Plasticity beyond Cancer Cells and the "Immunosuppressive Switch"

Zvi Granot1 and Zvi G. Fridlender2

Abstract

Tumor initiation, growth, and metastatic progression are complex processes that, in order to be successful, require extraordinary cellular plasticity. Accordingly, tumor cell plasticity and how it affects disease progression have been studied extensively. However, as our understanding of the tumor microenvironment deepens, we are confronted with the notion that functional plasticity in the context of cancer is not limited to tumor cells alone but is also commonly seen in normal stromal cells of the microenvironment, and specifically in immune cells. Here, we review the functional plasticity these cells exhibit in the context of cancer, highlighting the role of circulating and tumor-associated neutrophils. We further discuss how this plasticity supports or limits tumor progression, inducing an "immunosuppressive switch" to promote further tumor growth and development. Cancer Res; 75(21): 4441–5. ©2015 AACR.

Introduction

In the past several decades, much attention has been directed toward understanding how tumors grow and progress. Cancer research has focused on an in-depth characterization of tumor cells toward identifying weaknesses that could be exploited therapeutically. Most of the research was aimed at understanding tumor cell autonomous features that make a tumor—proliferation, avoiding cell death, acquiring migratory properties, etc. However, in recent years, it has become apparent that the tumor microenvironment, as well as other cells and factors accumulating in the host by virtue of tumor presence, plays a critical role in tumor growth and metastatic progression. The tumor-associated nonmalignant stroma consists of a wide variety of cells, including immune cells, whose function is modified by the interaction with the growing tumor to generate a favorable microenvironment and tumor-supportive general setting in the host. Intriguingly, on many occasions, these nonmalignant cells play a role that is strikingly different than they do under noncancerous conditions and actively act against the host to promote tumor growth and metastatic progression.

Concerning possible plasticity and polarization in cancer, most studies to date are focused on plasticity and epithelial to mesenchymal transition (EMT) of cancer cells with little emphasis on plasticity of stromal or tumor-infiltrating cells. One example of stromal plasticity that was described almost two decades ago and is widely recognized is that of the endothelial cells. Shortly after tumor initiation these cells change their phenotype as part of the "angiogenic switch" that is crucial for further tumor development (1). The angiogenic switch is a discrete step in tumor development occurring at different stages of tumor progression, depending on the nature of the tumor itself and its microenvironment. This initiation of angiogenesis has to occur to ensure further growth of the tumor (1). Following our recent work further elucidating and demonstrating functional plasticity in neutrophils (2), we will discuss these cells whose function is altered by the presence of a tumor and highlight the contribution of the alternatively activated cells. We will expand on this plasticity in immune cells with special emphasis on tumor-associated neutrophils (TAN), deliberately shorting on it in nonimmune cells, and suggest a new parallel motive in cancer development, the "immunosuppressive switch."

Plasticity in Immune Cells—"the Immunosuppressive Switch"

The immune system, in principle, acts to protect the host against a wide variety of threats, including not only exogenous threats such as microbial infections but also against the propagation of naturally occurring aberrant host cells. However, in the context of cancer, immune cells undergo a dramatic phenotypic change and are regarded as "alternatively activated." Instead of protecting the host, the "alternatively activated" immune cells act to promote tumor growth and progression. Here, we will highlight the roles played by immune cells that exhibit functional plasticity, which allows the switch toward a tumor-supporting phenotype. We believe that in fashion similar to the angiogenic switch, these changes get to a critical turning point, conferring an immunosuppressive environment.

Neutrophils

Neutrophils provide the first line of defense against microbial infections and take a pivotal role in inflammation. Their role in cancer, however, has long been a matter of controversy. As early as the 1970s, neutrophils were shown to be capable of efficiently killing tumor cells. Since then, a plethora of studies have
demonstrated various antitumor properties. Specifically, stimulating neutrophils with PMA (phorbol 12-myristate 13-acetate) or fMLP (N-Formylmethionyl-leucyl-phenylalanine) when cocultured with tumor cells was sufficient to induce significant cytotoxicity. However, the effect of neutrophil reaches beyond direct cytotoxicity, and neutrophils were also found to play a key role in antibody-dependent cell-mediated cytotoxicity (3). On the other hand, with the evolution of our understanding of the tumor microenvironment, neutrophils were shown to possess a significantly different set of traits. Neutrophils were shown to directly promote tumor growth by providing a variety of chemokines and cytokines. Neutrophil contribution to the microenvironment also involves active recruitment of other tumor-supporting cells to the tumor bed. TAN were shown to promote tumor angiogenesis and play a critical role in mediating the angiogenic switch. Neutrophils were also shown to promote tumor cell motility, migration, and invasion as they secrete enzymes that degrade and modify the extracellular matrix. Along these lines neutrophils are also thought to take part in priming of the premetastatic niche, possibly via modification of the extracellular matrix at the distant site making it easier for tumor cells to extravasate. Moreover, states of chronic inflammation, where proinflammatory neutrophils are abundant, were shown to be directly linked to tumor initiation. Finally, we and others (2, 4) have shown that neutrophils also take part in immune suppression, thereby supporting tumor growth and metastatic progression. This, together with the fact that IFNγ-stimulated neutrophils may upregulate PD-L1 and acquire the capacity to suppress T-cell proliferation (5), suggests that TAN may serve as a target for antitumor immunotherapies and may actually be affected by strategies targeting the PD1/PD-L1 pathway.

