Published in final edited form as:

Crit Rev Oncog. 2015 ; 20(3-4): 301–314.

Physiological, Tumor, and Metastatic Niches: Opportunities and Challenges for Targeting the Tumor Microenvironment

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Abstract

The primary tumor niche and the related but distinct premetastatic/metastatic niche comprise a number of essential players, including immune cells, stromal cells, and extracellular matrix. The cross-talk between these components is key to tumor progression. Many of these cell types and signaling pathways in the tumor microenvironment also are found in physiological and stem cell niches, such as the bone marrow, colonic crypt, and skin bulge. Here they play tightly regulated roles in wound healing and tissue homeostasis. Understanding the similarities and differences between these distinct niches may better inform our ability to therapeutically target the tumor microenvironment. In this review we discuss a number of tumor and metastatic niche components as they relate to stem cell niches and highlight potential therapeutic strategies in pediatric cancers.

Keywords

premetastatic niche; metastatic niche; metastasis; stem cell niche; bone marrow; tumor microenvironment; inflammation; wound healing; immune cells; stroma; stromal cells; extracellular matrix; cancer associated fibroblasts

I. INTRODUCTION

While much attention has been paid to the cellular and genetic events within tumor cells that contribute to cancer progression, it has become clear in recent years that interaction of a tumor with its local microenvironment and its systemic effects on the host is critical for this process. The bidirectional communication of tumor cells with their microenvironment has been demonstrated in murine and zebrafish models in which the cancer phenotype of transplanted tumor cells was reversed by the embryonic microenvironment.¹ The tumor microenvironment can be defined as a specialized niche comprising tumor cells, activated stromal cells, infiltrating immune cells, and extracellular matrix (ECM) that regulate tumor development and progression.² The interplay between tumor cells and nonmalignant cell types and their contribution to niche formation, resistance to therapy, and cancer spread^{3,4} are all currently active areas of research. Numerous studies now support the idea that the altered microenvironment as a whole, and not exclusively tumor cell genetic dysfunction,

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promotes tumor pathogenicity and progression. Related but distinct from the primary tumor niche is the premetastatic niche, a supportive environment at distant organ sites that promotes the survival and proliferation of newly metastatic cells.⁵ This specialized niche is established by tumor-secreted factors that direct a number of microenvironment players, including stromal cells and bone marrow-derived cells (BMDCs), into tumor-promoting roles. The observations that led to the premetastatic niche concept add to Stephen Paget's 1889 seed and soil hypothesis, which stated that the propensity of tumor cells to metastasize to distant organs depends on the ability of the host microenvironment, which he referred to as the "soil," to support the tumor cells, the "seed."⁶ Paget's influential observations were based on the examination of breast cancer autopsy records that indicated that metastatic sites were not random, but rather were preferentially localized to specific organ sites.

Many parallels have been drawn between the tumor niche and physiological niches, including those observed in inflammation/wound healing⁷ and development^{8,9} (Fig. 1). Stroma-modulating and immune cell-secreted paracrine factors, such as growth factors and interleukins (ILs), play significant roles in development and normal tissue homeostasis and become dysregulated in the tumor microenvironment. These factors play well-documented roles in tumor angiogenesis, fibrosis, and inflammation, directly affecting tumor cell survival within a particular environment. Further, tightly regulated decisions of cell fate observed in normal stem cell niches that dictate apoptosis versus survival and proliferation versus quiescence are co-opted in tumor niche biology.

In this review we focus on the cellular and extracellular components of tumor and premetastatic niches and how understanding the biology of tumor and microenvironment interactions can provide insight into tumor progression to uncover novel treatment strategies that complement traditional tumor cell-targeting approaches. Although less about the tumor microenvironment is known in pediatric cancers than in the adult setting, we discuss what is known in pediatric cancer, what can be easily extrapolated from what is known about more common cancers in adults, and what is unique and ultimately targetable about tumor microenvironment, stem cell niche, and tumor niche biology in pediatric cancers.

