Differentiation (2002) 70:469–472

EDITORIAL

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It's not my fault, blame it on my microenvironment

For decades, pathologists have recognized that the stroma surrounding tumors is in many cases altered with obvious changes in the cellular composition and the extracellular matrix (ECM). Over the years, descriptive studies have verified that gene expression is altered in carcinoma-associated stroma or "tumor stroma", and for some time, investigators have considered and explored the role of tumor stroma in the emergence and progression of carcinomas. A pivotal monograph in this area was the book edited by David Tarin entitled "Tissue Interactions in Carcinogenesis" published in 1972 (Tarin, 1972). Since then, steady progress has been made, and now the full implications of interactions between carcinoma cells and their associated stroma have become apparent. During development of this field, various terms have been used to describe the cells and ECM surrounding the tumor. The term "tumor microenvironment" was used initially in a series of papers in 1979–1980 (based upon a PubMed search) (Lord et al., 1979; Bicher et al., 1980; Dewhirst et al., 1980). These papers stressed the importance of tumor cell-host cell interactions and the potential of new treatment modalities based on tumor cell-host interactions.

Recognizing the importance and significance of cell-cell and cell-ECM interactions in malignant progression, the National Cancer Institute (NCI) stimulated the field in the mid 1990s with a RFA focussed on "microenvironmental" aspects of cancer development, which led to the funding of several grants. As a follow-up, an NCI workshop was held in September 2000 organized by Lynn M. Matrisian (Vanderbilt), Gerald R. Cunha (UCSF), and Suresh Mohla (NCI). The attendees consisted of 20 experts in the field as well as NIH representatives. The subject of the meeting was "Epithelial-stromal interactions and tumor progression", but also encompassed other topics within the general focus of the role of tumor microenvironment in carcinogenesis. The purpose of the workshop was to assess the status of the field with the long-range goal of accelerating progress in understanding the interrelationship between tumor cells and their microenvironment and applying this knowledge to the control of tumor progression. A short meeting report was published summarizing the workshop (Matrisian et al., 2001). This issue of *Differentiation* draws upon many of the attendees of the original NCI workshop to provide a series of reviews and primary papers focussed on the role of tumor microenvironment in development, progression, growth, and metastasis of carcinomas and other tumors.

The basic concept of this field is that carcinoma (and other tumor) cells do not exist in isolation but instead

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emerge and exhibit a range of behaviors regulated in part by host cells comprising the tumor stroma, which contains connective tissue cells, vasculature, cells of the immune system, and ECM. Cells of the tumor microenvironment and the spectrum of paracrine and autocrine mediators within the tumor microenvironment play critical roles in the biology of the malignant cells. Accordingly, therapeutic strategies targeted at the tumor microenvironment may provide novel means of controlling tumor growth and metastasis.

The reviews and primary papers embodied in this issue of *Differentiation* emphasize the potential reward to be gained by a greater understanding of the influence of tumor microenvironment on malignant epithelial cells. It is clear that the cellular and extracellular components of tumor stroma are integral parts of the tumor and contribute to some of the most destructive features of the tumor. Indeed, at least under some conditions, tumor stroma can exert a dominant force over the malignant phenotype of the tumor cells. It is now evident that there is much to be gained from a focus on the understanding of events mediating the interactions between the cancer cell and its immediate microenvironment, which influences the behavior of the cancer cell. Such an approach has great potential for therapeutic benefit as a result of the genetic stability of the cellular components and accessibility of the molecular targets within the tumor microenvironment. Manipulating host/tumor interactions has the potential of reverting the malignant phenotype and establishing normal control mechanisms.

