# Mini-Reviews

# Targeting the Tumor Microenvironment for Cancer Therapy

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**BACKGROUND:** With the emergence of the tumor microenvironment as an essential ingredient of cancer malignancy, therapies targeting the host compartment of tumors have begun to be designed and applied in the clinic.

**CONTENT:** The malignant features of cancer cells cannot be manifested without an important interplay between cancer cells and their local environment. The tumor infiltrate composed of immune cells, angiogenic vascular cells, lymphatic endothelial cells, and cancer-associated fibroblastic cells contributes actively to cancer progression. The ability to change these surroundings is an important property by which tumor cells are able to acquire some of the hallmark functions necessary for tumor growth and metastatic dissemination. Thus in the clinical setting the targeting of the tumor microenvironment to encapsulate or destroy cancer cells in their local environment has become mandatory. The variety of stromal cells, the complexity of the molecular components of the tumor stroma, and the similarity with normal tissue present huge challenges for therapies targeting the tumor microenvironment. These issues and their interplay are addressed in this review. After a decade of intensive clinical trials targeting cellular components of the tumor microenvironment, more recent investigations have shed light on the important role in cancer progression played by the noncellular stromal compartment composed of the extracellular matrix.

**SUMMARY:** A better understanding of how the tumor environment affects cancer progression should provide new targets for the isolation and destruction of cancer cells via interference with the complex crosstalk established between cancer cells, host cells, and their surrounding extracellular matrix.

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Neoplastic cells have been the focus of interest in cancer research for several decades. This approach led to the identification of key oncogenes or oncogenic signaling pathways targetable for therapeutic interventions. More recently, whole-genome sequencing, transcriptional profiling, and genome-wide epigenetic analyses have provided an avalanche of new data on (epi)genetic alterations associated with cancer. Despite the increased understanding of cancer cell transformation, current therapies are not fully or are only transiently efficient for most forms of cancer. Although genetic alterations in tumor cells are essential for tumor development, they are not sufficient to endow cancer cells with malignant properties. Infiltration of the tumor by different host cell types, leading to an evolving stromal compartment intermingled with tumor cells, is required to create a permissive environment for the invasion of (epi)genetically altered tumor cells *(1, 2)*. The tumor infiltrate is composed of a variety of leukocyte subtypes (immune and inflammatory cells), blood endothelial cells (BECs),[2] and lymphatic endothelial cells (LECs), leading to the (lymph)angiogenic switch, carcinoma-associated fibroblasts (CAFs), and bone marrow–derived mesenchymal stem cells (MSCs) that contribute to quantitative and qualitative changes in the extracellular matrix (ECM) *(3, 4)*. Desmoplasia, the fibrotic stromal reaction associated with most carcinomas, is characterized by the local deposition of different fibrillar collagen types and often correlates with adverse prognosis in carcinomas *(4, 5)*. In addition to quantitative changes in collagen deposition, the architecture of the collagen scaffold is also drastically affected during cancer evolution, both in primary tumors and in metastatic sites. Indeed, collagen cross-linking by lysyl oxidase (LOX) recently appeared as a key determinant of late-stage tumors and metastases *(6)*. Recognition of the importance of the tumor mi-

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[2] Nonstandard abbreviations: BECs, blood endothelial cells; MSCs, mesenchymal stem cells; LECs, lymphatic endothelial cells; CAFs, carcinoma-associated fibroblasts; ECM, extracellular matrix; LOX, lysyl oxidase; BMDCs, bone marrow–derived cells; NK, natural killer cells; MDSCs, myeloid derived suppressor cells; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; MMPs, matrix metalloproteases; MCs, mast cells; Th1, T-helper 1; NF-κB, nuclear factor κ–light-chain-enhancer of activated B cells; STAT3, signal transducer and activator of transcription 3; IL-1, interleukin 1; FAP, fibroblast activation protein α; ROCK, Rho/Rho-associated protein kinase.

croenvironment in cancer evolution led to a shift from a tumor-centered view of cancer development to the concept of a complex tumor ecosystem in which cellular and molecular components are as influential as cancer cells themselves for cancer evolution and metastatic dissemination. One feature of all ecosystems is that minor alterations in a single component may cause a dramatic reorganization of the whole system. As a consequence, the interference with any element of the tumor ecosystem provides an opportunity to counteract cancer progression.

