gradient and (2) internalization of specific receptors for that chemokine with the consequent desensitization of the capacity to respond to it [24]. In the case of IL-8, the receptors that are stimulated and desensitized are CXCR1 and CXCR2 [11, 25].

Chronic and continued exposure of neutrophils, or other strains of leukocytes and their myeloid hematopoietic precursors at high concentrations of IL-8, determines the functional desensitization of CXCR1 and CXCR2 receptors, or at least a disorientation in their chemotactical migration [4]. By disorientation in its migration, the high concentration of IL-8 in the whole organism determines a disruption of the concentration gradients that guide the chemotactic movement. These phenomena can occur in the organism of patients with advanced cancer, and it has a consequence that peripheral polymorphonuclear leukocytes will migrate with lower efficacy towards IL-8 gradients. Therefore, extravasation to infected/inflamed tissues will occur with less efficiency and accordingly, susceptibility to bacterial infections and their severity will be greater. Chemotherapy often determines neutropenia and therefore aggravates this situation if the migration to form pus is qualitatively altered.

An important role of IL-8 is the attraction of multiple lymphocyte populations to the same source of emission [26]. It is especially important in the regulation of the immune response for tumor development and may even be responsible in part for the suppression of this antitumor response [27, 28].

In this case, we have verified that IL-8 is able to attract both DC and neutrophils to the same place, where they are in close contact [17]. This allows a transfer of material between the cells that subsequently can trigger an immune response that favors tumor development.

Our experiments make evident complex relationship between PMN and DC. Physiologically, the PMNs are much more numerous than the DC and, therefore, could act as possible accumulators of antigens and microbial molecules for DC. DC can internalize the material present in the PMN and then modulate DC functions while transferring the antigens that PMN may carry [17]. It is within the possibility that this phenomenon may occur in the same manner by endogenous DC and could take special importance in the immune response against the tumor. However, exactly how relevant are these functions for the overall physiology of the immune system still remains to be seen.

3. NET Formation and Implications

The process of NET generation, also called NETosis, is a specific type of cell death, different from necrosis and apoptosis [29, 30]. NETs are formed by neutrophils upon contact with several bacteria or fungi as well as with activated platelets or under the influence of numerous inflammatory stimuli, and this process is associated with dramatic changes in the morphology of the cells [31]. The main components of NETs are DNA and granular antimicrobial proteins that determine their antimicrobial properties. Recent studies have shown that neutrophils are able to perform beneficial suicide to create a sophisticated and unique microbicide network composed of cellular content linked to the chromatic frame [32, 33]. The pathogens strapped in these NETs are killed by oxidative and nonoxidative mechanisms [30, 34].

Therefore, it is a powerful tool that primary serves as a protector from severe infections, but this effective defense tool is also a double-edged sword in the immunity [35, 36]. For this reason, overproduced NETs could provoke coagulation disorders, certain autoimmune diseases, and even cancer metastases [37].

On the other hand, several studies have discovered that chromatin and proteases released in the circulatory system during NET formation can regulate procoagulant and prothrombotic factors [34]. In the same way, they could take part in clot formation in blood vessels and might be cytotoxic for tumoral cells [38]. It is speculated that NET components like myeloperoxidase, proteinases, and histones possess antitumorigenic effects by means of actual killing of tumor cells. Therefore, its main function would be to inhibit their growth, activate the immune system, or scaffold directly tumor cells, preventing in this way their further dissemination. Furthermore, probably through histones, NETs can kill activated endothelial cells thus damaging tumor-feeding blood vessels [39, 40].

Alternatively, NETs which harbor potent proteases could be protumorigenic by degradation of the extracellular matrix [41]. So these structures would be able to promote extravasation and metastasis besides helping metastatic cells to evade the immune response as by forming a barrier between cancer cells and the immune system [42]. In this manner, NETs could help cancer cells to escape immune recognition.

Therefore, it is important to increase the knowledge about paths underlying NET formation and degradation processes if we want to efficiently fight with bacterial infections and certain diseases, as in cancerous processes [29, 43].

4. Polymorphonuclear Leukocytes and NET Production in Tumor Microenvironment

Neutrophils are the most abundant leukocyte type of the peripheral blood and play a crucial role in the defense against microorganisms [44]. Neutrophils are rapidly recruited to foci of acute inflammation where their main role is to induce the death of bacteria and fungi [45]. As we have commented previously, the microbicidal mechanisms that neutrophils use are mainly phagocytosis, degranulation of enzymes and bactericidal cationic peptides, and production of free oxygen radicals, as well as the capacity to protrude their nuclear DNA by forming networks [46–48].