There are many challenges to understanding the contribution and role of the stromal microenvironment to the tumor phenotype. "Stroma" is remarkably complex having molecular, mechanical, and cellular components. The ECM is composed of a complex array of proteins, glycoproteins, proteoglycans, and glycosaminoglycans that can provide structure, generate biological signals, store growth factors that can generate biological signals, and exert mechanical influences on epithelial cells. Cellular components that influence a tumor cell include other epithelial cells, fibroblasts, inflammatory cells, and endothelial cells. The stroma has both spatial and temporal complexity, is organ-specific and heterogeneous both within and between tumors, and changes with tumor progression. The specific molecules that are responsible for tumor-induced changes in the microenvironment and the reciprocal modifications of the tumor by the microenvironment are largely unknown but are currently under investigation (see reviews).

The stromal microenvironment is known to regulate normal epithelial differentiation, motility, growth, and function. Likewise, the tumor microenvironment plays a critical role in tumor differentiation, motility, growth, invasion, angiogenesis, and metastasis. Current investigation is now revealing the precise cells involved in these regulatory processes and at least some of the molecular effectors. Advances in each of these areas will surely reveal new therapeutic modalities with which to treat cancer. This collection of reviews provides an assessment of the current state of our understanding and provides summaries of the models that are available to pursue this line of research.

Studies from Cunha's group (UCSF) emphasized the important roles of mesenchymal-epithelial interactions in normal development and stromal-epithelial interactions in adult homeostasis. Experiments utilizing the recombination of immortalized human prostatic epithelial cells and stromal cells derived from human prostatic carcinomas demonstrated that tumor stroma can induce permanent carcinogenic changes in the epithelial cells. This induction of tumorigenesis in the parental non-tumorigenic cells resulted in further genetic alterations and defined changes in gene expression. Studies from Leland Chung's group (Emory University) emphasized that the unique prostatic cellular phenotypes are induced and maintained by interaction between epithelium and adjacent stroma through intimate intercellular signaling pathways. Emerging and established carcinomas respond to stromal signals that drive progression to malignancy via a vicious cycle mediated by soluble and insoluble molecules secreted by carcinoma cells and stroma. The review by Sung and Chung emphasized the roles of ECM, integrins, FGFs, PDGF, IGFs, VEGF, and cytokines as critical factors supporting and sustaining tumor colonization of bone.

The theme of stromal-epithelial interactions in both tissue homeostasis and progression to malignancy was further developed in models focussed on the epidermis and squamous cell carcinoma by Mueller and Fusenig (German Cancer Research Center, Heidelberg). An important aspect of the tumorigenic process is the differential modulation of stromal characteristics induced by epithelial skin tumor cells depending on their transformation stage. Thus, tumor cells regulate the development of a "tumor-stroma" via aberrant expression of growth factor signaling systems in the stromal compartment. Key players in these abnormal cell-cell interactions include cytokines (G-CSF and GM-CSF), growth factors (VEGF, FGFs, and PDGF), ECM, and matrix protease activity. Tumor vascularization is a key event in the overall process, controlling tumor invasion and expansion by distinct mechanisms, thus emphasizing the importance of the stromal compartment for the development and progression of carcinomas.

I.J. Fidler (M.D. Anderson Cancer Center) described the profound effects of the microenvironment on development of tumor cell metastasis. Fidler's studies emphasized that primary neoplasms are biologically heterogeneous and that the process of metastasis consists of a series of sequential, selective steps, in which the outcome of cancer metastasis depends on multiple interactions between cancer cells and the organ microenvironment. The specific organ microenvironment determines the ex-

tent of cancer cell proliferation, angiogenesis, invasion, and survival.

M.J. Bissell (Lawrence Berkeley National Laboratory) stressed the importance of cell-ECM interactions using a three-dimensional culture system of reconstituted basement membrane developed in her laboratory for growth of mammary epithelial cells. Modulation of surface receptors, including those for the extracellular matrix and growth factors, provide signals which can revert malignant mammary epithelial cells to a normal morphology and behavior, or, in reciprocal fashion, can convert cellular characteristics from benign to malignant. Bissell reinforced the often overlooked notion that a tumor is an organ, composed of multiple cell types and a structural framework that are intimately connected and inter-dependent. The dynamic influence of the stroma (cells, ECM, soluble, and tethered mediators) on tumor cells, at least those at an early stage of tumor progression, provides an opportunity to target stromal cells to control the malignant behavior of genetically-unstable epithelial cells.