Although the reports regarding neutrophil function in cancer seem conflicting, they can be well mitigated by looking at the functional plasticity neutrophils demonstrate. A groundbreaking study by Fridlender and colleagues (6) had demonstrated that TGFβ, available in high concentrations at the primary tumor site, can mediate the functional switch from proinflammatory, antitumor neutrophils (termed “N1”) to anti-inflammatory tumor-promoting neutrophils (termed “N2”). This finding has led to the hypothesis that neutrophil function in cancer is dictated in a “niche-dependent” fashion. This is well exemplified comparing the primary tumor, where TGFβ is abundant, with the premetastatic niche where TGFβ levels are low. Although neutrophils act to promote tumor growth at the primary site, they can concomitantly act to limit metastatic seeding via direct cytotoxicity (7, 8).

Niche-dependent neutrophil function may explain certain aspects of neutrophil function in cancer; however, there are several lines of evidence to suggest that the conflicting reports regarding neutrophil function in cancer may actually stem from the existence of multiple neutrophil subsets. It seems that the prevailing perception of neutrophils as a homogeneous population of cells needs to be revised. In a recent study (2), we demonstrated that there are at least three distinct neutrophil populations during cancer progression at the same niche (i.e., the circulation) in mice and human patients. These may be
roughly divided into mature high-density neutrophils (mHDN), mature low-density neutrophils (mLDN), and immature low-density neutrophils (iLDN). While mHDN present N1-like phenotype and have the capacity to kill tumor cells, mLDN are not cytotoxic, show impaired functionality but acquire suppressive properties usually associated with myeloid-derived suppressor cells (MDSC). Interestingly, unlike high density neutrophils, LDN are not a homogeneous population of cells but also consist of significant numbers of true MDSC, which are defined in mice as CD11b⁺Gr-1⁺ immature cells from either the granulocytic or monocytic lineage. These cells are propagated under various pathologic conditions and have the capacity to limit the expansion and activation of cytotoxic CD8⁺ T cells. In cancer, granulocytic-MDSC (G-MDSC) were shown to suppress anti-tumor immune responses and promote tumor growth and metastatic progression. At this point, we do not have clear information of the source of different TAN in the tumor microenvironment. A major question is whether neutrophil plasticity occurs only outside of the tumor, or can it happen inside the tumor microenvironment. It is possible, for example, that N2 TAN originate solely from LDN (or one of its subsets) and N1 TAN originate from HDN. Alternatively, it is possible that neutrophils enter the tumor where their functional plasticity is manifested in response to cues available in the tumor microenvironment (e.g., by TGFβ).

Collectively, the observations obtained thus far support the existence of multiple neutrophil subsets as well as the role played by the microenvironment in determining neutrophil functionality. The plasticization suggests that the use of neutrophil counts or the neutrophil to lymphocyte ratio as a prognostic factor may be significantly lacking. On the other hand, in-depth understanding of neutrophil plasticity and the factors controlling it may be used to shape a favorable neutrophil landscape and utilized therapeutically.

**T cells**

Adaptive immunity plays a key role in immune surveillance and protection of the host against cancerous cells. Cytotoxic T cells (CTL) have the capacity to identify specific epitopes expressed by tumor cells and to induce tumor cell apoptosis. Indeed, the presence of activated CD8⁺ cells in early tumors is a very good prognostic factor (9). However, although under certain conditions CTL show significant antitumor activity (Th1 response), tumor-infiltrating lymphocytes (TIL) most often lack any detectable antitumor activity (Th2 response). Currently, this lack of antitumor activity is thought to be the consequence of two distinct mechanisms that inactivate the CTL and suppress lymphocyte antitumor activity.