Several groups demonstrate desensitization of IL-8-induced migration of polymorphonuclear leukocytes from healthy individuals and cancer patients after preincubation with IL-8, while respecting migration to other stimulus chemistries such as E. coli bacteria irradiated with ultraviolet light [49]. Likewise, it has been verified that the exposure to IL-8 determines the internalization and decrease of the surface expression of CXCR1 and CXCR2, as can be seen by flow cytometry [49, 50]. Finally, numerous groups confirmed the presence of high circulating levels of IL-8 in a series of patients with advanced neoplasms [51, 52]. In this context, elevated serum concentrations of IL-8 are observed in patients with advanced cancer that are not observed in healthy volunteers [51]. It is well known that the synthesis of IL-8 is very abundant in human tumor cell lines both *in vitro* and *in vivo* [53–55].

IL-8 for its role in attracting polymorphonuclear leukocytes has a direct and indirect role in the stimulation of angiogenesis [25]. Genetic studies to clarify the role of IL-8 in cancer are complex since IL-8 is not conserved in rodents, and, for this reason, studies in transgenic or knockout mice cannot be performed. In addition, studies with IL-8 in tumor xenografts are difficult to interpret because they lack specific receptors for IL-8 in both leukocytes and endothelial cells of the human tumor-carrying mouse, although IL-8 exerts some activity on the mouse CXCR1 receptor [56].

As we have previously commented, the expression of IL-8 is frequent in human tumors and its plasma concentration in most cases correlates directly with the tumor size [51, 52]. In turn, we have been able to demonstrate the biological effects of IL-8 in the repression of the antitumor immune response. These pathogenic functions include disorientation in the migration of dendritic cells or the attraction of suppressive myeloid cells [49, 57]. In turn, we have shown that IL-8 induces NETs in granulocytic MDSCs in the same way that it induces them on neutrophils [57, 58].

The role of neutrophils in the evolution of cancer is not known in depth. Massive expression analyses using TCGA have associated a genetic signature of the presence

of polymorphonuclear leukocytes with an adverse prognosis in the development of the disease in several types of cancer [59]. Many studies suggest that neutrophils may acquire immunoregulatory capabilities by acquiring the expression of molecules such as arginase-1 that inhibit the T-lymphocyte-mediated immune response [60, 61]. In fact, a subpopulation of immature neutrophils has been found to be abundant in cancer patients and mice, which is called granulocytic myeloid-derived suppressor cells (Gr-MDSC) [62].

Preliminary data have demonstrated that numerous chemoattractant stimuli tested (CXCL1-8, LTb4, and formyl peptides) to date are able to induce the extrusion of NETs in neutrophils at high concentrations (Dr. A. Teijeira, personal communication, June 15, 2018). Neutrophils are able to polarize and migrate towards foci of tumor cells that express these chemotactic factors in abundance [63, 64]. Upon reaching the maximum production zone of the chemokine, the gradient of chemotactic concentration disappears. It is possible that it is upon reaching a high level of receptor occupancy when the chemotactic stimulus determines the extrusion of the DNA and the formation of NETs [65]. By means of intravital microscopy in tumors, previous studies observed that neutrophils present directional motility to the tumor and the formation of DNA NETs (personal communication). In the tumor context, these structures have been associated mainly with processes that favor metastasis [66–68]. An intravascular role of neutrophils is proposed, whose DNA favors the persistence and survival of tumor cells in the bloodstream [69, 70]. A recent study in mice also suggests that NETs favor the invasive capacity of tumor cells favoring their migration [71]. However, the extent to which NETs affect the function of other cells of the immune system in the tumor context has not been directly demonstrated.

5. IL-8 Derived from Tumors Contributes to the Chemotactic Recruitment of Myeloid-Derived Suppressor Cells

We have explored the relevance of the IL-8 attraction influence towards possible suppressive populations that are found in the tumor microenvironment [57].

