Title

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Authors

Werb, Zena
Lu, Pengfei

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Zena Werb, PhD,* and Pengfei Lu, PhD*†

Abstract: The tumor microenvironment plays an essential role in various stages of cancer development. This environment, composed of the extracellular matrix, fibroblasts, endothelial cells, and cells of the immune system regulates the behavior of and co-evolve with tumor cells. Many of the components, including the innate and adaptive immune cells, play multifaceted roles during cancer progression and can promote or inhibit tumor development, depending on local and systemic conditions. Interestingly, a strategy by which tumor cells gain drug resistance is by modifying the tumor microenvironment. Together, understanding the mechanisms by which the tumor microenvironment functions should greatly facilitate the development of new therapeutic interventions by targeting the tumor niche.

Key Words: Adaptive immunity, carcinoma-associated fibroblasts, chemoresistance, exosomes microvesicles, extracellular matrix, innate immunity, matrix metalloproteinases, tumor microenvironment

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The stroma consists of the extracellular matrix (ECM), which is composed of proteoglycans, hyaluronic acid, and fibrous proteins such as collagen, fibronectin, and laminin; growth factors, chemokines, cytokines, antibodies, and metabolites; and mesenchymal supporting cells (e.g., fibroblasts and adipocytes), cells of the vascular system, and cells of the immune system (Fig. 1). As tumors develop, the stroma also evolves.[1–6]

COMPOSITION OF THE STROMA

Cancer cells produce factors that activate and recruit carcinoma-associated fibroblasts, which are an activated fibroblast subtype (myofibroblasts).[7] Carcinoma-associated fibroblasts resemble mesenchymal progenitors or embryonic fibroblasts[8] and are able to stimulate cancer cell growth and invasion as well as inflammation and angiogenesis.[7,9,10] In some systems, they may also be tumor inhibiting.[7,11] Carcinoma-associated fibroblasts activated by the tumor microenvironment are largely responsible for tumor-associated changes in the ECM including increased ECM synthesis and remodeling of ECM proteins by proteinases, for example, matrix metalloproteinases (MMPs).[12,13] The altered ECM then influences tumor progression by architectural and signaling interactions.[14]

Several ECM proteins such as tenascin C and an alternatively spliced version of fibronectin expressed embryonically during organ development are re-expressed during tumor progression.[15] Fibrillar type I collagen also increases in tumors.[16] Fragments of type I collagen or laminin 332 produced as a result of MMP cleavage may be tumor promoting by stimulating cellular migration and survival.[17,18] The biophysical characteristics of tissues, such as stiffness, may affect cellular function. Mammary epithelial cells cultured in compliant collagen matrices form polarized acini, whereas in rigid matrices, they lose polarity and become proliferative and invasive.[4,19]

Inflammatory responses are associated with many cancers and may facilitate tumor progression.[20] Both adaptive and innate immune cells infiltrate into tissues and are critical players.[21–25] Whereas the innate immune compartment is primarily tumor promoting, the adaptive immune compartment (B and T cells) can be tumor suppressing.

The adaptive immune compartment (B and T cells) carries out immune surveillance, keeping initiated cancer cells in check.[22,26] Indeed, patients with a suppressed adaptive immune system have an increased risk of developing cancers.[27] CD4^{+} T cells are key regulators of the immune system and differentiate into various T-helper cell lineages: interferon γ–producing T_H1 cells that promote cell-mediated immunity and interleukin 4 (IL-4)–producing T helper 2 (T_H2) cells that support humoral immune responses.[28] Both T_H1 and T_H2 cells can enhance antitumor immunity by expanding the cytotoxic CD8^{+} T-cell (CTC) population. In contrast, regulatory T (Tregs) cells suppress antitumor immunity by inhibiting cytotoxic T cells. T_H17 cells secrete IL-17. Whereas T_H1 cells are primarily antitumor, T_H2 cells promote tumors through their cytokines, which polarize tumor-associated macrophages (TAMs) to promote cancer progression.[21] CD4^{+} Tregs are immune suppressive, directly suppressing antitumor immunity of CD8^{+} cytotoxic T cells via secretion of IL-10 and transforming growth factor β. Depletion of Tregs enhances tumor growth.[28] CD4^{+} T_H17 cells play roles in inflammation and tumor immunity.[29] T_H17 cells develop from naive CD4^{+} T cells in the presence of transforming growth factor β, IL-6, and IL-1β. Whether T_H17 cells adopt a protumorigenic or antitumorigenic role depends on the stimuli encountered by the cells.