In this review we describe the cellular components of the tumor microenvironment and the strategies designed to target them. We will then focus on recent research studies that have shed light on the ECM as an influential regulator of cancer cell and host cell properties.

## Key Cellular Players of the Tumor Microenvironment

The host cells infiltrating tumors at early stages of tumor development include, at a minimum, infiltrating inflammatory cells, CAFs, and bone marrow-derived cells (BMDCs) (hematopoietic progenitor cells and endothelial progenitor cells). CAFs can derive from local fibroblasts as well as from a plethora of progenitor cells including BDMCs, endothelial cells through endothelial-to-mesenchymal transition, and cancer cells through epithelial-to-mesenchymal transition (7, 8). Immune cells, including macrophages, lymphocytes, dendritic cells, and natural killer cells (NKs) are crucial for tumor suppression. However, this immune response is negatively regulated by myeloid-derived suppressor cells (MDSCs), which neutralize the activation of T cells, NKs, and macrophages (9). Accumulated evidence has demonstrated that leukocyte subsets of both myeloid and lymphoid origin that infiltrate tumor tissues induce chronic inflammation and secrete cytokines, chemokines, growth factors, and proteases that promote the formation of new blood vessels and elaborate a fertile tumor ecosystem for tumor growth and progression (3) (Fig. 1). It is now becoming clear that the contribution of stromal cells to cancer development is not limited to primary tumor growth, but also involves the establishment of premetastatic niches at distant organs and metastasis (1, 2). Targeting the cellular component of the tumor microenvironment has emerged as a promising approach and constitutes the basis for antiangiogenic and antiinflammatory therapies applied to different types of human cancer (3, 10). Therapies targeting cellular constituents of the tumor microenvironment, notably endothelial and inflammatory cells, have showed benefits but also have several limitations.