The suppressive myeloid cells (myeloid-derived suppressor cells (MDSC)) constitute a heterogeneous population of immature cells composed of macrophages, granulocytes, and other populations of myeloid origin in early stages of differentiation [72]. They especially have an important immunosuppressive component of T cells in cancer patients, as well as they are being able to promote the expansion of regulatory T cells [72, 73]. Currently, the factors capable of attracting this cellular subtype to the tumor microenvironment are poorly understood. We have verified in previous works [57] that IL-8 is a chemokine produced by cancer cells and whose serum concentration correlates with the tumor burden of patients and with a poor prognosis of the disease. We have shown that IL-8 produced by cancer cells attracts by chemotaxis to suppressive myeloid cells obtained from the peripheral blood of patients with advanced cancer and

that this chemotactic activity can be interrupted pharmacologically in tests in mice [57]. Surprisingly, it was also found that IL-8 activates granulocytic myeloid suppressor cells to produce the formation of extracellular neutrophil traps (neutrophil extracellular traps (NETs)). These mechanisms mediated by IL-8 could be relevant in the establishment of a tumor microenvironment that favors the attraction of leukocytes that help the tumor to evade the immune system [74]. Definitely, IL-8 produced by tumors contributes to the chemotactic attraction of suppressive myeloid cells and their functional control [25, 57].

6. Importance of Work in Oncology

The development of humanized monoclonal antibodies against CXC chemokines (such as ABX-IL-8), as well as drugs that inhibit CXCR1/2 receptors, allowed us to study the effect of suppressing the signaling by IL-8 or other ligands of these receptors in the tumor progression [75–77]. Thus, it has been seen that the administration of ABX-IL-8 to mice carrying xenografts of bladder cancer decreases their tendency to metastasize and progress, as it also happens in similar models of melanoma and prostate cancer [18, 78].

A possible option in the treatment of cancer would be the combination of effective immunotherapy strategies with treatments that interfere with neutrophil chemoattraction and NET extrusion [79]. Therefore, knowing the mechanisms by which tumors take advantage of this ability of neutrophils to form NETs is important in the search for new antitumor therapies and possible therapeutic combinations.

For this reason, it is necessary to analyze the mechanism through which CXC chemokines functionally damage human polymorphonuclear leukocytes, analyzing the correlation between IL-8 and leukocyte migration parameters as well as the propensity to severe infections in patients. It would be possible in this way to reveal a determinant and potentially treatable factor in the pathogenesis of the susceptibility to metastasize in patients with advanced cancer.

7. Final Conclusions

We have observed the implication of IL-8 as a biomarker in several tumors and as a chemoattractant of neutrophils and human myeloid suppressor cells. In conclusion, there could be a much defined axis where IL-8 plays a very important role in the recruitment of certain lymphocyte populations and tumor development, including the way in which tumors are capable of developing metastasis. The influence of IL-8 is like an actor who has different roles in the same tumor movie.

Although it is still early to unravel the true role of NETs in the organism, it seems evident that an antimicrobial role is something innate for PMNs as the first defense mechanism. The problem lies in the particular use by certain cell types or the exacerbation of this production that could cause different pathologies or even favor certain metastatic events. Future research should focus on the possibility that tumor cells take advantage of DNA networks extruded by polymorphonuclear leukocytes and their immunosuppressive effect to metastasize successfully.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] B. Moser and P. Loetscher, "Lymphocyte traffic control by chemokines," *Nature Immunology*, vol. 2, no. 2, pp. 123–128, 2001.

[2] D. J. Brat, A. C. Bellail, and E. G. Van Meir, "The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis," *Neuro-Oncology*, vol. 7, no. 2, pp. 122–133, 2005.

[3] M. Baggiolini, P. Loetscher, and B. Moser, "Interleukin-8 and the chemokine family," *International Journal of Immunopharmacology*, vol. 17, no. 2, pp. 103–108, 1995.

[4] R. Stillie, S. M. Farooq, J. R. Gordon, and A. W. Stadnyk, "The functional significance behind expressing two IL-8 receptor types on PMN," *Journal of Leukocyte Biology*, vol. 86, no. 3, pp. 529–543, 2009.

[5] W. E. Holmes, J. Lee, W. J. Kuang, G. C. Rice, and W. I. Wood, "Structure and functional expression of a human interleukin-8 receptor," *Science*, vol. 253, no. 5025, pp. 1278–1280, 1991.

[6] P. M. Murphy and H. L. Tiffany, "Cloning of complementary DNA encoding a functional human interleukin-8 receptor," *Science*, vol. 253, no. 5025, pp. 1280–1283, 1991.

[7] H. M. Schuller, "Regulatory role of G protein-coupled receptors in pancreatic cancer development and progression," *Current Medicinal Chemistry*, vol. 25, no. 22, pp. 2566–2575, 2018.