Myeloid-derived innate cells (e.g., macrophages, neutrophils, and mast cells) are largely responsible for inflammatory reactions (Fig. 2). Monocytes are polarized to M1 macrophages by cytokines secreted from T_H1 cells such as interferon γ, tumor necrosis factor α, and granulocyte-monocyte colony-stimulating factor; produce reactive oxygen and nitrogen intermediates and inflammatory cytokines; and are antitumor.[30,31] In contrast, monocytes exposed to cytokines secreted from T_H2 cells such as IL-4 and IL-13 become polarized toward the M2 macrophage phenotype. However, this classification does not accurately define the differentiated state of macrophages exposed to the complex in vivo environments. Tumor-associated macrophages mostly resemble M2 macrophages. Accumulation of TAMs is associated with poor prognosis.[32,33]

Neutrophils can inhibit or promote cancer development.[24,34] With increased tumor burden, activated neutrophils accumulate in bone marrow, spleen, and peripheral blood and, at the invasive tumor front,[24,35,36] promote cancer metastasis by inhibiting cytotoxic T cells and promoting angiogenesis. Mast cells can also drive tumor progression.[37,38]

Recruiting vasculature is a critical step in tumor development. Tumor-infiltrating myeloid-derived cells are major sources of proangiogenic factors.[39,40] These myeloid cells also regulate the resistance of tumors to antiangiogenic therapy.[41]

TUMOR-REGULATING MICROENVIRONMENTS

Tumors have specialized niches, or microenvironments regulate functions of the cancer cells. Genes involved in regulation of stem
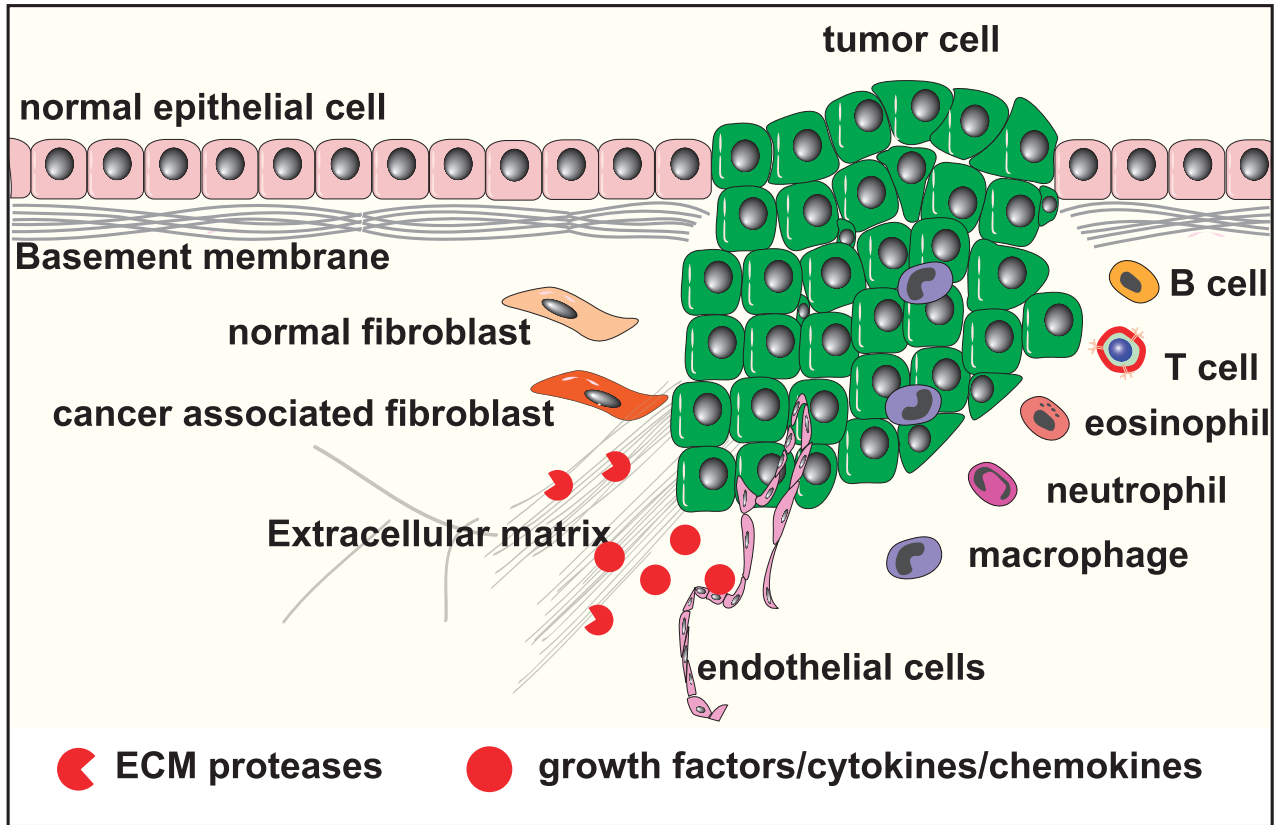
FIGURE 1. Composition of the tumor stromal microenvironment. The stroma consists of ECM, including proteoglycans, hyaluronic acid, and fibrous proteins such as collagen, fibronectin, and laminin, and stromal cells (e.g., fibroblasts and adipocytes); cells of the vascular system; and cells of the immune system.

