Adapt or Dye: Tumor Microenvironment, A Powerful Regulator of Cancer Progression

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Abstract

Tumorigenesis is a complex multistep process in which a plethora of tumor and stromal cells play important roles. From this point of view, the biology of a tumor can be elucidated by focusing on the crosstalk between tumor cells and the tumor microenvironment, comprised by stromal cells of different origins. Several lines of evidence demonstrate essential contributions of tumor stromal cells, which influence the growth, survival, invasiveness, and metastatic ability of neoplastic epithelial cells within these tumors. This review describes the role of the microenvironment during tumor progression, and suggests possible new therapeutic avenues.

Introduction

Arguably one of the most well-known and widely studied aspects of cancer biology is the genetic mutations that underlie primary tumor formation. Tumor initiation is the result of mutations in oncogenes and tumor-suppressor genes. Often these genes directly affect the rate of cell growth or cell death. These mutations are inherited by daughter cells and the expansion of these populations give rise to tumors that have the uncontrolled rates of proliferation that are typical of cancer (1).

Although clearly carcinomas arise from mutations in the epithelial cells that comprise the cancer, there is also evidence that the tumor microenvironment (TME) can play an important role in both initiation and promotion of cancer.

The tumor microenvironment encompasses all of the components that surround and support tumor cells, including the extracellular matrix, and a milieu of distinct cell-types, including endothelial cells of the blood and lymphatic circulation, pericytes, fibroblasts, myofibroblasts, adipocytes, and a variety of bone marrow-derived cells. Tumor cells can alter the microenvironment, and reciprocally, the microenvironment can affect how tumor cells grow and spread (2, 3).

The relevance of the TME was proposed almost 200 years ago, when in 1863 Virchow hypothesized that cancer may originate at sites of chronic inflammation. He based his hypothesis on the fact that some classes of irritants, which he called "promoters", enhance epithelial cell proliferation in response to tissue injury (reviewed by (4)). When tissues are wounded or

exposed to a chemical irritant, cell proliferation is enhanced to facilitate tissue regeneration or wound healing, thus maintaining homeostasis.

During the following years, several lines of research provided evidence for the existence and importance of the TME (5, 6). The first proof that the TME was critical came in 1961 when Scott and Reinertson showed that the behavior of human epithelial cells was determined by the stromal environment through the use of human autotransplants. In this study, they showed that basal cell epithelioma tissues transplanted with their adjacent stroma into clinically normal skin of the same patient survived after 5 weeks, with no histological alterations observed. However a transplant of mostly pure basal cell tumor tissue, free of stromal cells, transplanted into normal skin from the same patient, did not survive (7). A little over ten years later, Mintz et al. discovered that the environment that a cell inhabits has a direct effect on cellular identity and function. In this study, researchers transplanted teratocarcinoma cells into otherwise normal, healthy mouse embryos to show that the malignant properties of the tumor cells were repressed. This suggested that not only does the environment of a cancer cell assist in dictating its survival and aggressiveness, but also that cancer is programmable and capable of being manipulated (8).

In the 1970s, another important discovery in the field elucidated the importance of the TME during the tumor progression. Folkman and colleagues hypothesized that all tumors need a blood supply and discovered that some cancers can stimulate new blood vessel development via a process called "angiogenesis". He surmised that if a tumor could not form its own vasculature, it would wither and die (3).

Today the TME is thought to participate in many of the hallmarks of cancer delineated by Hanahan and Weinberg (9-11). It is widely accepted that as a tumor initiates and progresses, the surrounding microenvironment also evolves and becomes "activated" through continuous paracrine communication. In turn, this co-evolution of a tumor and its microenvironment creates dynamic signaling circuitry that promotes cancer growth, and ultimately leads to a fatal disease (2, 12-14).

Tumor Microenvironment at the site of the Primary tumor

Despite the accepted fact that carcinoma biology depends upon genetic and epigenetic changes in the epithelial cells, studies by Van Scott and Mintz et al. revealed that transformed cells (i.e., neoplastic cells with tumorigenic potential) are unable to form tumors if they are not in a permissive niche or tumor microenvironment (7, 8).

