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The Microenvironment in Cancer

Nicole N. Parker and Dietmar W. Siemann
Department of Radiation Oncology and University of Florida Shands Cancer Center, University of Florida, Gainesville, Florida, USA

1.1 Introduction

In the development of a cancer, the transformation of epithelial cells into a neoplastic and progressively invasive tumor occurs though the acquisition of several procancer characteristics that can take years or decades to develop. The particular stages of transformation have been established and a general consensus exists about the properties of a successful malignancy. While many therapeutics have been developed to combat these properties, these therapies are not universally successful, and their efficacy depends on the type and site of the primary tumor, its degree of vascularization, the proliferative compartment of the tumor, and in particular, the tumor microenvironment. The latter is the key support system of a cancer, and is an important source of critical protumorigenic factors that facilitate growth, invasion, angiogenesis, and metastatic ability. The focus of this book is to examine how the reliance of tumors on their microenvironments for development and preservation of key cellular functions is now recognized not only as a major contributor to cancer aggression and treatment resistance but also as a potential target for novel therapeutic intervention strategies.

1.2 A highly selective process is required to obtain the cancer phenotype

Many studies have focused on the predetermining factors that cause hyperplasia, or the hyperproliferation of cells within their normal environment. On the path to a cancer, regions of hyperplasticity must subsequently become dysplastic, or display a highly disordered pattern of proliferation with little or no growth regulation. Predisposing genomic lesions in various genes of these dysplastic cells

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confer a proliferative advantage over normal cellular counterparts. Therefore, the out-proliferation exhibited by dysplastic populations requires additional genetic instability or genomic modifications, and these cells are considered neoplastic: they have an advantageous rate of proliferation with a lack of regulation, and possess other procancerous features at the time of transformation. Oncogenic lesions, coupled with inhibition of tumor suppressors, together contribute to cellular transformation. Genomic, proteomic, post-translational, and epigenetic mutations are responsible for activating oncogenes and inhibiting tumor suppressor genes.

1.3 The cancer phenotype

Although a multitude of potential cancer etiologies may occur for cancer development, several essential characteristics are present in malignancies (Hanahan and Weinberg, 2000). A key element of malignant transformation is the loss of regulatory control mechanisms present in normal somatic non-stem cells that are growth arrested and do not divide. Cancer cells not only possess heightened rates of cell proliferation and aberrant cell cycle checkpoints, but also lose contact-inhibited growth regulation. As a result, unlike detached normal cells which die by anoikis or cell detachment-induced apoptosis, cancer cells continue to grow unabated, breaking through basement membranes and invading extracellular spaces around tissues and organs.

Extracellular matrix remodeling and cellular changes in adhesion molecules are both required for a cancer cell to become more motile. Rearrangement of the actin cytoskeleton facilitates cell motility and plasticity, as does downregulation of adhesion proteins that bind tightly to the extracellular matrix (Chapter 3). A cancer cell modifies its adhesive properties and implements a program of non-adhesion through multiple modifications.

The progressive growth of a tumor ultimately results in an inability of normal tissue blood vessels to oxygenate and provide nutrients to tumor cells most distal to the blood supply. As a consequence oxygen-deficient (hypoxic) regions develop within the tumor (Chapters 2 and 9). The ability of transformed cells to survive hypoxic conditions requires a switch from aerobic to anaerobic glycolysis, a major approach by which cancer cells circumvent the cytotoxic effects of oxygen deprivation (Gatenby and Gawlinski, 2003). As a result of glycolysis, lactic acid byproducts accumulate in cells undergoing this process. Although this acidification is generally toxic to cells, cancer cells upregulate acid transporter proteins and efficiently secrete acid products into the surrounding environment. A side effect of acid secretion is an increase in local extracellular acidity, fluid retention, and subsequently, an increase in interstitial pressure (Chapter 9). Still, the ability of cancer cells to metabolically adapt by preferentially undergoing glycolysis even in the presence of oxygen not only provides a survival advantage over non-transformed cells but also ensures the persistence of only the most successful cancer cells (Gatenby and Gillies, 2004).