cell niches also play a role in cancer.[42,43] Like stem cell–like niches that require Wnt signaling for self-renewal of intestinal stem cells, activation of the Wnt pathway by inactivating mutations in the APC gene results in colorectal cancers.[44] Alteration of the microenvironment through genetic mutation in SMAD4 in the stroma also results in gastrointestinal epithelial cancer.[45]
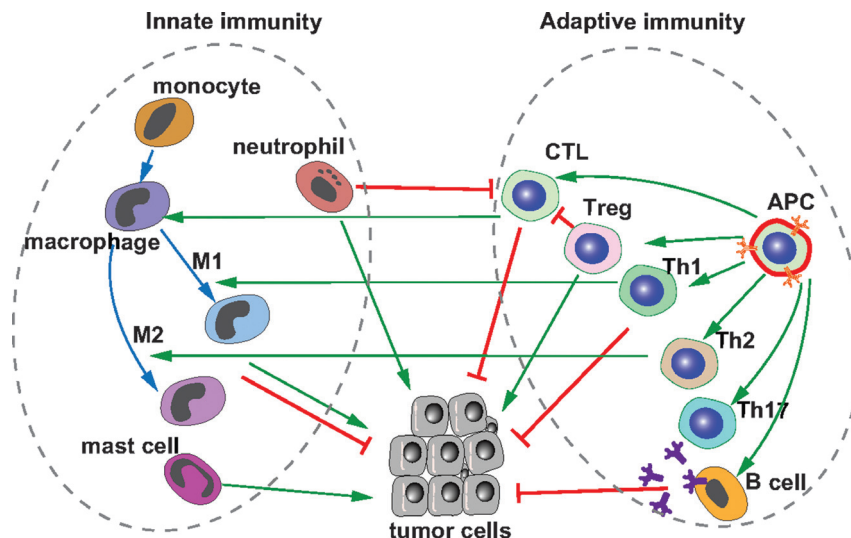