II. PHYSIOLOGICAL STEM CELL NICHES AND TUMOR NICHES

The stem cell niche is a specialized environment that regulates decisions of the fate of stem cells. The role of these physiological niches in mediating cell fate is critical to maintaining tissue homeostasis and repair. The niche environment is dynamic, and the information it senses and conveys reflects the state of the local tissue as well as distant sites. Although stem cell niches differ in tissue-specific composition and localization, there are commonalities to niche biology that explain how cell-cell interactions and ECM can form a milieu that controls cell fate. These key cellular constituents that are common to diverse niches include vascular cells, hematopoietic cells, and stromal cells (Fig. 1). The relative contribution of cell-intrinsic characteristics versus extrinsic environmental cues for the induction and maintenance of a stem cell phenotype is a topic of controversy, and the potential of a stem cell niche to induce a stem-like phenotype remains an issue of interest.¹⁰ A deeper understanding of the complex interactions between cells and their environment has the potential to provide powerful insights into tumorigenesis and niche biology in the cancer

setting. The environment of the tumor cell can dictate cell fate decisions and the genetic alterations specific to a tumor cell. Whether tumor cells arise from a preexisting stem cell or acquire cell-renewal capabilities based on alterations in stem cell morphogenic pathways is still under debate. No matter the origin, the tumor cell can be particularly responsive to niche cues, and understanding tumor–niche interactions can provide insight into tumor dormancy, therapeutic resistance and metastasis.^{3,6}

III. CELLULAR AND EXTRACELLULAR COMPONENTS OF TUMOR NICHES

A. Stromal Cell Contributions to the Tumor and Metastatic Microenvironments

Cancer-associated fibroblasts (CAFs) constitute the majority of stromal cells within the tumor microenvironment. They are thought to play a major role in matrix production and remodeling, similar to that in wound healing. CAFs are large, spindle-shaped mesenchymal cells that share characteristics with smooth muscle cells (SMCs) and pericytes, particularly when they become activated myofibroblasts expressing α -smooth muscle actin (α SMA).¹¹ Although no precise definition of CAFs exists, a number of markers have been proposed, including α SMA, vimentin, desmin, fibroblast specific protein-1, platelet-derived growth factor (PDGF) receptor α , PDGF receptor β , transforming growth factor (TGF)- β receptor 2, fibroblast activation protein (FAP), or some combination of these. Many of these markers, however, are transiently expressed by CAFs or shared by other mesenchymal cell types, making it difficult to accurately identify this population.¹²

Evidence suggests that metastasis-promoting CAFs differentiate from bone marrow–derived mesenchymal stem cells (MSCs),^{13–15} a multipotent cell population thought to be involved in wound healing. MSCs are characterized by their ability to differentiate into mesodermal cell types such as adipocytes, chondroblasts, osteoblasts, and myocytes, underscoring the ability of MSCs to contribute to tissue homeostasis by adapting to developmental and inflammatory microenvironments.¹⁴ In the developmental setting MSCs secrete ECM components, growth factors, and cytokines, through which cell-to-cell signaling and migration functions are activated in tightly regulated and well-coordinated systems.¹⁶ In inflammatory environments, MSCs can respond to various cytokines first to promote and then suppress immune inflammatory responses. For instance, during early acute stages of inflammation, MSCs promote the recruitment of neutrophils and macrophages and secrete proinflammatory cytokines.¹⁷ At later stages of inflammation, however, MSCs can become immunosuppressive, whereby they promote tissue homeostasis by polarizing macrophages to an M2 phenotype.¹⁸ M2 macrophages are characterized by their anti-inflammatory, protumorigenic behavior and ability to recruit regulatory T cells (Treg). These powerful functions of MSCs, when co-opted in tumor niches, promote tumor progression and metastasis through the development of CAFs and other pathogenic mesenchymal cell types.