The theme of cell-ECM interactions was further emphasized by D. Ingber (Harvard University), who stressed the importance of structural components in the ECM that influence cell shape and tension. He demonstrated that mechano-sensing of the environment plays a critical role in regulating properties such as cell migration and growth in tumors and during normal developmental processes. Ingber emphasized the complexity of the signal transduction pathways received by an epithelial cell from its environment that influences its physiological state.

The recurring theme of the role of the three dimensional extracellular matrix (ECM) and stromal cells such as fibroblasts and inflammatory cells was further developed in the review by Lynch and Matrisian (Vanderbilt University), which focussed on the matrix metalloproteinases (MMPs). Strict regulation of MMP expression is critical to maintain proper ECM homeostasis. In cancer, there is often a high level of MMP activity at the tumor-stroma interface, in which the stromal cells and not necessarily the carcinoma are primarily responsible. MMPs are implicated in a wide variety of roles and can assist tumor initiation, growth, migration, angiogenesis, selection of apoptosis resistant sub-populations, invasion, and metastasis. Matrisian and colleagues stress the idea that MMPs are part of an elegant communication system by which the tumor interacts with the stroma and drives cancer progression.

Van Kempen et al. from the laboratory of Lisa Coussens (UCSF) reviewed the role of MMP-9 in squamous carcinoma of the skin and its key role in angiogenesis and keratinocyte proliferation. Their studies demonstrated an influx of infiltrating leukocytes and mast cells during the carcinogenic process. These inflammatory cells are a known source of MMP-9 and chymase, which can convert pro-MMP-9 into its active form. While MMP-9 functionally contributed to cancer development, its major regulatory role may be in its ability to activate poorly diffusible and/or matrix-sequestered growth factors that regulate epithelial and/or endothelial cell growth. Most surprising of all was the observation that, although both epithelial cells and stromal cells can express MMP-9, it was the bone marrow-derived inflammatory cells that had the most dramatically affect pre-malignant progression.

Thoreson and Reynolds (Vanderbilt University) emphasized in their review the central role of alterations in E-cadherin and its cytoplasmic regulators, the catenins, in the transformation process since loss or perturbation of epithelial adhesion molecules determines in large part the ability of epithelial cells to maintain a cohesive tissue architecture. Their review focussed on p120-catenin, which is frequently altered and/or lost in tumors of the colon, bladder, stomach, breast, prostate, lung, and pancreas. Moreover, in some cases, p120-catenin loss appears to be an early event in tumor progression, possibly preceding loss of E-cadherin. Potential roles of p120-catenin as a tumor suppressor or metastasis promoter were discussed.

Growth factors certainly play critical roles in tumor progression and growth. Two reviews focussed on the TGFβ and the FGF families. A review by Dumont and Arteaga (Vanderbilt University) described the wide range of signaling networks implicated in mediating various biological responses induced by TGFβ. The mechanism by which latent TGFβ is activated may determine, in part, how cells and/or tissues respond to TGFβ. In addition to the extracellular matrix components regulated by TGFβ, effects of TGFβ signaling on resident immune cells within the stroma represent another important component of the tumor microenvironment that can be modulated by TGFβ. The complex interplay between TGFβ and various matrix proteins, receptors, and proteases highlights the bi-directional nature of reciprocal tumor-microenvironmental interactions that are an important determinant of tumorigenesis.