As mentioned above, one of the components of the TME are immune cells. These inflammatory cells can be subclassified into myeloid and lymphoid cells. Lymphoid lineage includes Natural killer, T and B-cells, whereas the myeloid lineage includes macrophages, TIE2-expressing monocytes, hemangiocytes, neutrophils, eosinophils, and mast cells (15). Traditionally, the presence of leukocytes in tumors was thought to be a consequence of a failed attempt at cancer cell destruction. However, tumors are not only effective in escaping immune-mediated rejection; they can also modify certain inflammatory cell types, rendering them pro-tumorigenic rather than tumor suppressive (16-20). Indeed, several studies have found correlations between particular immune cell infiltrates in primary tumors and patient prognosis (13).

Other relevant components of the TME are fibroblasts and myofibroblasts, which constitute the most abundant mesenchymal cells found within most carcinomas. They promote tumor progression in experimental models and are present in the histopathologic entity termed the desmoplastic response (14). Desmoplastic stroma is almost always observed in malignant human carcinomas and is used by pathologists as

a diagnostic parameter due to its association with invasiveness and poor prognosis (21, 22).

In addition to the contribution of stromal cell types, the extracellular matrix formed by mesenchymal cells is thought to regulate carcinoma cell growth and motility (23, 24).

Another constituent of the TME are endothelial cells. After the release of pro-angiogenic signals by tumor cells, endothelial cells arrive to the tumor mass and form neovasculature which is important in the development and growth of many solid tumors, and necessary for haematogenous dissemination of tumor cells (13, 25, 26).

TME components play a critical role into the communication with the tumor cells through the secretion of a large, ever-increasing number of cytokines, chemokines, and growth factors, such as VEGF, EGF, IGF-1, SDF1, IL6, IL8, TGFβ, OPN, or FGF, that have been found to promote tumor progression, affecting tumor cell proliferation, invasion and angiogenesis (2, 27-29).

TME during metastatic spread

In later stage tumors, several tumor cells at the primary tumor niche acquire an invasive phenotype and intravasate into the circulation.

A variety of terms are used to describe metastatic cells in blood and bone marrow. Tumor cells in the peripheral blood are termed circulating tumor cells (CTCs) (30). CTCs have been reported in 70-100% of patients with metastatic spread (54). These cells are highly relevant to the study of early metastatic spread and provide a diagnostic tool for patients with overt metastases (30). CTCs in the bloodstream must survive a variety of stresses such as sheer force of haematogenous flow and turbulences of the blood circulation, surveillance by immune cells, mainly natural killer cells (NK), in order to reach distant organ sites, which generally happens within a few minutes (31, 32). To avoid the physical forces and elude natural killer (NK) cell-mediated lysis, CTCs express receptors to activate the coagulation cascade and the formation of platelet-rich tumor cell-associated

microthrombi. The CTC-platelet interaction and aggregate formation is integral for adhesion to the endothelium. Platelets also release TGF-β into the circulation, allowing the inhibition of NK activity. Although platelets can create a "microenvironment" that protects CTCs in the circulation, other cell types such as innate immune cells (leukocytes) or fibroblasts could have similar effects, but have not been extensively investigated yet (31). CTCs in the bloodstream metastasize to organs in part by circulation patterns and blood vessel diameters, but also by active adhesion to the vasculature via specific proteins provided by CTCs themselves or by associated platelets, leukocytes or thrombi. Moreover, the presence of an activated endothelium (i.e., expressing P- and E-selectin, ICAM, VCAM) is required for efficient metastasis, since these factors favor the sequestration and adhesion of tumor cells. This activation involves the participation of soluble factors secreted by primary tumors which induce the formation of hyper-permeable foci at the endothelium. Furthermore, platelet secreted cytokines and growth factors (i.e., PDGF, TGF-β, CCL5, VEGF) interact with leukocytes which subsequently influence the activation of the endothelium (31, 33-35).

Role of Tumor microenvironment in metastasis

Once CTCs have lodged in the vasculature at distant organs, they initiate intra-luminal growth, forming a "micrometastasis" that can break the walls of surrounding vessels and cross the endothelial and pericyte layers, in a process called extravasation (36) (Figure 1). Tumor cells in visceral organs or bone marrow are most often referred to as disseminated tumor cells (DTCs) (30).

