CANCER IMMUNOLOGY

Tumor microenvironment on the move and the Aselli connection

Jeffrey J. Rodvold and Maurizio Zanetti*

The tumor microenvironment is involved in many activities that promote tumor cell growth, local spreading, and metastasis. In this issue of *Science Signaling*, Jung *et al.* found that lymphangiogenesis may result from the cooperation of two molecules, sphingosine-1-phosphate (S1P) and lipocalin 2 (LCN2), produced by tumor cells and macrophages, respectively. The new S1P-LCN2 axis stresses the importance of innate immunity in remodeling the tumor microenvironment and in lymphangiogenesis.

It is a great coincidence that Aselli is the name ancient astronomers gave to two stars in the constellation Cancer and is also the name of physician Gaspare Aselli, who in 1622 first identified lymphatic vessels of the intestine in dogs. The lymphatic vasculature is essential for immune function, tissue fluid homeostasis, and absorption of dietary fat. In cancer, the generation of new lymphatics, lymphangiogenesis, and remodeling of existing ones lead to greater lymphatic vessel invasion, which significantly increases the risk of tumor cell migration to draining lymph nodes and distal organ metastasis formation (*1*). Now, Jung *et al.* (*2*) identify new players linking mechanistically tumor microenvironment (TME) and lymphangiogenesis.

The TME in solid malignancies represents a heterogeneous population of cells, including tumor cells at various stages of differentiation mixed with fibroblasts, endothelial cells, and leukocytes. Although the proportion and number vary in different malignancies, leukocyte infiltrates can be broadly categorized as lymphocytes and myeloid cells. The accumulation of macrophages, a myeloid cell subset, has been shown to correlate with poor survival in several types of cancer including breast cancer, colorectal cancer, and glioblastoma. Once recruited to the TME, myeloid cells, including macrophages, promote tumor growth, spreading, neoangiogenesis, and metastasis via cell nonautonomous prosurvival signals. Large evidence now suggests that these tumor-enhancing functions include weakening the antitumor adaptive immune response. The success of immune checkpoint inhibitors therapy also leverages on the fact that tumor-infiltrating lymphocytes are functionally inert, likely the consequence of TME-borne cues. Undoubtedly, immune dysregulation in the TME is very complex and still to be regarded as a black box. The identification of a novel mechanism through which tumor cells co-opt innate immune cells to promote lymphangiogenesis, henceforth metastasis, sheds new light in a complex matter.

Paracrine molecules that originate in response to stimuli in the TME are actively studied, but the number of signaling messengers is still incomplete. In their study, Jung *et al.* treated human breast cancer cells with the protein kinase inhibitor staurosporine to induce cell death, and subsequently cultured primary human macrophages with the conditioned medium of the apoptotic cell cultures. Macrophages thus treated produced large amounts of the siderophore-binding protein lipocalin 2 (LCN2) in a manner partially dependent on signal transducer and activator of transcription 3 (STAT3). Conditional gene silencing confirmed the identity of the molecule responsible for the induction of LCN2 in macrophages as the signaling lipid sphingosine-1-phosphate (S1P). Thus, tumor-derived S1P, via S1PR1 (S1P receptor 1)–STAT3 engagement, induced macrophages to produce LCN2, which in turn enhanced lymphangiogenesis (in culture and in vivo) through the engagement of VEGFC (vascular endothelial growth factor C) and its receptor VEGFR3 (Fig. 1), hence contributing to mammary tumor lung metastasis in MMTV-PyMT mice. A role for the S1P-LCN2 axis in the TME had not been previously reported.

