Watch thy neighbor: cancer is a communal affair

Valerie M. Weaver* and Penney Gilbert

Department of Pathology and Institute for Medicine and Engineering, University of Pennsylvania, PA 19104, USA

*Author for correspondence (e-mail: vmweaver@mail.med.upenn.edu)

Journal of Cell Science 117, 1287-1290
Published by The Company of Biologists 2004
doi:10.1242/jcs.01137

Summary

Malignant transformation of an epithelium occurs within the context of a dynamically evolving tissue stroma that is composed of multiple cell types surrounded by an extracellular matrix. Because stromal-epithelial interactions regulate tissue homeostasis and can profoundly influence tumorigenesis it has been proposed that the stromal microenvironment is an epigenetic tumor modifier that can either positively or negatively regulate the malignant behavior of genetically aberrant cells. New work reported in this issue of *Journal of Cell Science* now provides compelling evidence that alterations in the stroma are necessary and also sufficient for induction of malignant behavior by genetically normal cells.

The multi-hit genetic model of cancer maintains that tumors arise through a combination of hereditary alterations and accumulation of incremental and sequential acquired changes in the genome of targeted cells. Central to this reductionist paradigm is the concept that cell transformation will ensue following accumulation of a sufficient number of mutations, amplifications, and/or deletions in key genes that are essential for tissue homeostasis. Malignancy arises because critical mutations theoretically release target cells from their normal growth and survival constraints and permit their invasion, growth and survival in the surrounding extracellular matrix (Kinzler and Vogelstein, 1996). Loss of genetic heterozygosity, however, has been detected in morphologically normal lobules adjacent to breast cancers (Deng et al., 1996), and promoter methylation (silencing) of tumor suppressors such as $p16^{INK4a}$ occurs in histologically normal human mammary epithelial tissue (Holst et al., 2003). Moreover, the rate of tumor penetration for hereditary germline mutations in tumor suppressors is variable, and malignant transformation of benign lesions often takes years before a clinically diagnosed malignancy emerges. Once tumors have formed, their behavior is often erratic and stochastic such that some tumors grow and invade aggressively, while others of similar grade can experience extended periods of dormancy (reviewed in Unger and Weaver, 2003). Such observations are consistent with the idea that tumorigenesis is an indolent and inefficient process that is probably more complex than initially appreciated. This realization has heightened appreciation of the role played by genetic modifiers and epigenetics in malignancy.

Epithelial tissues are multicellular, 3D structures that interact dynamically with multiple cell types, such as fibroblasts, adipocytes, infiltrating immune cells and endothelial cells, within the context of a proteinaceous microenvironmental network called the extracellular matrix (Fig. 1A, right). The fidelity of tissue development, adult tissue remodeling and tissue homeostasis all depend upon the strict maintenance of a complex spatial and temporal dialogue between the epithelium and the cellular and acellular components of the tissue stroma. Perturbations in stromal-epithelial interactions result in loss of tissue homeostasis and induction of pathologies such as malignancy. In fact, tumor development is associated with induction of a 'reactive or desmoplastic' response in the stroma that is characterized by proliferation and transdifferentiation of fibroblasts, infiltration and activation of inflammatory cells, induction of angiogenesis and altered deposition and degradation of the extracellular matrix. Indeed, the desmoplastic tumor stroma is strikingly similar to the stroma found in a wound (Fig. 1A, left). Because the wound stroma by necessity promotes epithelial cell growth and migration and fosters angiogenesis to drive healing, Bissell and co-workers hypothesized and thereafter experimentally demonstrated that the desmoplastic tumor stroma or wounded microenvironment is a tumor promoter. These and other similar observations by investigators including Cunha, Chung and colleagues heralded the study of tumorigenesis as a tissue-based disease in which malignant transformation is studied in the context of the tissue microenvironment (reviewed in Kenny and Bissell, 2003).

Over the past decade the epitheliocentric view of tumorigenesis has slowly been supplanted in favor of the tissue microenvironment concept of malignancy, where tumor pathogenesis is viewed as a 'tissue-phenomena' linked to alterations in stromal-epithelial interactions (Bissell and Radisky, 2001; Kenny and Bissell, 2003; Unger and Weaver, 2003). Key to this 'tumor microenvironment' perspective of malignancy is the idea that 'initiated' genetically primed or mutant target cells that give rise to epithelial tumors pre-exist or are acquired within a tissue. The theory asserts that genetically primed resident cells have a low pre-disposition to develop into a tumor and will probably remain dormant unless an exogenous stimulus, such as factors produced by the activated stroma, alters the kinetics of tumor progression to promote the probability of disease inception, through the creation of a favorable microenvironment. A key assumption is that the activated stroma acts as an auxillary factor or 'normal wound response' against a background of pre-existing genetically altered target tumor cells. In favor of this scenario is the following evidence: the demonstration that abnormal stromal fibroblasts can promote carcinogenesis in genetically abnormal but nontumorigenic prostate epithelial cells and fail to alter the behavior of genetically normal prostate cells significantly; the observation that co-culture of oncogene-expressing mammary epithelial cells (MECs) with fibroblasts significantly enhances their tumorigenicity in vivo; the demonstration that induction of a reactive stroma in the mammary gland following γ-irradiation drives tumorigenesis of a genetically aberrant mammary epithelium; the fact that factors secreted by infiltrating immune