[8] W. T. Peng, W. Y. Sun, X. R. Li, J. C. Sun, J. J. du, and W. Wei, "Emerging roles of G protein-coupled receptors in hepatocellular carcinoma," *International Journal of Molecular Sciences*, vol. 19, no. 5, 2018.

[9] D. J. J. Waugh and C. Wilson, "The interleukin-8 pathway in cancer," *Clinical Cancer Research*, vol. 14, no. 21, pp. 6735–6741, 2008.

[10] E. Cohen-Hillel, I. Yron, T. Meshel, G. Soria, H. Attal, and A. Benbaruch, "CXCL8-induced FAK phosphorylation via CXCR1 and CXCR2: cytoskeleton- and integrin-related mechanisms converge with FAK regulatory pathways in a receptor-specific manner," *Cytokine*, vol. 33, no. 1, pp. 1–16, 2006.

[11] Q. Liu, A. Li, Y. Tian et al., "The CXCL8-CXCR1/2 pathways in cancer," *Cytokine & Growth Factor Reviews*, vol. 31, pp. 61–71, 2016.

[12] J. J. Rose, J. F. Foley, P. M. Murphy, and S. Venkatesan, "On the mechanism and significance of ligand-induced internalization of human neutrophil chemokine receptors CXCR1 and CXCR2," *The Journal of Biological Chemistry*, vol. 279, no. 23, pp. 24372–24386, 2004.

[13] S. A. Jones, M. Wolf, S. Qin, C. R. Mackay, and M. Baggiolini, "Different functions for the interleukin 8 receptors (IL-8R) of human neutrophil leukocytes: NADPH oxidase and phospholipase D are activated through IL-8R1 but not IL-8R2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 13, pp. 6682–6686, 1996.

[14] H. Ha, B. Debnath, and N. Neamati, "Role of the CXCL8-CXCR1/2 axis in cancer and inflammatory diseases," *Theranostics*, vol. 7, no. 6, pp. 1543–1588, 2017.

[15] Y. Matsuo, N. Ochi, H. Sawai et al., "CXCL8/IL-8 and CXCL12/SDF-1α co-operatively promote invasiveness and angiogenesis in pancreatic cancer," *International Journal of Cancer*, vol. 124, no. 4, pp. 853–861, 2009.

[16] E. Feijoó, C. Alfaro, G. Mazzolini et al., "Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8," *International Journal of Cancer*, vol. 116, no. 2, pp. 275–281, 2005.

[17] C. Alfaro, N. Suarez, C. Oñate et al., "Dendritic cells take up and present antigens from viable and apoptotic polymorphonuclear leukocytes," *PLoS One*, vol. 6, no. 12, article e29300, 2011.

[18] B. M. Mian, C. P. Dinney, C. E. Bermejo et al., "Fully human anti-interleukin 8 antibody inhibits tumor growth in orthotopic bladder cancer xenografts via down-regulation of matrix metalloproteases and nuclear factor-κB," *Clinical Cancer Research*, vol. 9, no. 8, pp. 3167–3175, 2003.

[19] K. Inoue, J. W. Slaton, B. Y. Eve et al., "Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer," *Clinical Cancer Research*, vol. 6, no. 5, pp. 2104–2119, 2000.

[20] D. Huang, Y. Ding, M. Zhou et al., "Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma," *Cancer Research*, vol. 70, no. 3, pp. 1063–1071, 2010.

[21] K. V. I. Rolston, "Infections in cancer patients with solid tumors: a review," *Infectious Disease and Therapy*, vol. 6, no. 1, pp. 69–83, 2017.

[22] A. A. Justiz Vaillant and P. M. Zito, *Neutropenia*, StatPearls Publishing, Treasure Island, FL, USA, 2018.

[23] A. G. Beck-Sickinger and N. Panitz, "Semi-synthesis of chemokines," *Current Opinion in Chemical Biology*, vol. 22, pp. 100–107, 2014.

[24] R. M. Amarandi, G. M. Hjortø, M. M. Rosenkilde, and S. Karlshøj, "Probing biased signaling in chemokine receptors," *Methods in Enzymology*, vol. 570, pp. 155–186, 2016.

[25] C. Alfaro, M. F. Sanmamed, M. E. Rodríguez-Ruiz et al., "Interleukin-8 in cancer pathogenesis, treatment and follow-up," *Cancer Treatment Reviews*, vol. 60, pp. 24–31, 2017.