S1P is best known as an autocrine and paracrine signaling molecule in lymphocyte trafficking and immune cell survival. It is also implicated in inducing antimicrobial peptides as well as to exert direct inhibition of *Mycobacterium tuberculosis* intracellular growth through endosome acidification. As a tumor-derived factor, S1P has been previously implicated in angiogenesis and lymphangiogenesis (*3*). LCN2 is produced by diverse cell types, including myeloid cells, and is part of an innate immune response to microbial infection by effectively sequestering a subset of ferric siderophore complexes, thereby starving microbes of iron (Fig. 1). LCN2 has also been implicated in tumorigenesis through diverse mechanisms (*4*). These functions range from managing endogenous iron demand necessary for tumor cell replication to driving epithelial-to-mesenchymal transition (EMT) via the Slug pathway (*5*). Congruently, the new report (*2*) demonstrates that the deletion of *Lcn2* significantly increased the survival of MMTV-PyMT mice with mammary cancer and significantly reduced lymphangiogenesis and lung metastases. However, because these mice carry a germline and not a lineage-specific *Lcn2* deletion, the causative role of macrophage-derived LCN2 in orchestrating both lymphangiogenesis and metastasis in vivo remains unclear. Nevertheless, this study incriminates the TME and the innate arm of the immune response in repurposing two innate immunity molecules, S1P and LCN2, to benefit tumor growth and spreading.

Remarkably, the two mediators, S1P and LCN2, are also produced in response to microbial infection qualifying them as innate immune response molecules, suggesting an intriguing parallelism between the innate immune response to microbial infection and cancer (*6*). Signaling proteins, peptides, and small lipid molecules once thought to be exclusively used in response to pathogens are now being reassessed as tumor facilitators within the TME. For example, lactic acid produced by bacteria reportedly activates the production of transforming growth factor–β (TGF-β), interleukin-6 (IL-6), and IL-10 in dendritic cells (*7*). Tumor cells can also secrete lactic acid in concentrations (~25 mM) high enough to cause the differentiation of tumor-infiltrating myeloid cells to an immunosuppressive phenotype in a manner dependent on hypoxia-inducible factor-1α (HIF-1α) (*8*). Another example is prostaglandin E_2 (PGE_2), an important mediator of inflammatory responses produced by macrophages during Gram-positive and Gram-negative bacterial infection. PGE_2 is also produced by tumor cells and has been found to account

The Laboratory of Immunology, Department of Medicine and Moores Cancer Center, University of California, San Diego, 9500 Gilman Drive, La Jolla, San Diego, CA 92093-0815, USA.
***Corresponding author. Email: mzanetti@ucsd.edu**