Although 90% of mortality is caused by the spread of tumor cells from the primary tumor, the metastatic cascade is a highly inefficient process, and less than 0.01% of the disseminated cells will give rise to successful metastasis (12, 37-38)**.** Explaining this low efficiency, Paget described in 1889 the "Seed and Soil" hypothesis

of metastasis outgrowth; even though tumor cells are broadly disseminated during tumor progression, clinically detectable metastases only develop at those sites where the tumor cells are suitably adapted for survival and proliferation (39).

Thus, it is important to consider not only the intrinsic characteristics of tumor cells, but also the microenvironment in which they operate. Tumor cells that have extravasated into different organs need to establish a permissive niche that allows them to proliferate and give rise

metastasis, in a process called "colonization" (Figure 1).

Psaila and colleagues have used mouse models to show that primary tumors can prime the soil (the lung) and adapt it before the foreign seed arrives (breast cancer tumor cells). In their model, priming the lung and creating a permissive microenvironment was necessary for metastatic tumor formation (40). The lung microenvironment evolves into a metastatic niche that facilitates the formation of micrometastasis, and a subsequent transition into macrometastasis.
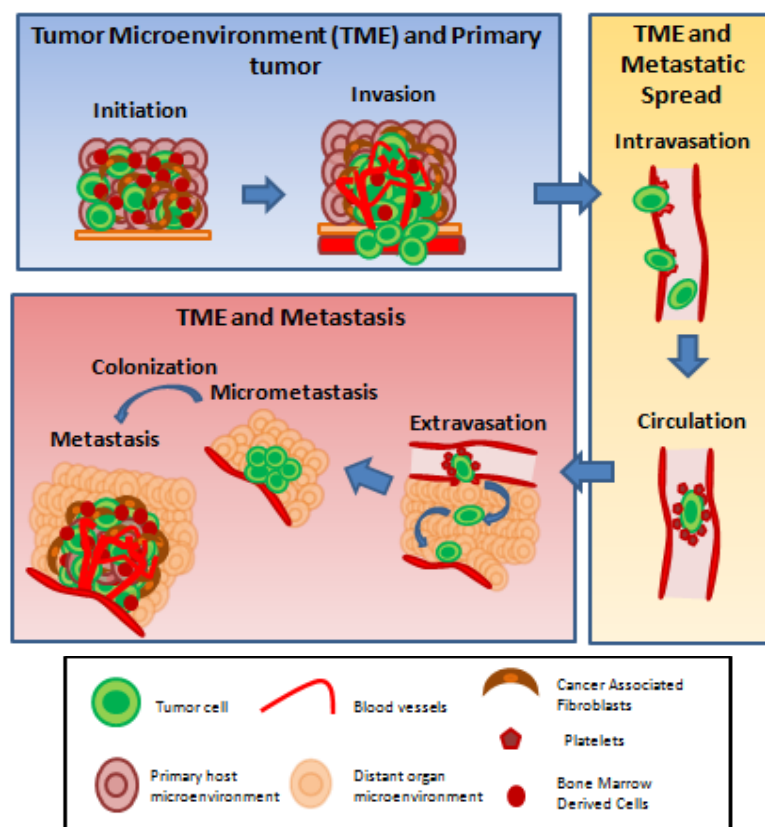

Figure 1. Effect of TME during the multistep process of tumorigenesis: from primary site to distant tissues. Tumors initiate as a result of successive genetic and epigenetic changes that confer growth advantage. During tumor progression, the tumor microenvironment, composed by a milieu of distinct cell-types, such as endothelial cells, fibroblasts, and bone marrow derived cells, are key players in this process (1). Tumor cells start invading surrounding extracellular matrix as a consequence of activation of aberrant genetic and epigenetic programs as well as a paracrine signaling between recruited and resident stromal constituents (2). Cancer cells intravasate into blood vessels and survive many stresses, aided mainly by platelet action (3). CTCs arrest and extravasate at distant organs (4). Disseminated Tumor Cells (DTCs) survive in a foreign microenvironment waiting for a permissive niche which allows their outgrowth (5). Metastatic microenvironment is adapted and DTCs reinitiate their proliferative programs (6). *Figure adapted from Zetter B.R., Annu. Rev. Med, 1998.*