Bone marrow–derived MSCs promote tumor survival and treatment resistance by secreting factors that suppress apoptosis in tumor cells.¹⁹ In neuroblastoma, MSCs, along with monocytes, produce high levels of IL-6, which is responsible for tumor cell survival, angiogenesis, and inflammation. In turn, neuroblastoma cells produce galectin-3 binding protein, which stimulates MSCs. This feedback loop may be a potential anticancer target in pediatric solid tumors.²⁰

Many studies have indicated that fibroblasts play a tumor-promoting role, which has spurred much interest in identifying and developing molecular targets to eliminate CAFs.^{11,12} Similar to the role fibroblasts play in matrix remodeling during wound healing, CAFs produce matrix proteins, secrete matrix-remodeling enzymes, and can support tumor cell growth and migration through these actions.¹² Tumor cells that are grown with CAFs result in larger, more angiogenic tumors than those grown with normal fibroblasts derived from non-tumor-bearing settings in a number of cancer models, including prostate, breast, ovary, and pancreas.^{12,21} Markers such as α SMA, FAP, and TGF- β receptor 2, which often are attributed to CAFs, are correlated with poor prognosis in patients; however, no one marker sufficiently identifies this population, nor distinguishes CAFs from other stromal cell types.^{22–24}

Despite the evidence that CAFs contribute to tumorigenicity and tumor progression, their precise role and function in the tumor environment remains poorly understood. A recent study demonstrated that elimination of α SMA-positive cells in a murine model of pancreatic cancer resulted, surprisingly, in increased tumor invasion and progression.²⁵ That study highlights the complex role that such mesenchymal cells might play in the tumor microenvironment. While many studies implicate FAP-expressing CAFs in tumor growth and angiogenesis, suggesting that they could be a good therapeutic target, preclinical studies of transgenic mice demonstrated that elimination of FAP-expressing cells results in cachexia and anemia.²⁶ Together, these studies indicate that fibroblasts or other α SMA- and FAP-expressing cells are essential in normal biology, and that additional unique molecular markers that distinguish CAFs from normal fibroblasts are necessary to successfully develop therapeutic strategies that target this cell population.

B. Tumor Angiogenesis in the Primary Tumor Microenvironment and Metastasis

Tumor angiogenesis is essential for tumor growth/expansion, immune cell trafficking, and tumor-derived factor secretion.²⁷ Vascular endothelial cells (ECs) from the local environment and bone marrow–derived endothelial progenitor cells (EPCs) are recruited to tumor niches by proangiogenic factors to initiate and contribute to vessel formation.^{11,28} Blood vessel formation is highly dysregulated in tumor niches, often exhibiting dense networks of ECs that form tortuous vessels that lack a normal hierarchical architecture.^{29,30} In contrast to ECs in normal biology, ECs in the tumor microenvironment are irregular in shape, with many projections into the vessel lumen and gaps at EC cell junctions that increase vessel permeability and permit both extravasation and intravasation of tumor and blood cells (Fig. 1). Perivascular SMCs and pericytes surround normal blood vessels and provide essential signals to ECs in response to environmental stimuli that dictate vessel diameter and permeability. In the tumor setting, however, SMC/pericyte localization around tumor vessels is impaired, resulting in immature, leaky blood vessels that have a larger diameter and inefficient nutrient and oxygen delivery.³⁰ This proangiogenic environment that fails to resolve into mature, functional blood vessels is influenced by tumor-secreted growth factors such as vascular endothelial growth factor (VEGF) and PDGF, as well as sustained signaling through hypoxia-induced factors (HIFs) such as HIF1 α .^{31–33} VEGF receptor (VEGFR)-2–expressing EPCs home in on the tumor microenvironment, where they can initiate neovasculogenesis, along with VEGFR-1–expressing BMDCs, which promote

angiogenesis and vascular stability.²⁸ Tumor-derived PDGF-BB stimulates pericyte recruitment to the tumor microenvironment but impairs pericyte maturation and function. Thus, tumor blood vessels lack the support of the SMCs/pericytes, resulting in increased vascular permeability.³⁴