## Targeting Angiogenesis

Tumor growth and expansion are dependent on oxygen and nutrients provided by the newly formed blood vessels. This capability to shift to a vascularized state, called the angiogenic switch, is dependent on cancer cell interaction with the local microenvironment (1). As initially proposed by Judah Folkman more than 40 years ago, targeting angiogenesis appeared as a unique opportunity for therapeutic intervention in cancer treatment. More than 1000 clinical trials have been conducted worldwide with antiangiogenic drugs. In the case of vascular endothelial growth factor (VEGF), the anti-VEGF antibody bevacizumab increases overall survival or progression-free survival of patients with metastatic colorectal cancer, non–small cell lung cancer, and breast cancer when given in combination with conventional chemotherapeutic regimens (10). Sunitinib, a multireceptor tyrosine kinase inhibitor, also offers a clinical benefit for patients with renal cell carcinoma and advanced gastrointestinal stromal tumors, a benefit that could be in part due to its c-KIT–inhibitory activity. Sorafenib, an antiangiogenic tyrosine kinase inhibitor that also targets Raf kinase activity, has been approved for the treatment of renal cell carcinoma and liver cancer. Overall, the survival benefits of antiangiogenic drugs have been rather modest so far, and surprisingly most cancer patients stop responding or do not respond at all to the antiangiogenic therapy [reviewed in (10)]. During the last 4 years, controversy has increased regarding antiangiogenic therapies because of the lack of response seen in the majority of patients and the divergence between preclinical and clinical data. Recently, these controversies have been fueled by intriguing preclinical reports from 2 leading angiogenesis laboratories showing that antiangiogenic drugs cause a switch to vasoinvasion of tumor cells, leading to increased metastasis and shortened life in mice (10, 11). Both studies highlight a possible role of microenvironmental defense mechanisms in drug failure, which may lead to a more aggressive and invasive tumor phenotype. Tumor-dependent and tumor-independent host-mediated resistance to antiangiogenic drug mechanisms are described and summarized in a nonexhaustive list in Table 1. Although phase III clinical randomized studies from 4205 cancer patients did not support the concept of cancer aggravation after cessation of anti-VEGF therapy (12), pertinent questions can be raised as to how to best treat cancer patients with antiangiogenic medicine in the future (10). Emerging challenges in the development of antiangiogenic drugs for cancer treatment have led to increased interest in the development and/or optimization of antiangiogenic drugs as adjuvant or neoadjuvant therapies combined with traditional cytotoxic
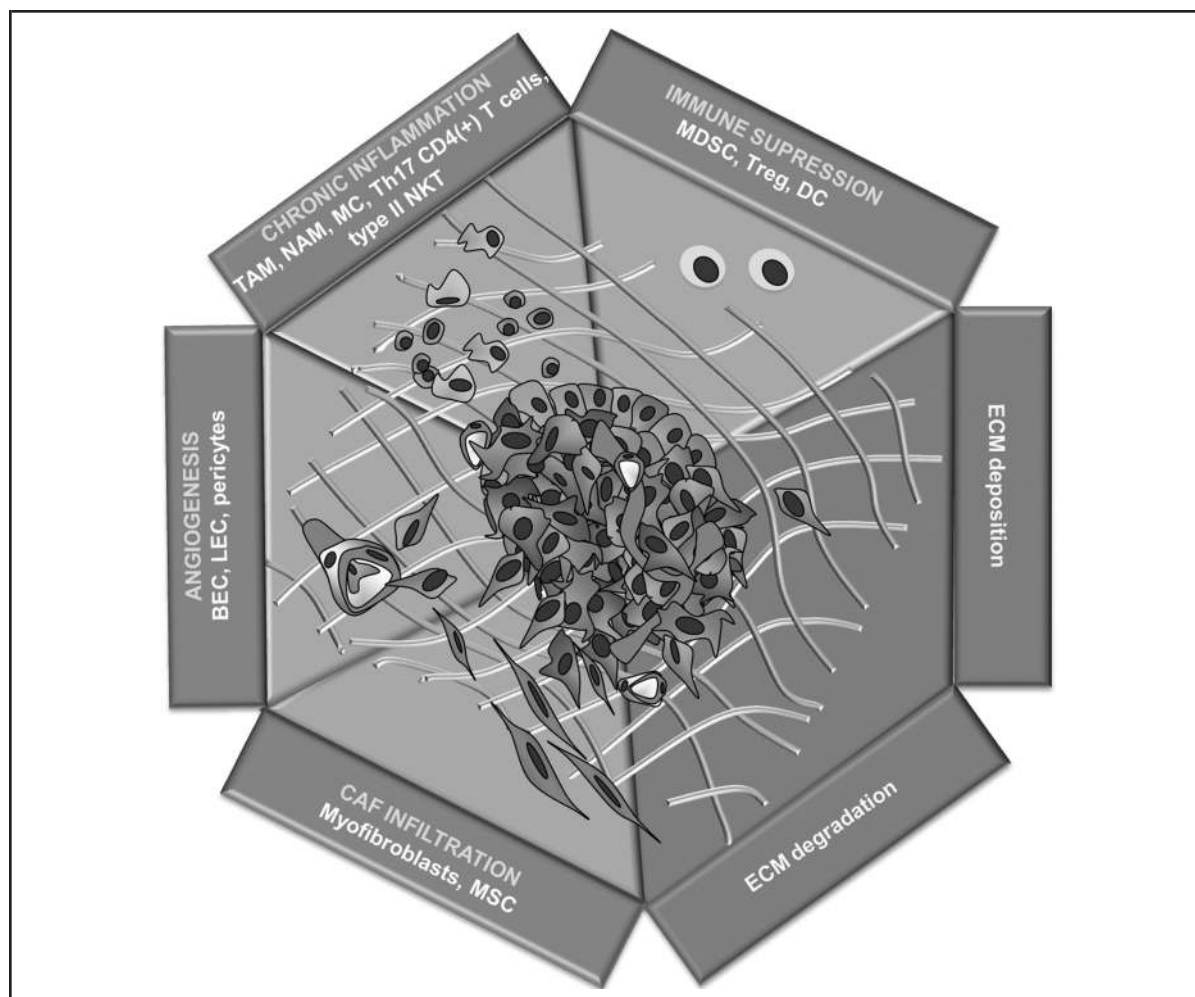
**Fig. 1. Cellular and noncellular elements of the tumor microenvironment.**