In the clinic, breast cancer patients show variable patterns of recurrence that span years and even decades, after the primary tumor has been surgically removed (41). This phenomenon demonstrates that the vast majority of DTCs that survive their initial encounter with the foreign and unfavorable tissue microenvironment persist in an apparent long-term dormancy or suffer slow attrition (32). The ability of DTCs to escape dormancy and start to proliferate may depend on non cell-autonomous mechanisms that are needed to transform the hostile microenvironment. McAllister and colleagues have shown that some indolent tumor cells can be stimulated to form overt metastasis by systemic factors, as tumor-secreted cytokines and bone marrow derived cells (BMDCs), influenced and mobilized by overt tumors at distant sites. In other words, the body's response to one tumor can aid the progression of other disseminated tumors that would otherwise remain dormant. The process by which one tumor (being a primary tumor, residual disease or metastatic colony) stimulates the distant outgrowth of micrometastasis is termed "systemic instigation" (17, 42, 43). In some cases, these activated BMDCs travel to the sites where disseminated tumors reside and induce a permissive tumor microenvironment through the activation of the resident fibroblasts, and in others, they aid formation of vasculature, which facilitates tumor outgrowth (17, 42, 44). The formation of macroscopic growing metastasis, or "colonization" as described earlier, represents the endpoint of the invasion-metastasis cascade.

These are some examples of how TME can regulate tumor progression. Although the complexity of the tumorigenesis has been acknowledged for many years, we are only beginning to understand the cross-talk between tumor cells and the microenvironments in which they reside.

Tumor Microenvironment: Therapeutic implications

Due to the heterogeneous mutations and chromosomal instability that characterize different tumors during cancer progression, the complexities of cancer subtypes are still being elucidated. For example, in breast cancer there are different gene expression-patterns that effectively distinguish tumor subclasses associated with clinical outcome (45). It is of vital importance to understand these differences in order to design personalized therapies to target the specific pathways altered in a given patient. However, it is still unclear as to whether designing personalized medicine is even feasible due to the high variability in patients that present in the clinic.

Another possible approach is to target distinct TMEs. The advantages to targeting tumor stroma include the fact that these cells are not as genetically unstable as cancer cells, and could be therefore less likely to develop drug resistance (46, 47). "Normalization" of the stromal environment, in theory, could repress tumor progression as has been suggested by Mintz and colleges (8). Another approach is to inhibit inflammatory responses in the TME. Inhibition of inflammatory cells and cytokines by treatment with non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to correlate with lower risk of colon and breast cancer and may prolong dormancy of disseminated tumor cells (42, 48).

In the last years it has been a huge emphasis into the discovery of growth factors secreted by the TME responsible to increase the tumorigenicity and metastasis ability of the tumor cells (49, 50, 51). Discoveries of these growth factors open a new window for therapies preventing paracrine interactions between tumor cells and their microenvironment. However, there have also been some disappointments when targeting the stroma for cancer therapy. Clinical trials of VEGF inhibitors, for example bevacizumab, and MMP inhibitors have shown little efficacy in patients suffering from advanced stages of cancer.

In fact these treatments have severe side effects and sometimes result in drug-resistant recurrence (51, 52).

To overcome these problems, the development of more specific inhibitors is now underway (15, 53). However, when developing microenvironment-based therapies, we need to keep in mind that targeting just one aspect of the TME, and doing these in patients with late-stage cancer is not likely to be successful. Therefore, the aim should be to combine drugs that target multiple regulators of the TME in addition to cytotoxic therapies that directly target tumor cells.

Acknowledgments

We acknowledge the great strides made by a large number of investigators in these areas of research and regret that we could not include all of them and their work in this review. We thank Drs. Sandra S. McAllister, Yuanbo Qin, Tim Marsh and Jessica Hawkins for helpful insights and critiques of this manuscript. Authors report no conflict of interest.

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