Published in final edited form as:

Curr Opin Genet Dev. 2008 February ; 18(1): 27–34.

Microenvironmental regulation of cancer development

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Abstract

Numerous studies have demonstrated that the tumor microenvironment not only responds to and supports carcinogenesis, but actively contributes to tumor initiation, progression, and metastasis. During tumor progression all cells composing the tumor undergo phenotypic and epigenetic changes. Paracrine signaling between epithelial and stromal cells is important for the regulation of the proliferation, invasive, angiogenic, and metastatic behavior of cancer cells. Better understanding the molecular mechanisms by which stromal cells exert these effects may open up new venues for cancer therapeutic and preventative interventions.

Introduction

The “seed and soil” hypothesis of tumor growth dates back as early as 1889 [1], but during the past decades advances in identifying aberrances in oncogenes and tumor suppressor genes within tumor epithelial cells resulted in the ignorance of the role of the microenvironment in tumorigenesis. However, a tumor is much more than clusters of transformed cells standing alone. Tumor epithelial cells can only thrive in an aberrant microenvironment composed of altered extracellular matrix (ECM) and various non-transformed cells (e.g., fibroblasts, myofibroblasts, leukocytes, and myoepithelial and endothelial cells) that play a role in the initiation and progression of neoplasms [2–5]. Cross-talk between epithelial and stromal cells is known to be essential for differentiation and development of normal organs and tissues as well as for the growth and progression of tumors [2,6,7]. Recent work has begun to address the importance of the microenvironment in supporting malignant growth and the molecular mechanisms by which stromal cells may contribute to tumorigenesis. In this review, we focus on the phenotypic, genetic, and epigenetic alterations found in cells composing the tumor microenvironment, the role of these cells in tumor progression, and the clinical implications of these findings.

The tumor microenvironment: from reactive neighborhood to active contributor

It was noted long time ago that changes in the microenvironment accompany tumor formation [8–10]. Increased fibroblast proliferation and ECM remodeling are often found adjacent to cancer cells. The tumor stroma in many aspects resembles wound healing and chronic inflammatory conditions, except that normal physiologic controls are not maintained [11].

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Furthermore, even fibroblasts outside of the immediate vicinity of neoplastic lesions can demonstrate phenotypic changes. For example, skin fibroblasts from a significant portion of patients with breast cancer were described to display altered migratory behavior due to the expression of a soluble migration-stimulating factor not made by normal adult cells [12].

Large amounts of data support the hypothesis that the stroma is not just a passive bystander that simply reacts to the transformed cells. Rather, interactions between mesenchymal and epithelial cells result in reciprocal influences, and the microenvironment is an active participant throughout cancer initiation, progression, and metastasis.

First, phenotypic and genotypic abnormalities in cancer epithelial cells cannot fully delineate tumor phenotypes and clinical behavior. cDNA microarray profiling and hierarchical clustering analyses have been utilized to classify breast cancer subtypes and predict clinical outcome [13,14]. However, comprehensive gene expression and genetic profiling studies comparing *in situ*, invasive, and metastatic breast carcinomas have failed to identify tumor stage-specific gene signatures [15–19], implying that besides the intrinsic malignant properties of tumor epithelial cells, other factors such as microenvironmental changes may regulate progression to invasion and metastasis.

Second, normal cellular microenvironment can inhibit tumor cell proliferation and cancer formation. This was first and most dramatically demonstrated by the lack of tumor development in chick embryos infected with the Rous sarcoma virus expressing a potent oncogene [20]. Normal myoepithelial cells have been shown able to suppress the growth, invasion, and angiogenesis of multiple different breast cancer cell types [21–23], whereas the effects of normal fibroblasts are more dependent on the ratio of stromal and epithelial cells and the degree of malignancy of the tumor cells [24].

Third, stromal cells can create a permissive microenvironment for tumorigenesis. In contrast to normal myoepithelial cells, cancer-associated fibroblasts and myofibroblasts have been demonstrated to promote tumorigenesis via enhancing angiogenesis, and proliferation, survival, invasion, and metastatic spread of tumor epithelial cells [2–5]. Bone marrow-derived cells were found to colonize at sites of distant metastases prior to the arrival of the tumor cells, establishing a “pre-metastatic niche” that facilitates tumor cell growth in distant organs [25–29]. Disruption of the normal microenvironment due to chronic inflammation or hereditary changes in genes regulating immune reactions and tissue remodeling may play similar “preparing the soil” roles in tumor initiation. Human epidemiologic data as well as experiments in model systems strongly support this hypothesis [30].