In parallel to its proangiogenic functions in the primary tumor, the growth factor VEGFA secreted by the primary tumor can promote the formation of the premetastatic niche by inducing the expression of S100A8 by vascular endothelial cadherin-expressing ECs and Mac1-positive myeloid cells. This has been demonstrated specifically in the lungs, and it promotes the recruitment of more myeloid cells and the adhesion of circulating tumor cells, resulting in metastatic colonization.³⁵ ECs can also express P-selectin and E-selectin in response to the tumor-derived cytokines IL-1 and tumor necrosis factor- α , enhancing BMDC and tumor cell attachment at metastatic sites.³⁶ Circulating VEGFR-2-expressing EPCs preferentially home in to premetastatic niche sites, where they localize with blood vessels. Their arrival coincides with the appearance of colonizing tumor cells and supports tumor cell survival and metastatic growth by promoting angiogenesis.^{28,37}

Although the majority of studies of tumor angiogenesis have been conducted in adult solid tumors, many parallels have been drawn in the pediatric solid tumor setting. Overexpression of $\text{Np63}\alpha$, a p63 isoform, in osteosarcoma and neuroblastoma results in stabilization of HIF-1 α expression, secretion of VEGF, and, in turn, angiogenesis.³⁸ Secretion of proangiogenic factors such as VEGF, fibroblast growth factor, and placental growth factor have been observed in a number of pediatric rhabdomyosarcoma and neuroblastoma tumor cell lines, and pretreatment serum concentrations of VEGF were higher in patients with osteosarcoma who relapsed in the first year of treatment.^{39–41} Brain-derived neurotrophic factor and its receptor TrkB are overexpressed in neuroblastoma and induce overexpression of HIF-1 α , which in turn induces the expression of VEGF.^{42,43} Hypoxia-induced erythropoietin expression by neuroblastoma, Ewing sarcoma, Wilms tumors, rhabdomyosarcomas, and hepatoblastoma tumors promote EC proliferation and migration.⁴⁴ Taken together, these results suggest that, as with adult solid tumors, vascular cells play a key role in the tumor microenvironment of pediatric solid tumors.^{44–46}

C. ECM Remodeling Contributes to Tumor Progression

The ECM comprises a variety of proteins, proteoglycans, and polysaccharides that together make up the basement membrane and the interstitium.⁴⁷ The biochemical and biomechanical properties of the various members of the ECM contribute to its diverse range of signaling capabilities. Given the close proximity of the ECM to the cell populations necessary for organ function, the ECM is well situated to act in multiple signaling and regulatory pathways throughout development and disease.

Matrix remodeling by tumor cells, activated stromal cells, and immune cells within the tumor microenvironment is often correlated with malignancy.⁴⁸ The secretion of remodeling enzymes, such as matrix metalloproteinases (MMPs), heparanases, and cathepsins, into the ECM is responsible for this remodeling^{12,24,49} and, together with increased deposition of ECM components, results in fibrosis. These changes have been hypothesized to direct tumor

architecture and confer altered biomechanical properties, such as enhanced stromal rigidity, which may augment tumor cell migration.^{50,51}

Matrix remodeling enzymes that are secreted by a tumor not only modulate the local tumor environment but also promote premetastatic niche formation at distant tissue sites. In particular, tumor-secreted lysyl oxidase can remodel premetastatic lung ECM by cross-linking collagens and elastins in a breast cancer model of metastasis and promoting BMDC adhesion and invasion into the lung basement membrane.⁵² Recruited BMDCs further remodel the local premetastatic environment through MMP2 and MMP9 secretion, which cleave cross-linked collagens and enhance invasion through the interstitial matrix. Tumor-secreted factors also enhance fibronectin expression in the premetastatic niche, which enhances the adhesion of VEGFR-1–expressing BMDCs via the integrin receptor $\alpha_4\beta_1$.⁵ Organ specificity of metastasis is regulated, at least in part, by alterations in the ECM. For instance, hyaluronan signaling via CD44 enhances CXCR4-CXCL12 signaling and results in preferential metastasis to the lung and bone.¹⁷ Another matrix protein, osteopontin, is also capable of binding to CD44 and is associated with metastasis through multiple mechanisms, including inhibition of apoptosis, adhesion, migration, and immune evasion.⁵³