FIGURE 2. Multifaceted roles of innate and adaptive immunity in cancer development. Whereas adaptive immunity, including T and B cells, is essential for inhibiting cancer development, innate immunity, including neutrophils, macrophages, and mast cells, may promote or inhibit cancer development depending on the local and systemic contexts. For example, macrophages can be polarized and activated by cytokines secreted by T$_H$1 cells and produce reactive oxygen and nitrogen intermediates and inflammatory cytokines. These proinflammatory M1 macrophages can inhibit tumorigenesis; by contrast, TAMs (or M2 anti-inflammatory) polarized by cytokines secreted from T$_H$2 cells are associated with poor prognosis. Regulatory T cells can inhibit cytotoxic T-cell function and thus promote tumorigenesis.
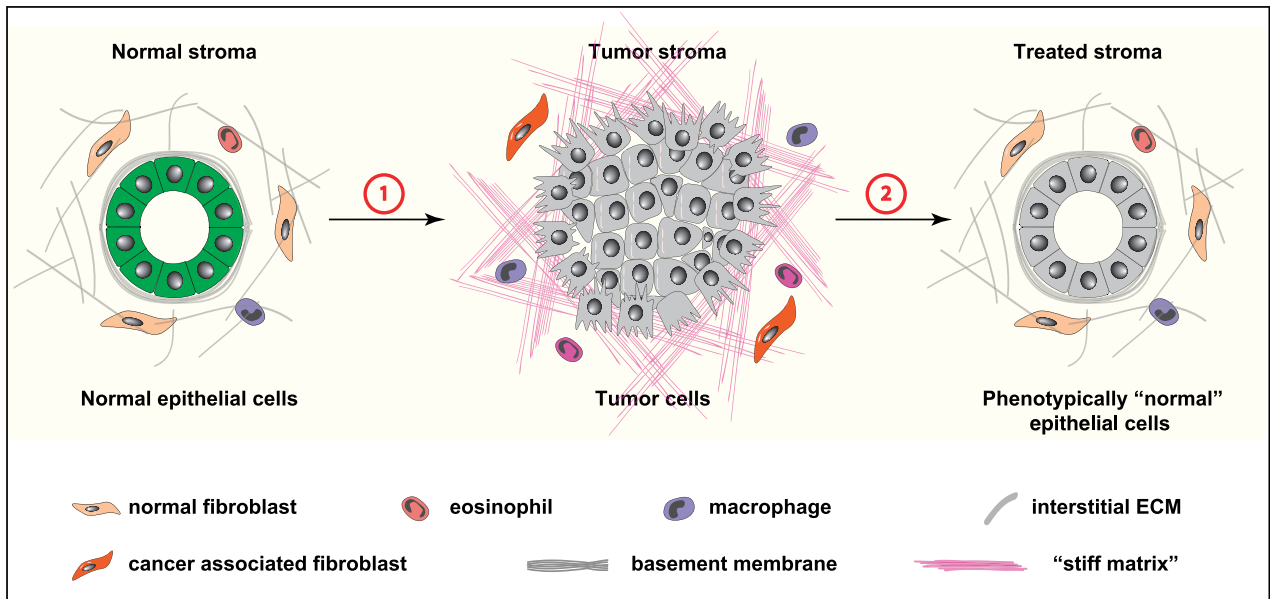
FIGURE 3. Targeting stroma in cancer therapeutics. Changes in the stromal microenvironment are an important aspect of cancer evolution. Tumor stroma undergoes concurrent changes with cancer cells and plays a causative role during initiation, progression, and metastasis of cancer development (1). In addition to promoting cancer development, tumor stroma is a major barrier to cancer drugs and plays a role in drug resistance. Importantly, forceful reversion of cancer stroma to the normal state restores normal behavior to cancer cells (2). As such, tumor stroma is a promising therapeutic target for cancer treatment.

How the microenvironment directs tumor development is just beginning to be elucidated. One clue is that the percentage of cancer cells that express stem cell or basal markers increases when the cells are grown on type I collagen.[46,47] Moreover, invasive cells are often associated with fibrillar collagen in vivo,[47] and cancer stem-like cells may be enriched at the invasive front where the highest levels of type I collagen are found.[48]

Certain microenvironments can restrict tumor progression. In the presence of a basement membrane–like matrix, breast tumor cells behave more like normal epithelial cells.[14] Antibodies to β1 integrins that increase in malignant cells can normalize the malignant phenotype of cancer cells both in culture and in vivo.[49] In essence, ECM molecules that maintain normal tissue architecture and cancer cells quiescence may be tumor suppressors (Fig. 3).

The organ specificity of metastasis correlates with specific gene expression of the disseminating cancer cells.[50] This raises the question of whether the specific organ microenvironments are matched to specific needs of cancer cells. Moreover, primary tumors alter the distant microenvironmental niches through secreted factors and exosomes, making them more amenable for colonization.[24,51,52] Because exosomes and other microvesicles may be taken up in a cell-specific manner, they may be involved in selecting specific metastatic sites.[53]

THE TUMOR MICROENVIRONMENT AND RESPONSE TO CHEMOTHERAPY

The tumor microenvironment is critical in the response chemotherapy.[54] Cancer cells may be more distant from blood vessels, impairing drug penetration. Tumors may have incompetent blood vessels and decreased lymphatics, resulting in increased interstitial fluid pressure, which inhibits diffusion of drugs into the tumor. The properties of the microenvironment including ECM composition and hypoxia may alter the phenotype of the tumor cells, resulting in decreased drug uptake. This raises the question of whether chemoresistance may be overcome by targeting the microenvironment. Altering vascular permeability by affecting MMPs may increase drug delivery.[55,56] Blocking fibroblast-activating protein, which is made by carcinoma-associated fibroblasts, results in reduced type I collagen and improved drug delivery.[57]

PERSPECTIVES

Components of the tumor microenvironment contribute to both the establishment of primary tumors as well as to the initiation, establishment, and growth of metastases. However, there may be different requirements for the primary tumor compared with the distant environment. In many ways, the tumor microenvironment resembles the microenvironments used in development and tissue repair. The tumor-associated stromal cells (e.g., macrophages and fibroblasts) are different from their counterparts in the normal tissue.[58] They may be newly recruited from the bone marrow and have more embryonic character, but they are also misregulated. Altering the tumor microenvironment therapeutically has promise for improving the cancer therapy on a widespread basis.

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