[26] J. M. David, C. Dominguez, D. H. Hamilton, and C. Palena, "The IL-8/IL-8R axis: a double agent in tumor immune resistance," *Vaccines*, vol. 4, no. 3, 2016.

[27] K. Inoue, C. G. Wood, J. W. Slaton, T. Karashima, P. Sweeney, and C. P. Dinney, "Adenoviral-mediated gene therapy of human bladder cancer with antisense interleukin-8," *Oncology Reports*, vol. 8, no. 5, pp. 955–964, 2001.

[28] N. Karin, "Chemokines and cancer: new immune checkpoints for cancer therapy," *Current Opinion in Immunology*, vol. 51, pp. 140–145, 2018.

[29] S. Yousefi, D. Stojkov, N. Germic et al., "Untangling "NETosis" from NETs," *European Journal of Immunology*, vol. 49, no. 2, pp. 221–227, 2019.

[30] B. G. Yipp and P. Kubes, "NETosis: how vital is it?," *Blood*, vol. 122, no. 16, pp. 2784–2794, 2013.

[31] B. G. Yipp, B. Petri, D. Salina et al., "Infection-induced NETosis is a dynamic process involving neutrophil multitasking *in vivo*," *Nature Medicine*, vol. 18, no. 9, pp. 1386–1393, 2012.

[32] G. Sollberger, D. O. Tilley, and A. Zychlinsky, "Neutrophil extracellular traps: the biology of chromatin externalization," *Developmental Cell*, vol. 44, no. 5, pp. 542–553, 2018.

[33] V. Brinkmann and A. Zychlinsky, "Neutrophil extracellular traps: is immunity the second function of chromatin?," *The Journal of Cell Biology*, vol. 198, no. 5, pp. 773–783, 2012.

[34] M. Zawrotniak and M. Rapala-Kozik, "Neutrophil extracellular traps (NETs) - formation and implications," *Acta Biochimica Polonica*, vol. 60, no. 3, pp. 277–284, 2013.

[35] M. J. Kaplan and M. Radic, "Neutrophil extracellular traps: double-edged swords of innate immunity," *Journal of Immunology*, vol. 189, no. 6, pp. 2689–2695, 2012.

[36] N. Thieblemont, H. L. Wright, S. W. Edwards, and V. Witko-Sarsat, "Human neutrophils in auto-immunity," *Seminars in Immunology*, vol. 28, no. 2, pp. 159–173, 2016.

[37] V. Papayannopoulos, "Neutrophil extracellular traps in immunity and disease," *Nature Reviews Immunology*, vol. 18, pp. 134–147, 2018.

[38] M. Zawrotniak, A. Kozik, and M. Rapala-Kozik, "Selected mucolytic, anti-inflammatory and cardiovascular drugs change the ability of neutrophils to form extracellular traps (NETs)," *Acta Biochimica Polonica*, vol. 62, no. 3, pp. 465–473, 2015.

[39] A. K. Gupta, M. B. Joshi, M. Philippova et al., "Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death," *FEBS Letters*, vol. 584, no. 14, pp. 3193–3197, 2010.

[40] N. M. Kazzaz, G. Sule, and J. S. Knight, "Intercellular interactions as regulators of NETosis," *Frontiers in Immunology*, vol. 7, p. 453, 2016.

[41] J. Cedervall, A. Hamidi, and A. K. Olsson, "Platelets, NETs and cancer," *Thrombosis Research*, vol. 164, Supplement 1, pp. S148–S152, 2018.

[42] I. Homa-Mlak, A. Majdan, R. Mlak, and T. Małecka-Massalska, "Metastatic potential of NET in neoplastic disease," *Postępy Higieny i Medycyny Doświadczalnej*, vol. 70, pp. 887–895, 2016.

[43] L. E. Muñoz, M. J. Kaplan, M. Radic, and M. Herrmann, "Editorial: NETosis 2: the excitement continues," *Frontiers in Immunology*, vol. 8, article 1318, 2017.

[44] C. Summers, S. M. Rankin, A. M. Condliffe, N. Singh, A. M. Peters, and E. R. Chilvers, "Neutrophil kinetics in health and disease," *Trends in Immunology*, vol. 31, no. 8, pp. 318–324, 2010.