The outgrowth of a tumor that is beyond the diffusion limits of nearby blood vessels, which supply nutrients and oxygen, leads to another critical phenotypic advantage of cancer cells: the ability to induce angiogenesis, or the process

of developing new blood vessels from existing vascular structures. Cancer cells accomplish this through the upregulation and release of proangiogenic factors that can destabilize endothelial cells and induce vascular outgrowth from normal blood vessels. Endothelial cells proliferate toward the source of the chemoattractant angiogenic factors to form a new capillary network for the tumor mass. However, unlike normal vasculature, which is extremely ordered, this newly developed tumor neovasculature is highly aberrant in structure, lacking organization and vessel integrity. Although many proangiogenic factors have now been identified, vascular endothelial growth factor (VEGF) is believed to be the major inducer of tumor angiogenesis. It has not only been implicated in many cancer types (Fukumura *et al*., 1998) but importantly, VEGF expression has been shown to correlate with tumor angiogenesis and aggression, poor patient outcome, and is a predictor for metastasis and high tumor grade in multiple cancer types (Brychtova *et al*., 2008). Interestingly, some studies have demonstrated that acidic microenvironments can induce vesicle lysing, thereby secreting VEGF into the tumor microenvironment and contributing to a feed-forward mechanism in which tumor-induced hypoxia and cellular acidification lead to the formation of neovasculature.

It is generally believed that tumors cannot grow to a size larger than a few cubic millimeters without inducing a neovasculature. Once cancer cells induce revascularization, thereby ensuring a more constant nutrient supply, this growth restriction is effectively removed. The requirement for additional tumor space necessitates the ability of cancer cells to invade into surrounding tissue. It is most advantageous for cancer cells to digest adjacent extracellular matrix and force the local reorganization of normal epithelia and surrounding stromal elements.

1.4 The extracellular matrix

The extracellular matrix is comprised of various cell types and secreted proteins that help maintain the organization of higher-order cellular structures. In addition to containing various cell types, the matrix is deposited as a mix of such proteins as collagens, fibronectin, laminins, hyoluronan, plasminogens, proteases, and numerous others, which collectively form an inflexible scaffold to which cells attach. In addition, other secreted cellular proteins such as cytokines and extracellular matrix remodeling proteins normally reside in the extracellular matrix (Chapters 3 and 4). These proteins are released when the matrix is degraded, and upon their release become activated due to proteases and other activating enzymes present in the extracellular environment, further contributing to the regulation of extracellular matrix turnover.

Many cell types are present in the extracellular matrix and the tumor milieu. Examples include fibroblasts, which are an integral inducer of matrix remodeling, as well as endothelial cells, hematopoietic-derived cells, and immune cells, which normally monitor this environment for foreign (i.e., non-host) bodies (Chapter 5). In cancer, particularly at the later stages of transformation and invasion, normal immune functions are subverted, leading to recognition of the tumor as part of the host, rather than as an invading foreign entity.

1.5 Motility, invasion, and metastatic ability

Successful and evolutionarily adapted cancer cells are motile, have no major attachments to extracellular substrata, and can more easily move through the extracellular and intracellular space due to decreased cell adhesion and an increase in factors which facilitate extracellular matrix degradation and remodeling. A natural effect of motility is that cancer cells invade into surrounding tissue, colonize and populate the area given a favorable microenvironment. Further, cancer cell motility facilitates the movement of the cancer cell through layers of endothelial cells surrounding blood vessels, enabled in part through cancer cell secretion of vascular destabilizing factors. As a result the vasculature is perturbated and cancer cells gain access to the circulation, which is the major mode of transport for cancer cells to reach distant organs.

In the metastatic cascade, the tumor microenvironments of both the primary tumor and the target sites colonized by cells shed from the primary tumor are of critical importance to the successful spread of neoplastic cells (Joyce and Pollard, 2009) (Chapters 6–8 and 12). The classic 'seed and soil' mechanism describes a situation in which only permissive target microenvironments enable the attachment and subsequent proliferation by a metastatic cell. In addition, cells in the primary site of a cancer shed factors and progrowth signals that contribute to the tumor microenvironment at the secondary sites of tumor formation (Chapter 8). In this way, metastatic tumor cells establishing new colonies continue to receive progrowth support signals while they are colonizing the secondary site and during subsequent phases of secondary tumor growth.

In addition, the evolution of the microenvironment can impact premetastatic cells in a manner that leads to an invasive, advanced, and evolutionarily favored metastatic phenotype that can survive extravasation, intravasation, and can establish new tumors at distant sites. Accumulating evidence suggests that the hypoxic conditions that select for successful tumor types also contribute to the metastatic potential of that tumor (Chapter 14). Therefore, eradication of the hypoxic regions of a cancer has short-term and long-term benefits (Chapters 16–18), in that both tumor bulk and metastatic capability are reduced.