Tumor progression requires a cooperative interplay between host and cancer cells and reciprocal feedback between ECM molecules, host cells, and cancer cells. Host cells of the tumor microenvironment are mainly inflammatory cells such as tumor-associated macrophages (TAM), MCs, tumor-associated neutrophils (TAN), BECs, LECs, CAFs, and bone marrow-derived MSCs. Depending on their polarization status, immune cells can exert antitumor or protumor functions, for instance Th1 vs Th17 subsets of CD4(+) T cells, type I vs type II NKT cells, M1 vs M2 macrophages, and N1 vs N2 neutrophils. To escape the adaptive immune response, tumor cells recruit inflammatory cells that suppress T-cell responses. These include MDSCs, regulatory T cells, and tolerogenic dendritic cells (DC). ECM that is intensively remodeled (deposition and degradation) during the whole process of cancer progression has a direct effect on host cell differentiation and on cancer cell malignancy.

chemotherapies. It is worth noting that most of the clinical trials with antiangiogenic drugs were conducted during advanced stages of tumor development, whereas the most promising preclinical tests were conducted in animal models at early stages of tumor development. Moreover, antiangiogenic drugs used in the clinic are centered on the blockade of the VEGF-signaling pathway, whereas VEGF-independent angiogenic factors such as fibroblast growth factor, angiopoietins, placental growth factor (PlGF), matrix

metalloproteases (MMPs), and ECM molecules are worth considering *(6, 13)*. In addition, new multitarget antiangiogenic drugs may have great potential for the future of antiangiogenic therapy.

**Targeting Inflammation**

It is now well established that chronic inflammation contributes to cancer development. Clinical and experimental data indicate that the presence and activation

| | **Table 1.** Mechanisms of resistance to antiangiogenic therapies (nonexhaustive list). | |
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| | **Potential mechanisms of resistance to antiangiogenic therapy** | **References** |
| Tumor-dependent mechanisms | Increased expression of prometastatic proteins, for instance IL-8 | Huang et al. *(14)* |
| | Initiation of tumor epithelial–mesenchymal transition | Hammers et al. *(15)* |
| | Differentiation of cancer cells to cancer cell–derived endothelial cells | Wang et al. *(16)* |
| | Activation of compensatory pathways by cancer cells (for instance VEGF-A, -B, -C; PlGF; VEGF receptor 1) | Fan et al. *(17)* |
| Nontumor host-dependent mechanisms | Stimulation of angiogenesis by cancer associated fibroblasts involvement of PDGF-C[a] | Crawford et al. *(18)* |
| | Upregulation of circulating compensatory proangiogenic factors by host cells, for instance VEGF, PlGF, G-CSF, osteopontin, angiopoietin 2, PDGFA, and SDF1$\alpha$ | Ebos et al. *(10)* |
| | BMDC mobilization of myeloid suppressor type and upregulation of G-CSF and Bv8 (prokineticin) | Shojaei et al. *(19)* |

[a] PDGF-C, platelet-derived growth factor-C; G-CSF, granulocyte colony-stimulating factor; PDFGA, platelet-derived growth factor subunit A; SDF1$\alpha$, stromal cell-derived factor-1$\alpha$.

of chronic innate immune cell types, e.g., neutrophils, macrophages and mast cells (MCs) promote cancer development. Therefore, whereas the past point of view was that host immunity was protective against cancer, it is now clear that some subsets of chronically activated innate cells promote the growth and/or facilitate the survival of neoplastic cells *(3)*. Advances in tumor immunology have highlighted a high diversity in tumor-infiltrating leukocyte subsets that can play antagonist functions. Depending on their polarization status, immune cells can exert either antitumor or protumor functions, for instance T-helper 1 (Th1) vs Th17 subsets of CD4(+) T cells, type I vs type II NKT cells, M1 vs M2 macrophages, and N1 vs N2 neutrophils, respectively. Chronically activated and polarized immune cells such as M2 macrophages *(20)* and N2 neutrophils *(21)* produce or carry a myriad of chemokines, cytokines, growth factors, and proteases leading to tissue remodeling, angiogenesis, cell proliferation, genomic instability, and expansion of neoplastic cells into ectopic tissue, i.e., malignant conversion and cancer development. Preclinical evidence supports the use of antiinflammatory drugs in cancer prevention and therapy. Several tumor-promoting inflammation inhibitors are designed to: (*a*) inhibit signal transducers and transcription factors that mediate survival and growth, such as nuclear factor $\kappa$-light-chain-enhancer of activated B cells (NF-$\kappa$B) or signal transducer and activator of transcription 3 (STAT3); (*b*) inhibit tumor-promoting chemokines and cytokines that promote tumor infiltration by inflammatory cells such as interleukin 1 (IL-1), IL-6, tumor necrosis factor-$\alpha$, or receptor antagonists targeting C-C chemokine receptor types 2 and 4 and C-X-C chemokine receptor type 4; (*c*)

