Tumor-induced neutrophil extracellular traps—drivers of systemic inflammation and vascular dysfunction

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ABSTRACT
Neutrophil extracellular traps (NETs) are part of the innate immune defense against microbes, but their contribution to several non-infectious inflammatory conditions has recently been unraveled. We demonstrate that NETs accumulate in the peripheral circulation in tumor-bearing mice, causing systemic inflammation and vascular dysfunction in organs not affected by tumor cells.

A close interplay between tumors and innate immune cells is well documented. For example, while the classically activated M1-like subtype of macrophages exerts tumor-killing effects, the alternatively activated M2-like macrophages represent a major component of the tumor stroma and can promote tumorigenesis via immunosuppression and stimulation of angiogenesis. Similar to macrophages, subpopulations of neutrophils with either tumor-promoting or tumor-inhibitory properties have been identified. During infection, neutrophils constitute the first line of defense against microbes in our body. Neutrophils are the most abundant leukocyte and can exert bacterial killing via secretion of anti-bacterial peptides and phagocytosis. In addition, NETs were identified in 2004 as a novel neutrophil defense mechanism against microbes. NETs are formed in a spectacular way when neutrophils externalize their nuclear DNA, including histones and antimicrobial proteins, such as elastase. Together with platelets, a meshwork is formed that can trap and kill bacteria. During recent years, formation of NETs (NETosis) has also been identified in several non-infectious inflammatory conditions such as cancer, thrombosis, autoimmunity and diabetes, further highlighting the involvement of innate immunity in various pathological conditions. NETs can cause tissue damage due to the endothelial cytotoxicity exerted by extracellular histones.

In a recent study by Cedervall et al., we demonstrate that NETs also accumulate in the peripheral circulation in mice with cancer and cause systemic inflammation and significantly reduced vascular function in organs that are not sites for either primary or metastatic tumor growth (Fig. 1). Tumor-bearing mice displayed an average reduction in renal perfusion by 50% compared to mice without tumors. Since NETs have a high content of extracellular DNA, they can be dissolved by DNase treatment. Administration of DNase restored the vascular function in the kidney and heart to levels seen in non-tumor-bearing mice, and also suppressed the inflammatory response. The impact of DNase treatment was remarkable and strongly implicates NETs as a cause of the systemic inflammation and impaired peripheral vessel function that we observed in mice with cancer.

It is well recognized that cancer produces a variety of collateral effects in patients beyond the malignancy itself, including threats to distal organ functions. However, the basis for such effects, associated with either primary or metastatic tumors, is generally poorly understood and studies of tumor-induced effects on distant organs have primarily focused on tissues that represent metastatic sites. Our findings now point to NETs as a possible mediator of the general organ failure commonly seen in cancer patients. It is possible that this process may be more or less pronounced depending on tumor type-specific expression of soluble factors promoting NET formation. To firmly establish which tumor-secreted factors that contribute to NETosis will be of clinical value. Plasma biomarkers predicting the risk for developing intravascular NETs would be an advantage, since treatment aimed at dissolving NETs could then be initiated early, and optimally protect peripheral organs from NET-induced damage.

Potentially, tumor-induced NETosis could also constitute a risk factor for metastasis. We found that the adhesion molecules ICAM-1, VCAM-1 and E-selectin, as well as the pro-inflammatory cytokines IL-1ß, IL-6 and the chemokine CXCL1, is significantly upregulated in kidney tissue from tumor-bearing mice compared to healthy individuals. Hypothetically, this pro-inflammatory state with upregulated adhesion molecules on the peripheral endothelium could enhance tumor cell extravasation in distant organs. We are currently addressing this issue in our lab and if this is indeed the case, it would strongly motivate further investigations on NET-inhibiting drugs as prophylactic tools for metastasis in the clinical management of cancer. Encouraging news in this direction was recently published, showing that newly developed chemical
inhibitors of the enzyme protein-arginine deiminase 4 (PAD4) prevent mouse and human NETosis. PAD4 is mainly expressed in granulocytes and catalyzes citrullination of arginine residues, a process needed for NET formation. In agreement, mice genetically deficient for PAD4 cannot form NETs and were reported to be more susceptible to bacterial infection. However, the necessity of PAD4 expression for combating bacterial infections was recently challenged by data from Martinod et al., showing that the lack of NETosis in PAD4−/− mice does not increase host vulnerability to bacterial infections. Instead, PAD4-deficient mice were partially protected from septic shock. Importantly, other types of bacterial killing performed by the neutrophils remained intact.

Altogether, the data from our and several other groups highlight the potential benefit of suppressing NET formation during a number of pathological conditions. We believe that the findings in Cedervall et al. add new knowledge about tumor-induced systemic effects on distant organs, which are not directly affected by growing tumor cells. This information could optimally improve therapeutic intervention that prevents tumor-induced systemic effects from becoming irreversible in an individual with cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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