Fourth, the stroma may be a crucial target of carcinogens. In one study, neoplastic transformation of rat mammary epithelial cells occurred only when the mammary fat pad stroma was exposed to chemical carcinogens, regardless of whether or not the epithelial cells were treated [31]. However, similar experiments by another group failed to reproduce these results and pointed out that carcinogen treatment of the epithelial cells is required for tumor formation [32].

Finally, targeting the tumor microenvironment may be a feasible therapeutic approach for cancer treatment and prevention. In contrast to genetically unstable tumor cells, the genetically stable cells composing the microenvironment are less plastic and less likely to acquire drug resistance, and therefore, potentially make better targets for cancer therapy. In line with this, anti-angiogenic drugs have been developed to target tumor endothelial cells as a means of cancer treatment [33]. Elimination of carcinoma-associated macrophages in murine models of metastatic breast, colon, and non-small cell lung cancers decreased tumor angiogenesis, growth, and metastasis [34]. Similarly, targeting tumor-associated fibroblasts using a vaccine-based approach in multi-drug resistant murine colon and breast carcinomas suppressed primary

tumor growth and metastasis, decreased the expression of collagen type I, and increased the uptake of chemotherapeutic drugs [35]. The results of these studies demonstrate the feasibility and success of combined targeting of cancer cells and their microenvironment using various immuno- and chemotherapeutic approaches.

Phenotypic and molecular alterations in the tumor microenvironment

To define the molecular basis underlying microenvironmental changes in tumorigenesis, Allinen *et al.* characterized the comprehensive gene expression profiles of several major cell types from normal human breast tissue, ductal carcinoma *in situ* (DCIS), and invasive breast carcinomas, using SAGE (Serial Analysis of Gene Expression) [36]. The results of this study demonstrated that dramatic gene expression changes occur in all cell types, including tumor epithelial, endothelial, and myoepithelial cells, myofibroblasts, fibroblasts, and leukocytes, during breast tumor progression. Similarly, gene expression [37] and enzyme activity [38] profiles of human breast cancer cell lines grown in three different ways: (1) *in vitro* cultures, (2) primary tumors in the mammary fat pad, and (3) distant metastases in different organs, showed changes in both epithelial cells and neighboring host stroma, suggesting reciprocal interactions during tumor formation and metastasis.

Despite the dramatic and universal changes of gene expression in all cell types during tumor progression, clonally selected genetic alterations were restricted in tumor epithelial cells and could not be found in any of the stromal cells analyzed by aCGH (array comparative genomic hybridization) and SNP (Single Nucleotide Polymorphism) arrays [36]. Controversy arises when data from other groups suggested that genetic aberrances also happen in breast tumor stroma, including gene copy number changes, LOH (loss of heterozygosity), microsatellite instability (MSI) and point mutations in tumor suppressor genes and oncogenes [39–45]. Because the analysis of small numbers of cells from archived tissue samples using PCR based methods is prone to yield erroneous results, determining if these findings are reproducible using fresh tissue samples and alternative approaches would be imperative. Indeed, recent results obtained using fresh or frozen tissue samples, and primary cultured cells indicate that LOH and copy number alterations are extremely rare in breast and ovarian carcinoma-associated fibroblasts (Qiu *et al.*, unpublished data).

The presence of gene expression alterations, but lack of genetic abnormalities point to epigenetic changes including DNA methylation and chromatin modification being responsible for the relative stability of the abnormal phenotypes of cancer-associated stromal cells. To explore this possibility, Hu *et al.* characterized the comprehensive DNA methylation profiles of epithelial and myoepithelial cells, and fibroblasts isolated from normal and neoplastic breast tissues using MSDK (Methylation-Specific Digital Karyotyping) [46^{**}]. DNA methylation changes were detected in all three cell types in DCIS and invasive tumors, supporting the hypothesis that the phenotypic changes observed in tumor stromal cells are at least in part due to epigenetic modifications. Studies in HER2+ breast cancer [47] and prostate tumors [48] also demonstrated distinct methylation patterns of selected genes in tumor epithelial and surrounding stromal cells. A significant fraction of genes aberrantly methylated in cancer cells (both epithelium and others) encode transcription factors with known function in development and differentiation [46]. It is proposed that tumor-associated myofibroblasts and fibroblasts develop from bone marrow-derived mesenchymal stem cells recruited to the stroma of developing tumors [49]. Thus, their abnormal phenotypes and epigenetic profiles may reflect their abnormal differentiation due to factors present in tumors.