In a review on the pathogenesis of mouse prostate, Foster et al. from the laboratory of Norman Greenberg (Baylor Medical School) presented transgenic models of perturbing FGF signaling in the mouse prostatic epithelium by targeting expression of human fibroblast growth factor-7 (FGF-7) directly to prostatic epithelium of transgenic mice. This strategy converts FGF-7 from a paracrine to an autocrine factor. Another approach used by Greenberg was the targeting a truncated FGFR2iiib receptor to prostatic epithelium to functionally abrogate endogenous FGF-7 signaling. Both of these models resulted in hyperplastic and/or dysplastic changes in the prostate and, in some cases, disorganization of the stroma. Greenberg's studies clearly emphasize the role of FGF signaling in the develop of prostate cancer.

In a review focussed on melanoma, Hsu et al. from the laboratory of Meenhard Herlyn (Wistar Institute) demonstrated that regulatory signals governing melanocyte cell growth and differentiation originate from the surrounding host cells either directly through physical contacts or indirectly through soluble factors and extracellular matrix molecules. Melanoma-host interactions via short-range tumor-microenvironment interactions contribute to the oncogenic process. The intercellular molecular dialogues mediating tumor- microenvironmental interactions are discussed, which may provide key information for the development of novel treatment strategies that target the functional units of stroma and tumor.

Vito Quaranta (Scripps Research Institute) emphasized the idea that the microenvironment provides the cues for motility involved in epithelial morphogenesis, tissue remodeling, and wound healing. In the tumor microenvironment, these same motility cues may lead to invasion and metastasis. In this review, some of the molecular players, whether soluble factors or ECM substrates, are drawn from the cell biology of tissue remodeling and from the genetics of epithelial organogenesis and are discussed in relation to normal development, tissue homeostasis and tumor invasion. An example is the ECM macromolecule laminin-5, found both in normal epithelia and in carcinomas, whose cleavage by MMPs generates cell migration stimuli in the microenvironment.

While microenvironment-based therapies are under development, it is important to recognize that microenvironment-mediated therapy could itself generate drug resistance. Yu et al. from the laboratory of Robert Kerbel (Sunnybrook and Women's College Health Sciences Center, Toronto) reviewed this sobering idea. Because some tumor cells are highly vessel-dependent, whereas some are significantly less so, it is possible that variant tumor cells that are less vessel-dependent may therefore be selected over time by successful anti-angiogenic drug therapies. This results in loss of response or attenuated responses to the therapy. For this reason, consideration should be given to the combined use of bio-reductive hypoxic cell cytotoxic drugs and angiogenesis inhibitors to prolong the efficacy of anti-angiogenic therapeutics.

Included also in this Microenvironmental issue of *Differentiation* is a timely primary paper by Wong and Tam (University of Hong Kong). Their paper focussed on a rat model of prostatic carcinogenesis, which demonstrates in the course of hormone-induced prostatic carcinogenesis that the differentiation state of cells of the stroma become progressively altered. In the normal rat prostate, the predominant cell of the stroma is smooth muscle, which is in intimate association with the epithelium. During prostatic carcinogenesis, there is a progressive loss of smooth muscle differentiation markers associated with the acquisition of fibroblastic differentiation markers. Thus, the carcinogenic process is association with a dramatic change in the cellular differentiation state of the stroma.

It is hoped that this Microenvironmental issue of *Differentiation* will heighten awareness of the opportunities available to make significant advances in controlling cancer through the manipulation of tumor cell-host microenvironment interactions. The intimate relationship between the tumor cell and the stroma and the crucial contribution of tumor microenvironment to tumor development and progression needs to be brought to the forefront of cancer research. Elucidation of the molecular mechanisms that underlie these interactions should be a high priority of cancer research. Current indications suggest that the approach of targeting genetically stable components of a tumor's microenvironment may provide significant new advances in therapeutic intervention. With appropriate support, fertile new territories of research can be rapidly charted and harnessed for the treatment and management of cancer.

Acknowledgements Supported in part by NIH grants CN-15114-MAO, CA89520, CA84294, CA59831, DK52708, DK47517, DK45861, and CA64872.

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