1.6 Impact of the tumor microenvironment on the control of cancer

The tumor microenvironment is a growing target for consideration of cancer therapeutics due to its varied influence on the cells and on the physical aspects of chemotherapeutic delivery (Chapters 2 and 15). Several drawbacks to traditional chemotherapies that do not account for the microenvironment are: the tumor vasculature, which is highly disordered and leaky; tumor core hypoxia, which confers radiation resistance on tumor cells in this state; cells furthest from blood vessels become growth-arrested, preventing efficacious chemotherapeutic inhibition of proliferating cells; and the upregulation of acid transporter and other transporter proteins, which efficiently excrete chemotherapeutics from cancer cells and

hinder successful cancer treatment (Chapters 2, 9, and 15). Importantly, offspring of chemotherapeutic survivors can pass this genetic property to daughter cells, making subsequent populations of tumor cells highly resistant to subsequent therapy. One further consideration regarding the tumor microenvironment is the stem cell population, a slow-growing subset of cancer cells, which is inherently resistant to therapies targeting cells that are actively cycling. Stem- or stem-like cancer cells are pluripotent, highly plastic, and dedifferentiated entities that easily and steadily repopulate tumors following therapy. Considering these scenarios, targeting the tumor microenvironment becomes an increasingly logical and attractive therapeutic option in cancer management.

1.7 Targeting the tumor microenvironment

Classical anticancer therapies including radiotherapy and chemotherapy are toxic to cancer cells but such treatments are typically also associated with inadvertent damage to critical normal tissues. Newer and more specific therapies have become more prevalent in the treatment of specific cancers as the molecular mechanisms of carcinogenesis become better characterized. The approach of uncovering molecular etiologies of cancer coupled with the development of targeted therapies that exploit essential signaling pathways (Chapters 10, 13 and 17) will undoubtedly contribute to the future arsenal of anticancer therapeutics.

Because the microenvironment of tumors not only severely impairs the treatment efficacy of conventional anticancer therapies but also differs significantly from those found in normal tissues, research is beginning to focus on the tumor microenvironment as a separate cancer-associated entity that may be targeted (Chapters 10, 13 and 17). Indeed, several strategies have already been identified that exert an anticancer effect through the specific targeting of the tumor microenvironment. Oxygen-poor cells display greater resistance to radiotherapy, and methods for reversing the radioprotective effects of hypoxia in order to enhance the treatment efficacy of radiotherapy have received considerable attention (Chapters 9 and 16). The other compartment of the tumor microenvironment that has been extensively targeted is the tumor vasculature (Chapter 18). As an essential part of tumor survival, such a strategy seeks to deprive the tumor of critical nutrients and means to spread. The use of antiangiogenic and vascular disruptive therapies provide powerful adjuncts to conventional anticancer treatments. All such potential therapeutic interventions will be critically dependent upon the establishment of novel approaches to non-invasive imaging of the tumor microenvironment (Chapter 11).

1.8 Summary

The microenvironment of tumors creates a significant hindrance to the control of cancers by conventional anticancer therapies. The physical conditions present are imposing and manifold, and include elevated interstitial pressure, localized extracellular acidity, regions of oxygen and nutrient deprivation, and contraction

of the extracellular matrix. No less important are the functional consequences experienced by the tumor cells residing in such environments: adaptation to hypoxia, cell quiescence, modulation of transporters, and enhanced metastatic potential. Together these factors lead to therapeutic barriers that may render the chance of tumor elimination as minimal.

However, the aberrant nature of the tumor microenvironments also offers unique therapeutic opportunities. Reducing tumor hypoxia can improve drug delivery and enhance radiotherapy. The inhibition of fibroblasts and other cell types exploits the tumor's reliance on the microenvironment for various factors and properties necessary for its survival. Targeting the tumor vasculature would destroy the nutritional support network of the tumor. These approaches and many others directed against the tumor microenvironment are under active investigation in the laboratory and the clinic.

Because the molecular underpinnings of cancer development are becoming increasingly well characterized, future studies will undoubtedly identify distinct molecular markings that are characteristic of the tumor microenvironment. Such advances will lead to the development of targeted therapies that will selectively impair the neoplastic cell populations residing in these environments. Ultimately, by combining such therapies with conventional anticancer treatments it may be possible to bring cancer growth, invasion, and metastasis to a halt.

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