Although much of the work on ECM in the tumor microenvironment has been studied in adult tumors, there is evidence suggesting that similar mechanisms play a significant role in pediatric tumors. For instance, ECM rigidity regulates neuroblastoma cell proliferation and phenotypic changes associated with differentiation in vitro and reduces expression of N-Myc, a key factor in disease progression.⁵⁴ Heparanase concentrations in the serum of patients with Ewing sarcoma are elevated compared with normal controls and correlate with poor response to treatment.⁵⁵

D. Host Immune Cells in the Tumor Microenvironment and Metastasis

Tumor-infiltrating lymphocytes are frequently found within the microenvironment of a primary tumor, suggesting activation of an immune response. Indeed, higher rates of tumor-infiltrating lymphocyte in solid tumors are associated with improved clinical outcome, including diminished metastatic recurrence.⁵⁶ Tumor cells have the potential to be highly immunogenic as a result of the expression of mutated proteins, immune-privileged proteins (e.g., testicular or ocular genes), or viral proteins.⁵⁷ Exploiting these tumor antigens to activate the immune system is a key goal in the field of cancer immunotherapies. The primary tumor, however, often expresses immune-inhibitory molecules and secretes factors that promote the development of local and distant immune-suppressive cell subsets. Immunosuppressive cell types such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), Tregs and regulatory B cells, and many secreted factors afford immune protection to a growing tumor and may also promote tumor growth, invasion, and metastasis.

TAMs are associated with poor prognosis and are correlated with tumor progression and metastasis through their ability to secrete cytokines and factors that support tumor growth. Analysis of tumor-infiltrating leukocytes in pediatric cancers compared with adult cancers identifies CD68-positive macrophages as the predominant immune cell infiltrate.⁵⁸ TAMs are recruited to the tumor microenvironment early in tumor development because of their

role in innate immunity and inflammation.⁵⁹ These cells have also been shown to respond to HIF1- α , colony-stimulating factor 1, and CXCL12.¹⁷ TAMs are a very plastic and diverse population; as such, the function of particular TAM subsets changes depending on the location within the primary tumor and the stage of tumor development. For instance, TAMs isolated from the leading edge of a tumor, adjacent to other stromal cells, demonstrate a proangiogenic function and promote ECM remodeling through secretion of MMP2 and MMP9.⁶⁰ These TAMs can also promote tumor cell invasion and dissemination. TAMs located within the hypoxic region of a tumor seem to play a greater role in suppressing infiltrating T cells.⁶¹ Similar to TAMs, M2 macrophages have been identified by transcriptional profiling and have demonstrated immunosuppressive behavior in tumors. They also have strong proangiogenic properties related to their ability to both secrete and respond to VEGF, and they are found in hypoxic regions of a tumor.⁶²

MDSCs are a heterogeneous population derived from myeloid precursors that normally develop into granulocytes, dendritic cells, or macrophages but remain immature and immune-suppressive in the tumor setting. MDSCs derived from patients with cancer express the markers CD11b, CD33, and CD34 and are negative for human leukocyte antigen DR.⁶³ MDSCs are found within the primary tumor and are increased in blood. In tumor-bearing mice, MDSCs also were increased in distant tissues, including the bone marrow and premetastatic tissues^{2,57,62}.

MDSCs play a critical role in tumor progression by suppressing both the adaptive and innate arms of the immune system, as well as contributing to tumor angiogenesis and lymphangiogenesis. MDSC numbers and level of immunosuppressive activity is driven by tumor burden, and several tumor-secreted factors that foster the development of MDSCs in the tumor microenvironment have been identified, including VEGF and CXCL12, the latter of which is a crucial retention signal for immune cells within tissue. In the premetastatic niche, upregulated S100A proteins, CXCL12, and HIF1- α result in MDSC recruitment and impaired maturation of other myeloid lineages such as dendritic cells.

MDSCs can promote immune suppression through multiple mechanisms. Secretion of reactive oxygen species and reactive nitrogen species and the depletion of L-arginine by MDSCs impair the signaling pathways that control T-cell proliferation in response to antigen stimulation.⁵⁷ MDSCs can also induce the de novo generation of Tregs.⁶⁴ The ratio and interaction of immunosuppressive cells with effector lymphocytes is a key balance between a productive tumoricidal immune response and immune protection.