**Cell-mediated suppression**

Cell-mediated immune suppression is mediated by several distinct cell types. The first is another component of the adaptive immune system—Foxp3⁺ regulatory T cells (Treg). Treg are involved in immune tolerance, and their absence leads to a variety of autoimmune responses (10). Treg are abundant in many tumors and take an active part in generating an immune suppressive microenvironment. They have the capacity to limit the propagation and anti-tumor activity of TIL, thereby playing a key role in immune evasion. The other cell types involved in suppression of cytotoxic T cells are the MDSC. Until recently, these cells were characterized as immature cells of the monocytic (M-MDSC) or granulocytic (G-MDSC) lineages; however, recently mature neutrophils were also shown to acquire suppressive properties. MDSC were detected in multiple pathologies and are thought to play an anti-inflammatory role. In the context of cancer, MDSC limit the propagation and cytotoxic activity of cytotoxic T cells.

**The chemokine milieu or the microenvironment**

Like most immune cells, T cells respond to cues in their microenvironment. For example, although IFNγ plays a stimulatory role and promotes an antitumor T-cell immune response, other factors, such as IL10 and TGFβ, suppress these responses and promote tumor growth (11).

**Dendritic cells**

Dendritic cells (DC) are the most potent of all antigen-presenting cells and play a critical role in the activation and education of T cells. Many tumors are immunogenic and hence the presentation of tumor antigens by DC is a key component of antitumor immunity and immune surveillance. Accordingly, the presence of tumor-infiltrating DC is associated with prolonged patient survival in several different human malignancies (12). Importantly, naïve T cells were shown to infiltrate tumors and acquire effector functions within the tumor, suggesting an important role for tumor resident DC (13). However, correlating well with the reduced antitumor T-cell activation, several lines of evidence suggest that with tumor progression, the numbers of tumor-infiltrating DC are significantly reduced. In fact, tumor-secreted factors, such as gangliosides, neuropeptides, and nitric oxide, were shown to promote DC apoptosis, providing a plausible explanation for the declining numbers of tumors infiltrating DC. Furthermore, tumor-infiltrating DC show a dramatic reduction in their functionality, do not present tumor-derived antigens, and fail to induce the proliferation of tumor-specific CD4⁺ and CD8⁺ T cells. This is in part due to the preferential recruitment of immature DC to the tumor microenvironment; however, significant numbers of dysfunctional mature tumor-infiltrating DC may also be detected. Finally, regulatory DC, secreting immunosuppressive molecules such as TGFβ may be detected in the tumor microenvironment, suggesting a functional switch toward a tumor-promoting phenotype (14).

**Macrophages**

A major type of immune cells affecting the tumor microenvironment are tumor-associated macrophages (TAM; refs. 15, 16). TAM, which are the major component of the infiltrate in most tumors, the idea of plasticity and tumor-induced polarization, have been extensively studied (17). In early tumors, TAM have an inflammatory, tumoricidal (M1, “classically activated”) phenotype. These TAM are cytotoxic, phagocytic, present antigens well, and act to limit tumor growth (15, 17). They may also indirectly promote cytotoxicity by producing Th1-type cytokines and by activating other cells of the immune system, such as natural killer cells and T cells (18). In general, M1 TAM express high levels of IL12 and IL23 and are efficient producers of effector molecules, such as reactive oxygen and nitrogen intermediates and inflammatory cytokines,
participating as inducers and effectors cells in Th1 responses (17). However, as the tumor progresses, macrophages polarize toward an "alternatively activated" or M2 phenotype, differing from M1 TAM in receptor expression, antigen-presenting ability, function (i.e., arginine metabolism), and cytokine production. These M2 TAM exhibit a protumor, angiogenic, and immuno-inhibitory phenotype (18), making these cells an important factor in tumor immunology (15). M2 TAM are characterized by high IL10 expression and have variable capacity to produce inflammatory cytokines, with high levels of scavenger mannose and galactose-type receptors (17).

M1 macrophages have long been known to be induced by IFNγ alone or combined with lipopolysaccharide (LPS) or cytokines (e.g., GM-CSF). The alternative M2 phenotype was initially found to be induced by IL4 and IL13 (19). Many other cytokines have been later shown to govern M2 polarization, such as IL33 and the Th2-associated cytokine IL21 (17). TAM in mouse and human tumors generally display an M2 phenotype and are sometimes referred to as M2-like TAM (17). This polarization to the M2 phenotype of macrophages that have been recruited into tumors is induced by signals derived from tumor-infiltrating T cells (e.g., M-CSF) or from the tumor cells themselves (e.g., IL10 and TGFβ; ref. 20). In general, TAM become polarized based on distinct signals deriving from the particular microenvironment in which they reside (21, 22), resulting in production of a wide spectrum of TAM phenotypes with tumor-regulating properties (23).