deplete the tumor-promoting immune and inflammatory cells that promote tumor development and progression such as MDSCs and macrophages. Notably, macrophage depletion with anti–macrophage colony-stimulating factor receptor antibody in a murine model of osteosarcoma efficiently suppressed tumor angiogenesis and lymphangiogenesis, whereas it did not affect healthy vascular and lymphatic systems outside tumors. It is worth noting that in contrast to anti-VEGF therapy, the targeting of macrophage colony-stimulating factor did not lead to tumor regrowth after treatment withdrawal *(22)*.

Several antiinflammatory drugs have been found to reduce tumor incidence when used as prophylactics, and to slow down tumor progression and reduce mortality when used as therapeutics, such as cyclooxygenase 2 inhibitors in colorectal cancer and in breast and colorectal cancer resistant to chemotherapy *(23)*; nonsteroidal antiinflammatory drugs in breast, colorectal, and prostate cancer; and the antiinflammatory steroid dexamethasone in brain tumors. However, nonsteroidal antiinflammatory drugs are not specific and usually have side effects that prevent their long-term administration. Advances in preclinical research have highlighted the fact that resistance to chemotherapy as well as radiotherapy are caused by the chronic activation of NF-$\kappa$B *(24)*. Several inhibitors of NF-$\kappa$B or STAT3 have been reported to enhance the effect of therapeutic agents in the treatment of bone metastasis in prostate cancer. However, sustained NF-$\kappa$B inhibition can result in severe side effects caused by immune deficiency, leading to neutrophilia, enhanced acute inflammation due increased IL-1$\beta$ secretion, and liver damage *(25)*. These side effects have hampered the progress of

NF-κB and IKKβ (NF-κB kinase subunit β) inhibitors in clinical development. Clinical trials evaluating the efficacy of drugs targeting cytokines such as anti-IL-6 and anti–tumor necrosis factor-α as single agents in various cancers have shown a modest therapeutic benefit with a partial response (26). Blocking antibodies against chemokines, including receptor antagonists targeting C-C chemokine receptor types 2 and 4 and C-X-C chemokine receptor type 4, have also been evaluated in vivo (26). In recent preclinical studies, anti-RANKL (anti–receptor activator of NF-κB ligand) antibodies inhibited bone metastasis in prostate and breast cancer (27), and IL-1 inhibition blocked myeloma progression (28). Although targeting single cytokines, chemokines, or transcription factors has led to interesting results in preclinical assays, their potential as single agents in the treatment of human cancers is limited. Combinations with other targets are needed in clinical trials to ensure their efficacy and to limit their side effects. On the other hand, the finding that Gr1+CD11b+ MDSCs neutralize antitumor immunity of T cells suggests an interesting target to enhance T-cell–mediated cancer therapy (9).

## Targeting CAFs

CAFs are involved in cancer progression and metastasis through their ability to enhance tumorigenecity, angiogenesis, and metastatic dissemination of cancer cells compared with normal fibroblasts (4). It is of particular interest that CAFs express a membrane-bound serine protease called fibroblast activation protein α (FAP) that is not detected in normal fibroblasts. FAP expression has been associated with an overall poorer prognosis in several cancer types, including colon, ovarian, pancreatic, and hepatocellular carcinoma, but not in breast cancer (5). Immunohistochemical studies have demonstrated that FAP is mainly localized in the stroma adjacent to tumor cells but not in the stroma of normal tissue, making it a very attractive candidate for tumor-targeted therapies (7). However, several phase I and II studies targeting FAP with a humanized monoclonal antibody (sibrotuzumab) failed to produce clinical benefits in colon and non–small cell lung cancer. Furthermore, attempts to block the enzymatic activity of FAP with small molecule inhibitors combined with docetaxel have resulted in lowered survival rates of lung cancer patients. Based on such conflicting data generated by directly targeting FAP in the tumor microenvironment, an alternative could be to use the enzymatic activity of FAP localized specifically in the tumor stroma to activate cytotoxic prodrugs. This strategy is expected to enhance drug efficacy delivered to the tumor microenvironment (5). In addition, one cannot exclude the possibility that different fibroblastic

subsets display opposite effects on tumor progression that might contribute to the failure of strategies aiming to target only 1 specific antigen of CAFs. Further studies are required to test this hypothesis.