Before the arrival of tumor cells, bone marrow-derived VEGFR1⁺ hematopoietic progenitor cells (HPCs) home to the premetastatic niche, where they cluster and recruit VEGFR-2-expressing EPCs.²⁸ VEGFR-1⁺ HPCs express the integrin receptor $\alpha_4\beta_1$, which enables these cells to localize preferentially to sites of high fibronectin expression, such as the primary tumor microenvironment and the premetastatic niche. These HPCs express the hematopoietic stem cell markers c-Kit and Sca-1. However, they also begin to express the marker CD11b, suggesting that these cells differentiate into immature myeloid cells within the premetastatic tissue.⁵

Many of the same factors that contribute to immune cell-mediated tumor promotion in adult tumors have been observed in the pediatric setting as well. In neuroblastoma, both TAMs and MDSCs infiltrate the tumor microenvironment and promote tumor progression through a number of mechanisms. Immune cell infiltration such as CD68+ TAMs are increased in samples from patients with Wilms tumor, and they coincide with increased levels of the inflammatory protein COX-2 in the tumor.⁶⁵ Several studies showed that myeloid cells and ECs are increased in the circulation of pediatric patients with cancer.⁴¹

In normal biology Tregs are mediators of immune tolerance to self-antigens. Tregs are characterized by their expression of CD4, CD25 and FoxP3, the latter of which is a key transcription factor for the development of Tregs and their immunosuppressive capabilities.⁶⁶ Two main subsets of Tregs have been identified, including natural Tregs, which are produced in the thymus, and adaptive Tregs, which are induced from naïve T cells in the periphery in response to chronic exposure to antigen. Induced Tregs are prominent in the tumor microenvironment, where they expand in response to VEGF, CXCL12, IL-10, and TGF- β and are capable of producing a number of immunosuppressive signals, including adenosine and prostaglandin E₂.^{67,68}

While the majority of studies investigating Treg expansion examined the primary tumor environment, similar mechanisms seem to occur in premetastatic tissue. Treg expansion is induced by TGF- β and IL-10, which are produced by MD-SCs recruited to premetastatic organs.⁶⁹ Indeed, just as has been observed in numerous adult cancers, higher levels of circulating Tregs are found in patients with metastatic Ewing sarcoma when compared with patients with localized disease.⁷⁰ Partial depletion of Tregs using anti-CD25 antibody treatment in a murine model of neuroblastoma increased tumor immunity and enhanced cell-based vaccination therapy, suggesting that Treg targeted therapies could be useful in the pediatric solid tumor setting.⁷¹

IV. POTENTIAL THERAPEUTIC TARGETS OF THE TUMOR MICROENVIRONMENT IN PEDIATRIC SOLID TUMORS

Many commonly used therapies in pediatric cancers capitalize on the observation that tumor cells proliferate at a higher rate than the surrounding tissue. These therapies have been effective in many pediatric cancers despite their lack of selectivity and associated toxicities. There remains, however, a great need for novel treatment approaches that target the cross-talk between tumor cells and their microenvironment. Metronomic chemotherapy, in which chemotherapeutics are given in a low, continuous dose, has demonstrated efficacy in targeting tumor angiogenesis and has also been shown to target specifically cancer stem cells.⁷² As data suggesting that both high-dose, intermittent chemotherapy and radiation can induce BMDC mobilization, rebound angiogenesis, and chemoresistance accumulate, metronomic chemotherapy has emerged as a potential alternative that may avoid these pitfalls.⁷³

In addition to metronomic chemotherapy, antiangiogenic therapies represent a recent major focus of clinical trials because of the widely accepted paradigm that a tumor cannot grow beyond the limits of its vascular supply without the induction of a new vascular supply.⁷⁴