Signaling networks between the microenvironment and tumor epithelial cells

Stromal cells influence epithelial cell behavior by secreting various ECM proteins, chemokines, cytokines, growth factors, proteases, and protease inhibitors. A large proportion

of genes differentially expressed in epithelial and stromal cells during breast tumor progression encode for secreted proteins and cell surface receptors [36]. An extensive network of cross-talks between cancer cells and the host was identified including regulations of cell-ECM interaction and growth factor signaling using a lung adenocarcinoma xenograft model [50]. Cell adhesion is an important regulator of neoplastic behavior, particularly that of invasion and metastasis [51,52]. Thus, the modulation of the expression of genes involved in cell adhesion may be another mechanism by which stromal cells regulate tumor cell invasion and metastasis.

Co-injection of lethally irradiated fibroblasts with human cancer cells increased the tumorigenic potential of prostate, bladder, and breast cancer cell lines, and cells derived from the ascites fluids of patients with metastatic renal or prostate cancers [53], supporting a role for paracrine signaling between fibroblasts and tumor epithelial cells. Injection of fibroblast-conditioned medium at the inoculation site of tumor cells enhanced tumor growth, suggesting the involvement of soluble factors secreted by fibroblasts [54]. Inhibiting TGF- β signaling in fibroblasts by specifically deleting the TGF- β type II receptor in these cells, enhances the growth and oncogenic potential of adjacent epithelia via activation of TGF- α , MSP (macrophage-stimulating protein), and HGF (hepatocyte growth factor) pathways [55,56^{**}, 57]. Similarly, the CXCL14 and CXCL12 (CXC motif chemokine ligand 14 and 12) chemokines, overexpressed in DCIS myoepithelial cells and myofibroblasts, respectively, promote tumor cell proliferation, migration, invasion, angiogenesis, and metastasis [36,58, 59].

Clinical observations have indicated that tumor cells metastasize to distant organs in a non-random manner, although they scatter ubiquitously [60]. To investigate the molecular mechanisms underlying homing and preferential tumor cell growth at certain sites, the gene expression profiles of variants of the MDA-MB-231 breast cancer cell line with elevated metastatic activity to particular organs such as bone or lung were characterized [61,62^{*},63]. Distinct groups of secreted and membrane proteins are found to be associated with organ preference for metastatic colonization and growth, suggesting the involvement of tumor-host interactions in these processes in part mediated by chemokines/cytokines and their receptors. In a separate study, co-injection of bone marrow-derived mesenchymal stem cells enhanced the metastatic potency of MDA-MB-231 human breast carcinoma cells growing at subcutaneous sites via upregulating CCL5-CCR5 signaling [64^{*}]. Another study on the other hand proposed that bone marrow-derived stem cells are mobilized due to tumor growth and arrive to sites of future distant metastasis prior to tumor epithelial cells [25–29]. Thus, the local microenvironment created by these circulating stem cells prepares a “niche” for metastases. Therefore, paracrine signaling between tumor epithelial cells and the host/organ microenvironment appears to have major influence on tumor progression to metastatic disease (Fig. 1). Overall, cells composing the microenvironment can regulate epithelial cell behavior multiple different ways including direct and indirect mechanisms.

Role of stromal cells in the *in situ*-to-invasive carcinoma progression

The transition of *in situ* to invasive carcinoma is a key event in breast tumor progression that is poorly understood. Phenotypic, genetic and epigenetic changes have been detected in tumor epithelial cells during this transition step yet stage-specific molecular signatures could not be identified [16–19,36,46]. Meanwhile, alterations in gene expression and DNA methylation occur in the non-transformed cells of the tumor microenvironment as well [36,46], implying that tumor-stromal communication may play a role in this progression step. Two models of the *in situ*-to-invasive carcinoma transition have been proposed, focusing on the “seed” or the “soil”, respectively [23]. The “escape” model hypothesizes that genetic changes and clonal selection in combination will give rise to a subpopulation of tumor epithelial cells with an ability to invade out of the duct and into the surrounding tissue. The “release” model proposes

that the abnormal microenvironment such as phenotypic changes of myoepithelial cells, infiltration of leukocytes, and accumulation of myofibroblasts, will lead to the disruption of the basement membrane (BM) and let the tumor epithelial cells freely spread in the stroma. Because changes in the tumor epithelial cells are likely to induce microenvironmental changes, the combination of the two models (changes in both the epithelial and stromal cell compartments) is what really explains progression to invasion. Multiple recent publications support the “release” hypothesis. Capillary blood vessels may invade and breach the BM in DCIS, creating an escape route for the cancer cells [65]. Focal breakdown of myoepithelial cell layer and BM at sites of white blood cell infiltration have also been observed in DCIS [66]. Emphasizing the necessity of changes in both in “seed” and “soil” for progression, epithelial cell clusters overlying the disrupted myoepithelial layers were different from adjacent cells within the same duct with respect of ER (estrogen receptor) status, frequency or pattern of LOH and/or MSI, and expression of tumor progression related genes, normal stem cell and proliferation markers, and showed invasion into the stroma and blood vessels-like structures [67–69]. Since tumor-stromal interactions are bi-directional, identification of the initiating events requires further study.