## Noncellular Tumor Microenvironment

Host and cancer cell interactions occur within a dense ECM network that governs and influences the properties of both cancer and host cells. The noncellular compartment of the tumor microenvironment is now recognized as an important regulator of cancer evolution. We next highlight the crosstalk between the cellular and noncellular part of the tumor microenvironment, a key determinant in cancer progression and metastatic dissemination. The noncellular environment comprises not only ECM molecules, but also includes physical and chemical parameters such as pH, oxygen tension, interstitial pressure, and fluid flux. Herein, we discuss the effect of ECM change as part of the tumor environment that influences cancer cell behavior. The ECM is no longer viewed as a static structure that simply maintains tissue morphology but is now recognized as a dynamic element of the tumor microenvironment. ECM molecules and their metabolites are known to regulate cell proliferation, migration, angiogenesis, and cancer metastasis. Any perturbation of ECM synthesis, degradation, density, and rigidity can considerably influence the capacity of the tumor microenvironment to promote cancer cell proliferation, migration, and invasion, as well as modulate inflammatory responses and lymphangiogenesis (6, 29, 30).

### ECM STIFFNESS, DENSITY, AND TOPOGRAPHY REGULATE CANCER CELL BEHAVIOR

The crosstalk between stromal and tumor cells is known to be mediated by chemical signals issued from ECM components and by ECM organization. The noncellular part of the tumor microenvironment is subjected to chronic rearrangement, leading to newly formed ECM, which in turn greatly affects fundamental cell properties. The term neo-ECM used herein refers to any ECM abnormality in diseased organs appearing through its synthesis, accumulation, degradation, density, and/or stiffness. Increased matrix deposition (known as desmoplasia) is a consequence of altered gene expression of CAFs, which leads to a dynamic evolution of the tumor stroma (4). CAFs recruited in the tumor form an active stroma that influences ECM plasticity and architecture, resulting in cancer malignancy (4). Remarkably, ECM density and stiffness can affect tumor cell invasive phenotype. Mammographically dense breast tissue has been linked to an increased risk of breast carcinoma (31). Breast density is associated with up to 30% of breast cancer,
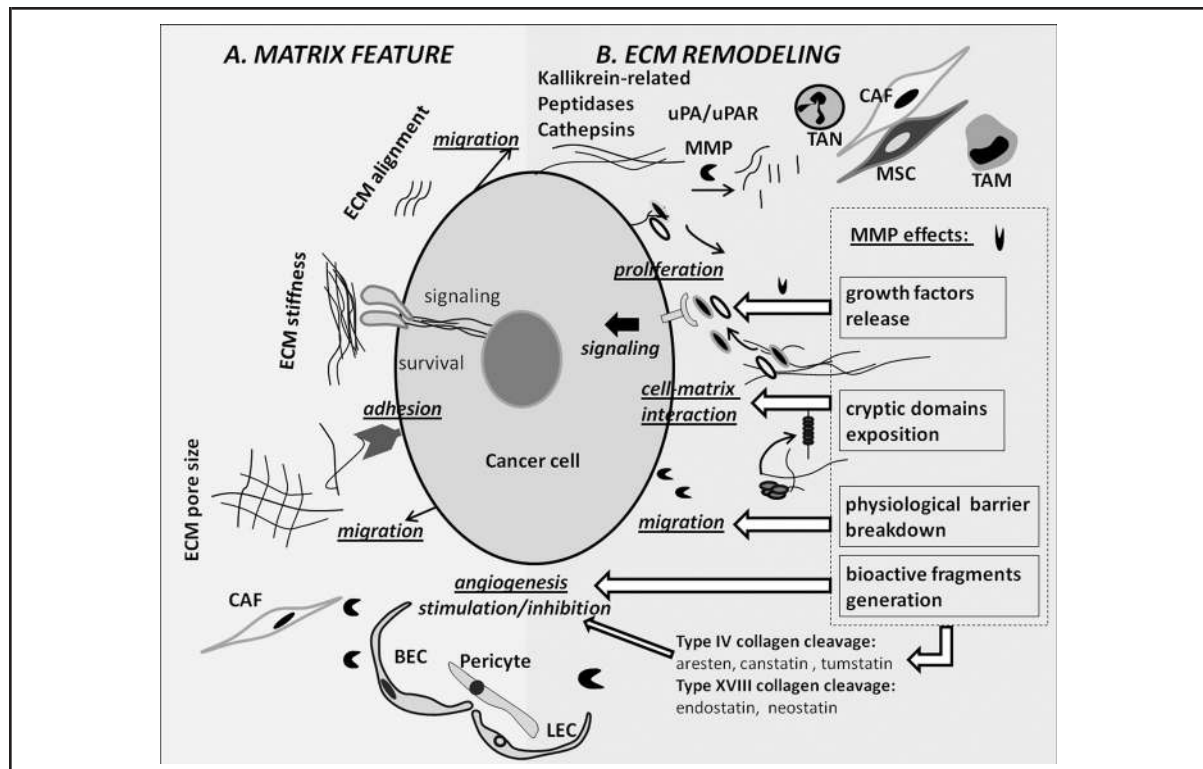
**Fig. 2. Regulation of cancer cell behavior by ECM features.**