VEGF is the best-characterized proangiogenic factor for which higher levels in many pediatric tumors have been correlated with more aggressive disease.³² The preclinical rationale in neuroblastoma and other pediatric solid tumors suggests that antiangiogenic therapy in combination with chemotherapy can limit tumor progression. In addition to a phase I trial of bevacizumab, which targets VEGFA, in pediatric patients with advanced solid tumors, several VEGFR-2 tyrosine kinase inhibitors, including aflibercept, sorafenib, sunitinib, pazopanib and cediranib, have been used in phase I clinical trials in pediatrics.^{75–77} The most common antitumor effect observed with these treatments has been stable disease, although objective responses have occasionally been observed. A phase II trial of bevacizumab in combination with chemotherapy including irinotecan has shown objective responses in low-grade astrocytoma. Combination therapy studies that combine bevacizumab with chemotherapy, including cyclophosphamide and vinorelbine for rhabdomyosarcoma and soft-tissue sarcomas and temozolimide for high-grade gliomas, are ongoing.⁷⁸ The current trials with antiangiogenic therapies in which stabilization of disease is the best response and regrowth after removal of drug often occurs, suggest that new antiangiogenic treatment strategies need to be developed to assess the true value of targeting this critical component of the microenvironment in cancer progression.

Immune therapies with cancer vaccines, genetically engineered T cells against a specific antigen, autologous activated T cells/dendritic cells, and checkpoint inhibitors, including PD-1, PDL-1, and CTLA4 blockade, are emerging as effective therapies in liquid tumors and melanoma.^{79–82} Forays in the treatment of solid tumors with these strategies also suggest great potential benefit; however, significant caveats remain in T-cell-mediated immune therapy because of the heterogeneity of tumor antigen expression and the coexpression of most candidate targets on normal cells. In addition, immune-suppressive cells such as M2 macrophages, MDSCs, and Tregs can derail the efficacy of these therapies in solid tumors, which tend to cultivate a heavily immunosuppressive environment. One potential strategy to mitigate the tumor suppressive microenvironment is to target trafficking of immune-suppressive macrophages to the tumor microenvironment by treating with chemotaxis inhibitors of colony-stimulating factor-1 receptor or CXCR2.^{83–85} Other strategies target the immunosuppressive molecules produced by MDSCs, such as COX-2, inducible nitric oxide synthase, and arginase.^{86–89} Another emerging approach to altering the immunosuppressive milieu of MDSCs is to promote their differentiation into mature non-immune-suppressive cells by treatment with all-trans retinoic acid and 5-azacytidine.^{90,91} Together, these drugs hold great promise in redirecting the immune system to promote an effective antitumor immune response by putting the brakes on T-cell-regulatory feedback loops and targeting immune suppressive cells.

Another promising approach involves specifically targeting morphogens that are known to control stem cells as well as the regeneration and repair processes. These include many of the key players in the Notch and Wnt pathways, which have demonstrated some potential, particularly in leukemias, specific brain tumors, and sarcomas, including Ewing sarcoma.⁹² Wnt monoclonal antibodies and soluble Wnt receptor antibodies are currently in development. Another approach targets β -catenin, part of the Wnt pathway, through nonsteroidal anti-inflammatory drugs and ICG-001 (cAMP-binding protein β -catenin), which may play a beneficial role in pediatric cancer treatment. Notch inhibitors, including

gamma secretase inhibitors and Notch soluble receptor decoys, possibly target not only the cancer-initiating cell but also the critical crosstalk between a tumor cell and its stem cell niche environment.^{93–96} Hedgehog inhibitors are being developed to target the hedgehog pathway that plays a key role in medulloblastoma development and the perivascular niche involved in tumor recurrence.⁹⁷ Small-molecule Smoothened inhibitors are in clinical trials for medulloblastoma as well as other solid tumors.⁹⁸ Although these agents are all in the early stages of development, there is biological rationale for their therapeutic benefit.