Nevertheless, myoepithelial cells are essential regulators of DCIS-to-invasive carcinoma transition because they produce the BM and form a physical barrier around tumor epithelial cells. Normal myoepithelial cells have been recognized as “natural tumor suppressors” and function as gatekeepers of tumor progression [21–23]. DCIS-associated myoepithelial cells demonstrate altered gene expression or DNA methylation profiles including loss of differentiation markers and augmented levels of pro-angiogenic and invasive genes [36,46]. Tumor myoepithelial cells were also unable to influence the polarity of breast epithelial cells in 3-dimensional collagen cultures due to their lack of production of BM constituent laminin 1 [70]. Myoepithelial cells of high grade DCIS exhibit strong expression of PAI-1 (plasminogen activator inhibitor type-1), which may resolve the interaction between uPAR (urokinase plasminogen activator receptor) present on the myoepithelium and vitronectin in the BM, and result in the detachment of the myoepithelial cells from the BM followed by tumor cell infiltration [71]. More importantly, tumor-associated myoepithelial cells express higher levels of several BM-degrading enzymes including MMPs, compared to their normal counterparts [36]. Therefore, contrary to the role of normal myoepithelial cells in BM synthesis and maintenance, progressive changes in the DCIS myoepithelial cells may lead to gradual degradation of BM. On the other hand, the basement membrane induces and maintains myoepithelial cell differentiation [72], therefore forming a positive feedback loop. Similarly, high levels of MMPs in myofibroblasts and fibroblasts can also contribute to ECM remodeling and indirectly regulate myoepithelial cell differentiation. Thus, molecular and cellular components of the microenvironment may shift the balance between BM synthesis and degradation, and control the *in situ*-to-invasive carcinoma transition (Fig. 2).

Conclusions

Tumor initiation and progression are determined by the molecular and phenotypic alterations arising in the tumor epithelial cells as well as in their microenvironment. Thus, combined targeting of both the “seed” and the “soil” may be a more effective approach for cancer prevention and treatment.

Acknowledgements

Studies in this laboratory are supported by Novartis, NIH (CA89393, CA94074, and CA116235), DOD (W81XWH-07-1-029), ACS (RSG-05-154-01-MGO), and Avon Foundation grants to KP, and Susan G. Komen Foundation fellowship (PDF042234) to MH.

Disclosure Statement

K.P. receives research support from and is a consultant to Novartis Pharmaceuticals, Inc. K.P. also receives research support from Biogen Idec, Inc. and is a consultant to and stock shareholder of Aveo Pharmaceuticals, Inc. K.P. and M.H. are also co-inventors of a patent application on DNA methylation changes occurring in the tumor microenvironment.

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Figure 1. The dual role of bone marrow derived mesenchymal stem cells (BMD-MSCs) in cancer metastasis

1. Primary tumor cells send signals to the bone marrow mobilizing BMD-MSCs. 2. BMD-MSCs are recruited to the primary tumor (2a), and also mobilized to other organs such as lungs (2b), creating a pre-metastatic niche prior to the dissemination of the cancer cells. 3. The interaction between cancer cells and BMD-MSCs within the primary tumor enhances the motility, invasive and metastatic capacity of tumor cells via paracrine interactions (e.g., CCL5-CCR5). In addition, BMD-MSCs can differentiate into myofibroblasts and other stromal cell types that further support the growth and progression of the tumor. Furthermore, disseminated cancer cells preferentially grow at sites where BMD-MSCs are localized forming distant metastases.

Figure 2. Events involved in the *in situ* to invasive breast carcinoma transition

Progression to invasion is marked by the disappearance of myoepithelial layer, disruption of the basement membrane, accompanied by infiltration of leukocytes, accumulation of myofibroblasts, and intrusion of capillary vessels. High levels of MMPs produced by DCIS associated myoepithelial cells, fibroblasts, myofibroblasts, and leukocytes all contribute to the degradation of the basement membrane and tumor progression.