Several decades ago, even before the concept of immunosuppressive TAM was formally popularized, investigators used LPS and LPS analogues to try to activate TAM (24). Although effective, LPS was quite toxic with significant adverse systemic effects. A variety of anti-TAM therapies have been further proposed and tested in preclinical models, including TAM depletion, differentiation, reprogramming, and activation (25). In a recent study, we found that polarizing tumor macrophages from an M2 to an M1 phenotype could be an important adjunct to vaccine immunotherapy, enabling them to reduce tumor growth in preclinical studies (26).

Examples of Plasticity in Stromal Nonimmune Cells

Fibroblasts

Tumor- or cancer-associated fibroblasts (CAF) are one of the most abundant stromal cell types in different carcinomas, such as breast and prostate (27). Fibroblasts have been generally considered as structural cells contributing to the extracellular matrix in connective tissues, and responsible for tissue repair (28). In past several years, it became clear that these cells have major effects on their surroundings, and specifically can support the adjacent epithelial cell layers and affect immune cells (29). Tumors are often perceived as wounds that never heal and as such maintain the CAF in a state of constant activation (30).

There are no clear selective markers for fibroblasts (29). However, it is now clear that several subtypes of fibroblasts do exist and that CAF comprise a heterogeneous cell population in the tumor microenvironment (27, 31). Classicaly, CAF are recognized as having protumorigenic effects stimulating tumor growth and progression (27, 28, 32). This includes direct stimulation of cancer cells promoting their proliferation, migration, invasion, and induction of EMT (27, 33). Furthermore, CAF secrete some proinflammatory factors, recruiting immunosuppressive cells (34). On the other hand, recent studies have shown that tumor-residing fibroblasts can have a similar degree of plasticity as other cell types in the stroma. Although the potential tumor-inhibiting effect of CAF is by far less studied, there are data showing that CAF can, depending on context (e.g., type of tumor, as well as effects of other stromal and immune cells) and source of their origin, acquire tumor-inhibitory properties (27, 35).

Endothelial cells and pericytes

Substantial heterogeneity can be found in the tumor vasculature, and there are several phenotypes of the tumor angiogenic process, reflecting different phenotypes of the cells involved in that process, i.e., the endothelial cells (36) and the pericytes (37, 38). The angiogenic switch refers to the initiation of angiogenesis that has to occur to ensure further growth of the tumor, and is dependent on changes in the phenotype of the endothelium and the perivascular cells, allowing new vessel formation and maturation (1). The pericytes are cells that surround the endothelium, sharing a common basement membrane with them (37). These cells appear to be multipotent, with the ability to differentiate into vascular smooth muscle (and possibly endothelial cells; ref. 38). Importantly, the presence of the angiogenic switch is determined by the overall microenvironmental balance between pro- and antiangiogenic factors (1, 36, 38).

Concluding Remarks

Malignant progression is associated with the acquisition of new traits by tumor cells. However, in the past decade, the contribution of the nonmalignant stroma that surrounds tumor cells was brought into focus. The normal cells that make the nonmalignant tumor stroma generate a unique, immune-suppressed tumor-promoting microenvironment where the nonmalignant cells’ function is altered (Fig. 1). One of the major questions regarding the alternative state of activation is whether it represents the true functional plasticity these cells possess or does it represent the existence of distinct cell populations. We believe these possibilities are not mutually exclusive and while altered TIL function may be due to the effect of Treg, neutrophils may acquire an N2-like phenotype in response to TGFβ. The preferential recruitment of unique T cells or neutrophil subtypes to the tumor bed may play an equally important role. It is now clear that future anticancer therapies will have to tackle not only the tumor cells but also the nonmalignant cells that support tumor growth and mediate immune evasion. Furthermore, we believe that the plasticity of stromal cells, and especially that of the immune cells, brings the microenvironment to a critical turning point, conferring an immunosuppressive environment. We would therefore like to suggest that during tumor progression there is, in addition to the angiogenic switch, another important change, that is “the immunosuppressive switch” (Fig. 1).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received June 1, 2015; revised June 28, 2015; accepted June 29, 2015; published OnlineFirst October 16, 2015.
References


Plasticity beyond Cancer Cells and the "Immunosuppressive Switch"

Zvi Granot and Zvi G. Fridlender


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-15-1502

Cited articles
This article cites 37 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/21/4441.full#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/75/21/4441.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/75/21/4441.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.