[45] S. M. Chatfield, N. Thieblemont, and V. Witko-Sarsat, "Expanding neutrophil horizons: new concepts in inflammation," *Journal of Innate Immunity*, vol. 10, no. 5-6, pp. 422–431, 2018.

[46] S. Costa, D. Bevilacqua, M. A. Cassatella, and P. Scapini, "Recent advances on the crosstalk between neutrophils and B or T lymphocytes," *Immunology*, vol. 156, no. 1, pp. 23–32, 2019.

[47] A. Manda, M. P. Pruchniak, M. Araźna, and U. A. Demkow, "Neutrophil extracellular traps in physiology and pathology," *Central European Journal of Immunology*, vol. 39, no. 1, pp. 116–121, 2014.

[48] V. Brinkmann and A. Zychlinsky, "Beneficial suicide: why neutrophils die to make NETs," *Nature Reviews Microbiology*, vol. 5, no. 8, pp. 577–582, 2007.

[49] C. Alfaro, N. Suárez, I. Martínez-Forero et al., "Carcinoma-derived interleukin-8 disorients dendritic cell migration without impairing T-cell stimulation," *PLoS One*, vol. 6, no. 3, article e17922, 2011.

[50] S. Kredel, M. Wolff, P. Gierschik, and R. Heilker, "Phenotypic analysis of chemokine-driven actin reorganization in primary human neutrophils," *Assay and Drug Development Technologies*, vol. 12, no. 2, pp. 120–128, 2014.

[51] M. F. Sanmamed, O. Carranza-Rua, C. Alfaro et al., "Serum interleukin-8 reflects tumor burden and treatment response across malignancies of multiple tissue origins," *Clinical Cancer Research*, vol. 20, no. 22, pp. 5697–5707, 2014.

[52] M. F. Sanmamed, J. L. Perez-Gracia, K. A. Schalper et al., "Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients," *Annals of Oncology*, vol. 28, no. 8, pp. 1988–1995, 2017.

[53] A. M. Bates, M. P. Gomez Hernandez, E. A. Lanzel, F. Qian, and K. A. Brogden, "Matrix metalloproteinase (MMP) and immunosuppressive biomarker profiles of seven head and neck squamous cell carcinoma (HNSCC) cell lines," *Translational Cancer Research*, vol. 7, no. 3, pp. 533–542, 2018.

[54] M. Idorn, S. K. Skadborg, L. Kellermann et al., "Chemokine receptor engineering of T cells with CXCR2 improves homing towards subcutaneous human melanomas in xenograft mouse model," *Oncoimmunology*, vol. 7, no. 8, article e1450715, 2018.

[55] Z. Niu, W. Tang, T. Liu et al., "Cell-free DNA derived from cancer cells facilitates tumor malignancy through toll-like receptor 9 signaling-triggered interleukin-8 secretion in colorectal cancer," *Acta Biochimica et Biophysica Sinica*, vol. 50, no. 10, pp. 1007–1017, 2018.

[56] X. Fan, A. C. Patera, A. Pong-Kennedy et al., "Murine CXCR1 is a functional receptor for GCP-2/CXCL6 and interleukin-8/CXCL8," *The Journal of Biological Chemistry*, vol. 282, no. 16, pp. 11658–11666, 2007.

[57] C. Alfaro, A. Teijeira, C. Oñate et al., "Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs)," *Clinical Cancer Research*, vol. 22, no. 15, pp. 3924–3936, 2016.

[58] S. Najmeh, J. Cools-Lartigue, B. Giannias, J. Spicer, and L. E. Ferri, "Simplified human neutrophil extracellular traps (NETs) isolation and handling," *Journal of Visualized Experiments*, vol. 98, no. 98, 2015.

[59] A. J. Gentles, A. M. Newman, C. L. Liu et al., "The prognostic landscape of genes and infiltrating immune cells across human cancers," *Nature Medicine*, vol. 21, no. 8, pp. 938–945, 2015.

[60] O. A. Forrest, S. A. Ingersoll, M. K. Preininger et al., "Frontline science: pathological conditioning of human neutrophils recruited to the airway milieu in cystic fibrosis," *Journal of Leukocyte Biology*, vol. 104, no. 4, pp. 665–675, 2018.

[61] J. Pillay, T. Tak, V. M. Kamp, and L. Koenderman, "Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences," *Cellular and Molecular Life Sciences*, vol. 70, no. 20, pp. 3813–3827, 2013.