(A), Neo-ECM properties, including molecular composition, stiffness, and density, influence cancer cell proliferation and migration. Collagen fiber alignment and ECM pore size determine cell migration direction and cell invasiveness. (B), ECM remodeling is dependent on the action of protease families such as urokinase plasminogen activator/urokinase plasminogen activator receptor (uPA/uPAR), kallikrein-related peptidases, cathepsins, and MMPs. MMPs are produced by CAFs, tumor-associated macrophages (TAM), tumor-associated neutrophils (TAN), MCs, BECs, LECs, and bone marrow–derived MSCs. The MMP-mediated breakdown of ECM barriers promotes cancer cell migration and increases the bioavailability of growth factors and cytokines sequestered either at the cell surface or in the matrix. ECM proteolysis exposes the cryptic domain within ECM molecules and generates bioactive fragments that can promote cell migration or regulate angiogenesis.

whereas *BRCA1* (breast cancer 1, early onset)[3] and *BRCA2* (breast cancer 2, early onset) mutations account for only 5% of breast cancers *(32)*. An excess in LOX activity has been correlated with ECM stiffness and poor prognosis in breast, head and neck, colorectal, and prostate cancer *(6, 31)*. The effect of LOX enzyme on the lateral cross-linking of collagen fibers and other ECM molecules, such as elastin, has been shown to be a major cause of cancer malignancy in several preclinical mouse models *(6)*. Although the LOX family has been long considered to include tumor-suppressor genes in mammary epithelial transformation dependent

on *HRAS* (v-Ha-ras Harvey rat sarcoma viral oncogene homolog) and *ERBB2* [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)] (also known as *HER2* and proto-oncogene Neu), it also promotes cancer metastasis through the induction of neo-ECM formation. There are conflicting views on the use of LOX inhibitor in cancer treatment [issue reviewed in *(6)*]. The ECM stiffness and density molecules are sensed by integrins and adhesion molecules that link ECM fiber to the cellular cytoskeleton (Fig. 2). ECM deposition and increased tissue stiffness have been noted to enhance tumor progression through altering integrin signaling, focal adhesions, Rho/Rho-associated protein kinase (ROCK) pathway activation, and actomyosin- and cytoskeletal-dependent cell contractility *(33)*. The ROCK-activation–dependent tissue stiffness leads to

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[3] Human genes: *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset; *HRAS*, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; *ERBB2*, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian).

β-catenin activation, which in turn induces epidermal hyperplasia and tumor growth. The activation and clustering of integrins dependent on ECM elasticity lead to a mechanosensitive signaling cascade (Fig. 2). Interestingly, targeting integrins by a drug that blocks αVβ3-integrins has been shown to inhibit bone metastasis of breast cancer cells and enhance the efficacy of radiation treatment in mice bearing mammary adenocarcinoma *(34)*.