Another approach involves targeting potential metastatic environments to prevent the spread of disease. Many pediatric cancers metastasize and grow preferentially in bone and bone marrow microenvironments; thus, targeting this niche may benefit this patient population. Indeed, the use of bisphosphonates to target osteoblasts, a key cell type within this stem cell niche, has shown some promise to inhibit bone metastasis, and possibly pulmonary metastases as well, in pediatric sarcomas.^{99–101} Integrins that control communication among the ECM, cells of the microenvironment, and tumor cells have also been investigated as potential therapeutic targets. Blocking antibodies that target $\beta 1$ -, $\alpha v\beta 3$ -, and $\alpha v\beta 5$ -integrins have demonstrated some early response in clinical trials of adult solid tumors.^{102,103}

Efforts to target stromal cells in the tumor microenvironment have had mixed results. Although fibrosis is clearly demonstrated as a poor prognostic sign in many solid tumors, the elimination of fibroblasts, the presumed inciting cell inducing the fibrosis, has been associated with enhancement of metastasis and potential for alterations in immune surveillance.²⁵ Such findings only emphasize the need for further study. Defining more precisely the different mesenchymal cells contributing to tumor microenvironments and their functional role in tumor progression is required to better understand their relationship with tumor and infiltrating immune cells before better treatments can be devised.

Last, the advent of molecularly targeted therapies has emphasized the need to understand better the impact of these tumor-specific therapies on microenvironment cell populations. Such agents may have direct off-target effects on cells of the microenvironment. Moreover, as tumor cells are modified by targeted therapy, the tumor's interactions with its microenvironment also are altered, and the ramifications of this is not yet fully appreciated. Future studies will help to define logical combination strategies that take advantage of the potential off-target, on-cell effects.

Although we have provided a cursory overview of potential therapeutic targets, given the diversity of the cellular and molecular constituents of the tumor microenvironment it is clear that there exist many avenues to effectively develop therapies that can complement current effective antitumor treatments. Considering the tumor within its neighborhood can lead to new approaches to treating cancer.

V. CONCLUSION

The tumor microenvironment comprises a complex and dynamic interplay among tumor cells, stromal cells, ECM components, and immune cells. Many of the same cellular and extracellular players observed in tumor niches have significant, tightly regulated roles in

normal physiological stem cell niches, where cell fate decisions are critical to maintaining tissue homeostasis—a feature that is dysregulated in the tumor niche. Investigating interactions between the cells of this specialized niche may provide greater insights into tumor progression and potentially reveal novel therapeutic targets. Thus, understanding how tumors can co-opt niche biology can provide clarity to the aberrant tumor and metastatic niches.

Although daunting, such work may prove very fruitful as an adjunct to traditional tumor-targeting therapies and may be particularly successful in preventing metastasis, the most devastating complication of cancer. Targeting the interactions of a tumor with its microenvironment is only in its infancy and requires exploration of diverse cell types in different microenvironments in multiple tumor types and models in order to draw robust conclusions. If this work is performed strategically and incrementally, however, it can provide new approaches to cancer treatments. Despite the work that remains to investigate these concepts, there is some evidence that targeting both tumor and tumor niche players together may prove successful in pediatric cancers; thus, the tumor microenvironment as it relates to stem cell niche biology remains an intriguing area of study.

ABBREVIATIONS

αSMA	α -smooth muscle actin
BMDC	bone marrow–derived cell
CAF	cancer-associated fibroblast
EC	endothelial cell
ECM	extracellular matrix
EPC	endothelial progenitor cell
FAP	fibroblast activation protein
HIF	hypoxia-inducible factor
HPC	hematopoietic progenitor cell
IL	interleukin
MDSC	myeloid-derived suppressor cell
MMP	matrix metalloproteinase
MSC	mesenchymal stem cell
PDGF	platelet-derived growth factor
SMC	smooth muscle cell
TAM	tumor-associated macrophage

TGF	transforming growth factor
Treg	regulatory T cell
VEGF	vascular endothelial growth factor

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FIG. 1.

Commonalities of pathological and physiological niche environments. The primary tumor niche and the related but distinct premetastatic/metastatic niche comprise a number of key players that promote tumor progression. Many of these molecular and cellular components also are found in physiological niches such as the bone marrow, colonic crypt, and skin bulge, where they play tightly regulated roles in wound healing and tissue homeostasis. Soluble factors such as growth factors and interleukins that play a key role in these stem cell niches also are produced by the tumor and can influence cells of the primary tumor niche

and premetastatic/metastatic niche to promote tumor cell proliferation, migration, and spread. Understanding the similarities and differences between these distinct niches may better inform the ability to therapeutically target the tumor microenvironment.

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