[62] A. Salminen, K. Kaarniranta, and A. Kauppinen, "The role of myeloid-derived suppressor cells (MDSC) in the inflammaging process," *Ageing Research Reviews*, vol. 48, pp. 1–10, 2018.

[63] P. O. Azevedo, A. E. Paiva, G. S. P. Santos et al., "Cross-talk between lung cancer and bones results in neutrophils that promote tumor progression," *Cancer Metastasis Reviews*, vol. 37, no. 4, pp. 779–790, 2018.

[64] E. Weiss and D. Kretschmer, "Formyl-peptide receptors in infection, inflammation, and cancer," *Trends in Immunology*, vol. 39, no. 10, pp. 815–829, 2018.

[65] P. Skendros, I. Mitroulis, and K. Ritis, "Autophagy in neutrophils: from granulopoiesis to neutrophil extracellular traps," *Frontiers in Cell and Development Biology*, vol. 6, p. 109, 2018.

[66] J. Cools-Lartigue, J. Spicer, B. McDonald et al., "Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis," *The Journal of Clinical Investigation*, vol. 123, no. 8, pp. 3446–3458, 2013.

[67] J. Park, R. W. Wysocki, Z. Amoozgar et al., "Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps," *Science Translational Medicine*, vol. 8, no. 361, article 361ra138, 2016.

[68] J. Pasquier, F. Vidal, J. Hoarau-Véchot et al., "Surgical peritoneal stress creates a pro-metastatic niche promoting resistance to apoptosis via IL-8," *Journal of Translational Medicine*, vol. 16, no. 1, p. 271, 2018.

[69] D. J. van der Windt, V. Sud, H. Zhang et al., "Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis," *Hepatology*, vol. 68, no. 4, pp. 1347–1360, 2018.

[70] J. D. Spicer, B. McDonald, J. J. Cools-Lartigue et al., "Neutrophils promote liver metastasis via Mac-1-mediated interactions with circulating tumor cells," *Cancer Research*, vol. 72, no. 16, pp. 3919–3927, 2012.

[71] S. Tohme, H. O. Yazdani, A. B. al-Khafaji et al., "Neutrophil extracellular traps promote the development and progression of liver metastases after surgical stress," *Cancer Research*, vol. 76, no. 6, pp. 1367–1380, 2016.

[72] S. Sendo, J. Saegusa, and A. Morinobu, "Myeloid-derived suppressor cells in non-neoplastic inflamed organs," *Inflammation and Regeneration*, vol. 38, no. 1, p. 19, 2018.

[73] T. Fujimura, Y. Kambayashi, and S. Aiba, "Crosstalk between regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) during melanoma growth," *Oncoimmunology*, vol. 1, no. 8, pp. 1433-1434, 2012.

[74] S. Najmeh, J. Cools-Lartigue, R. F. Rayes et al., "Neutrophil extracellular traps sequester circulating tumor cells via β1-integrin mediated interactions," *International Journal of Cancer*, vol. 140, no. 10, pp. 2321–2330, 2017.

[75] M. Tabrizi, B. Wang, H. Lu et al., "Population pharmacokinetic evaluation of a fully human IgG monoclonal antibody in patients with inflammatory diseases," *Inflammation & Allergy Drug Targets*, vol. 9, no. 4, pp. 229–237, 2018.

[76] C. Bizzarri, A. R. Beccari, R. Bertini, M. R. Cavicchia, S. Giorgini, and M. Allegretti, "ELR$^+$ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets," *Pharmacology & Therapeutics*, vol. 112, no. 1, pp. 139–149, 2006.

[77] S. Fu, X. Chen, H.-J. Lin, and J. Lin, "Inhibition of interleukin 8/C-X-C chemokine receptor 1,/2 signaling reduces malignant features in human pancreatic cancer cells," *International Journal of Oncology*, vol. 53, no. 1, pp. 349–357, 2018.

[78] R. Salcedo, M. Martins-Green, B. Gertz, J. J. Oppenheim, and W. J. Murphy, "Combined administration of antibodies to human interleukin 8 and epidermal growth factor receptor results in increased antimetastatic effects on human breast carcinoma xenografts," *Clinical Cancer Research*, vol. 8, no. 8, pp. 2655–2665, 2002.

[79] J. Cools-Lartigue, J. Spicer, S. Najmeh, and L. Ferri, "Neutrophil extracellular traps in cancer progression," *Cellular and Molecular Life Sciences*, vol. 71, no. 21, pp. 4179–4194, 2014.