ECM REMODELING

ECM is quantitatively and qualitatively deregulated in cancer in which increased deposition, altered organization, enhanced proteolytic activity, and ECM turnover regulate tumor progression. These capabilities are acquired by cancer and stromal cells through the increased expression and/or activity of proteolytic enzymes, especially the serine proteases, including the urokinase-type plasminogen activator/urokinase-type plasminogen activator receptor/plasminogen/plasmin system *(35)*, kallikrein-related peptidases *(36)*, cathepsins, and MMPs *(37)*. These different proteolytic systems are interconnected. For instance, serine proteases such as plasmin can convert most pro-MMPs into active enzymes. Remarkably, increased expression of interstitial collagens, the main ECM components of the stroma, and many of their remodeling enzymes such as MMP1, MMP2, MMP11, and MMP13, are frequently detected in gene signatures associated with poor prognosis in cancer patients *(29)*. MMP1 belongs to the gene expression signature in breast cancer patients and may be considered as a valuable biomarker to predict distant metastasis *(38)*. MMP13 is viewed as a potential tumor marker for breast cancer diagnosis, and its expression is correlated with metastasis formation *(39)*. It is a stromal mediator of cancer progression *(8)* that regulates the release of angiogenic factors and metastatic dissemination in experimental models *(29)*. In breast cancer, MMP11 derived from cancer-associated adipocytes or fibroblastic cells exerts type VI collagenolytic activity *(40)*. More recently, MMP2 has been shown to influence lymphangiogenesis through its interstitial collagenolytic activity *(30)*.

MMP-mediated collagen remodeling can regulate tissue architecture through different mechanisms (Fig. 2). These can generate extracellular space for cell migration and unmask cryptic sites within ECM molecules, thereby modifying cell-to-matrix adhesion. By promoting the release of matrix-associated growth factors or cytokines, they modulate the activity or bioavailability of signaling molecules during vascular response to physiological or pathological stimuli. In addition to this MMP-driven ECM degradation process, MMP activities also result in the generation of matrix fragments displaying novel biological activity. It

is now well recognized that collagen proteolysis may release a number of endogenous angiogenesis inhibitors, including type IV (arresten, canstatin, tumstatin), type V (restin), and type XVIII (endostatin, neostatins) collagen fragments among other fragments of ECM proteins that may display antiangiogenic activity *(29, 37)* (Fig. 2). These bioactive fragments become released upon proteolysis of both the interstitial matrix and the vascular basement membrane. These molecules can be found both in the circulation and sequestered in the ECM surrounding cells. These angioinhibitory fragments regulate primarily endothelial cell proliferation and apoptosis by interfering with integrins. Although the role of matrix-derived angiogenesis inhibitors has been well studied in animal models of cancer, their role in human cancers is less established. The ECM-derived inhibitors have a potential use as cancer therapeutic agents and biomarkers.

Although high levels of MMPs correlate with poor prognosis in cancer patients, and modulation of MMP activity changes tumor phenotype, MMP inhibitors have failed clinically *(35)*. Several reasons can explain this failure, such as the broad-spectrum activity of the inhibitor used, the late stage of the disease at which inhibitors were administered, and the opposite effects exerted by MMPs on cancer progression, some of them boosting and others inhibiting it. This unexpected lesson from clinical trials helped shed light on the complexity of the diverse proteolytic systems involved during different steps of cancer evolution and paved the way for new discoveries of underappreciated functions of proteases.

## Conclusions and Perspectives

The emerging picture arising from these studies reveals a complex interplay between tumor cells, host cells, and the ECM. Specific modifications of the tumor microenvironment during cancer progression endow cancer cells with malignant properties leading finally to metastatic dissemination, which remains the major cause of death in cancer patients. In the past decade, we have advanced our knowledge of the mechanisms by which cancer cells, inflammatory cells, endothelial cells, and fibroblastic cells interact. Owing to the complex nature of tumor cell–host cell interactions, as well as cell–ECM interactions inside a tumor, a better understanding of this complex ecosystem will be required to improve cancer therapies. The time is right to decipher each pathway to reinforce the efficacy of cancer therapeutics. It is unlikely that the targeting of any single molecular pathway or cell type will lead to efficient anticancer therapies and avoid the acquisition of resistance to treatment. A combination of classical chemo- and radiotherapy with antiinflammatory and antian-

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giogenic strategies targeting the tumor microenvironment is required to reach long-term efficiency. Future therapies should also take into consideration the ECM perturbation that affects stromal and cancer cell behaviors.

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