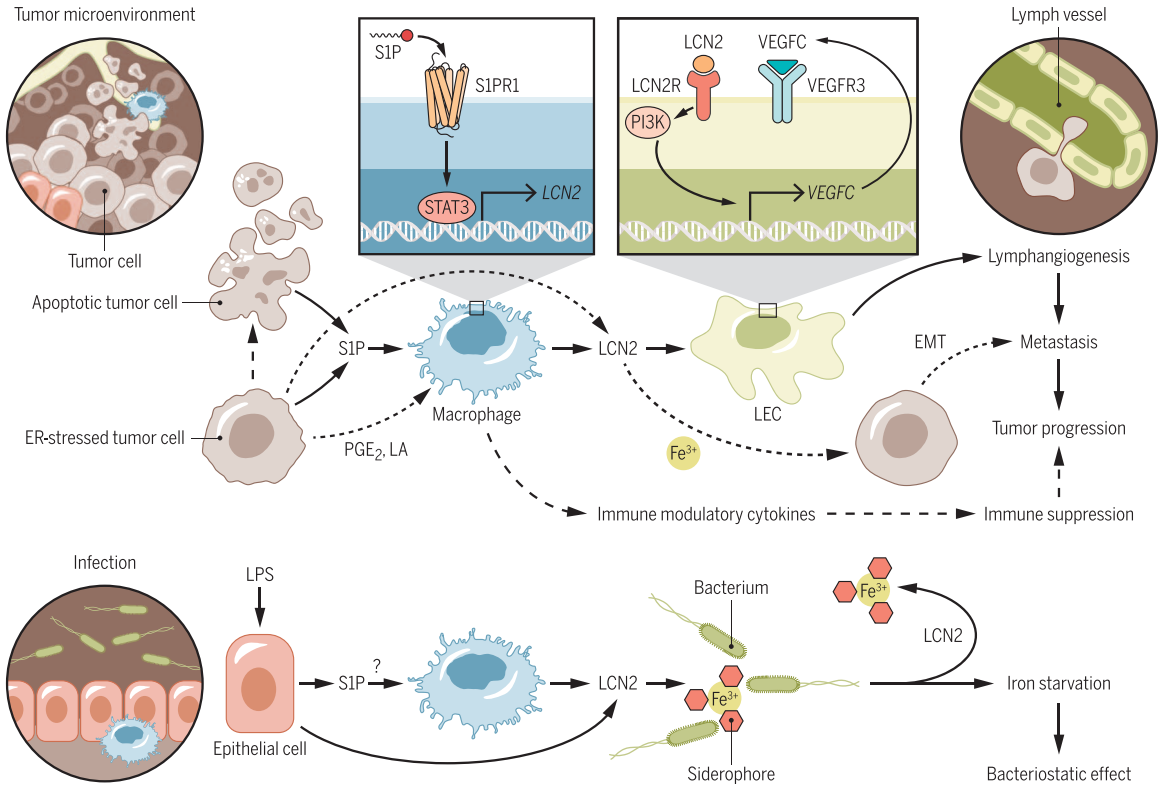

Fig. 1. A common innate immune cell signaling mechanism orchestrates the reorganization of the TME and the response to microbial infection. Tumor cells (top) or normal epithelial cells (bottom) respond to independent stimuli to produce the sphingolipid S1P, among other factors. In a tumor, S1P is produced in response to ER stress or apoptosis caused by hypoxia, nutrient starvation, or the accumulation of lactic acid (LA) in the TME. In the gut or other epithelial barriers, S1P is produced in response to bacterial products, such as lipopolysaccharide (LPS) released by Gram-negative bacteria. In both contexts, S1P activates resident innate immunity myeloid cells (such as macrophages), leading to the transcriptional activation and secretion of LCN2, a siderophore-binding protein. Macrophage-secreted LCN2 promotes lymphangiogenesis (*2*), and LCN2 has been shown to promote EMT (*5*) and tumor growth through iron scavenging, which also limits bacterial growth. In their role to resolve inflammation, macrophages also release immunosuppressive cytokines, which enables tumor progression. Thus, conserved innate immune mechanisms—and specifically macrophages—are important in promoting cancer metastasis and limiting bacterial infection, two seemingly unrelated events. PI3K, phosphatidylinositol 3-kinase.

for chemoresistance (*9*) and interference with T cell function through negative modulation of macrophages and dendritic cells (Fig. 1).

What TME stimuli could possibly initiate tumor cell–mediated co-option of myeloid cells? Because staurosporine is not naturally produced in the TME, for Jung *et al.*'s report to be physiologically relevant, other noxae need to be identified that can trigger the production of S1P in cancer cells. A plausible possibility is endoplasmic reticulum (ER) stress, which was recently shown to induce S1P (*10*). TME hypoxia, nutrient deprivation, and aneuploidy trigger ER stress in cancer cells. The outcome of the cell response to ER stress, also termed the unfolded protein response (UPR), can result in either cell survival or apoptosis, depending on its severity and duration. Tumor cells under ER stress conditions also release molecules that propagate the UPR to macrophages and dendritic cells, which produce VEGF and the immunosuppressive factor Arginase1 (*11*). Notably, ER stress can also trigger the production of LCN2 in cancer cells (*4*). It is then possible that ER stress in the TME, whichever its origin might be, is the common denominator setting in motion a remodeling of the TME through the production of signaling molecules that alone or in combination, as shown for the S1P-LCN2 axis, enhance tumor growth and spreading.

In the past decade, there has been a crescendo of interest in the role of innate immune cells in remodeling the TME for tumor promotion. The study by Jung *et al.* reveals a new facet of this complex equation and shows that players involved in a protective response against microbial pathogens are called into action to set the stage for lymphangiogenesis and tumor metastasis. These findings emphasize the importance of conserved innate immunity mechanisms in seemingly unrelated processes, cancer promotion and bacterial control. Notwithstanding this parallelism, it is not known whether S1P regulates *LCN2* expression in macrophages also during infection. Finally, because ER stress in the TME can activate the S1P-LCN2 axis, as well as other signaling molecules that can co-opt innate immune cells, future therapeutic interventions may also need to consider targeting these events and their specific mediators.

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