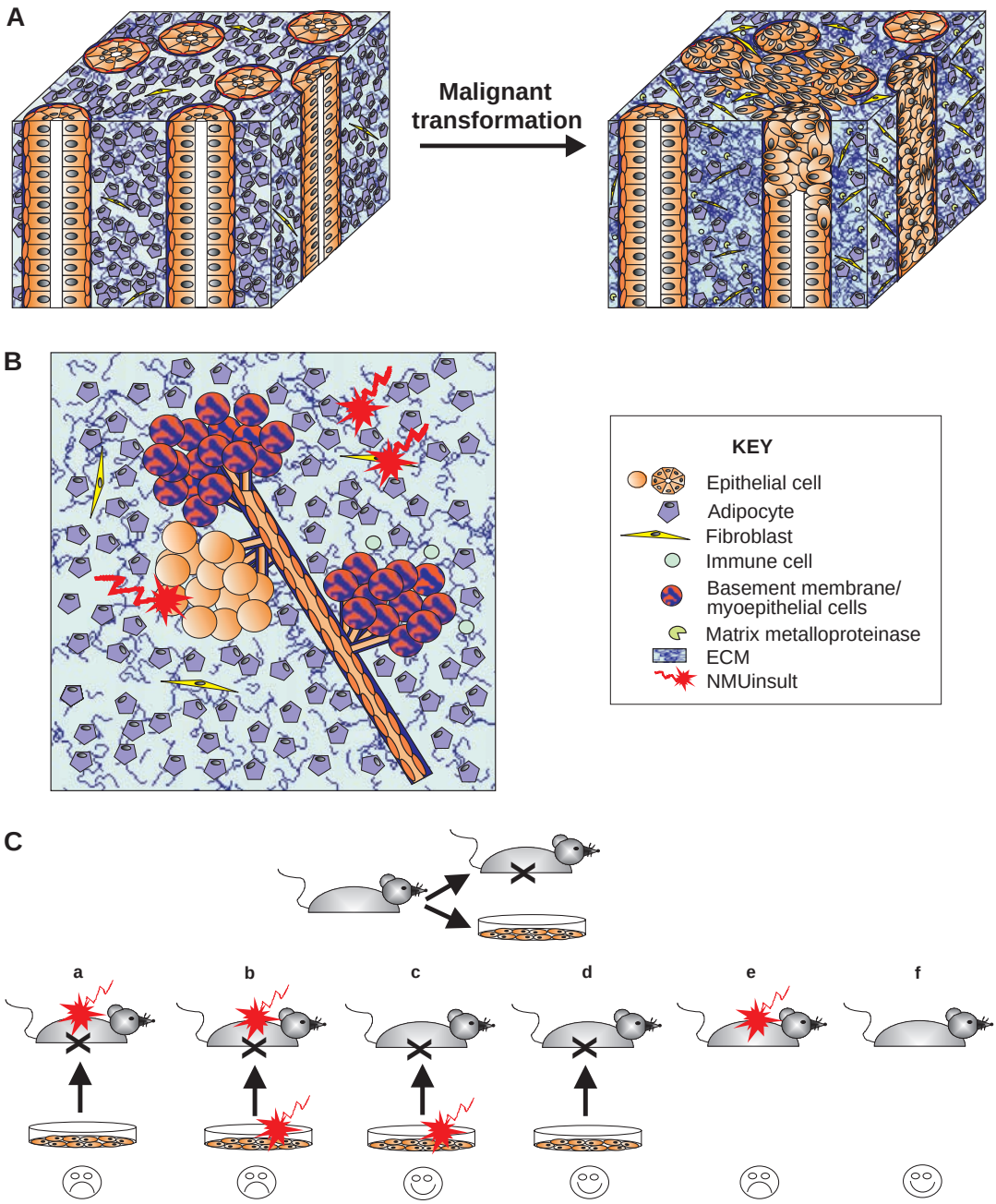

Fig. 1. (A) Malignant transformation of an epithelium occurs within the context of a three dimensional tissue that is accompanied by (1) fibroblast proliferation and transdifferentiation, (2) extracellular matrix deposition and remodeling, (3) increased matrix metalloproteinase expression and activity, (4) infiltration of immune cells, and (5) angiogenesis. The tumor microenvironment therefore is a dynamically evolving microenvironment that fosters tumor cell invasion, survival and growth. (B) Diagram showing potential NMU targets in the mammary tissue in vivo. Although the epithelium has been classically viewed as the critical mutagenic target of chemical carcinogens, cells within the stroma and the extracellular matrix may also constitute viable chemical targets. (C) Experimental scheme used by Maffini and colleagues to test the tissue organization field theory of carcinogenesis. Animal manipulations included: (a) stromal fat pad treated with NMU and reconstituted with vehicle treated MECs, (b) stromal fat pad and MECs both treated with NMU, (c) normal stromal fat pad reconstituted with NMU-treated MECs, (d) stromal fat pad and MECs treated with vehicle only, (e) intact mammary gland treated with NMU, and (f) intact mammary gland treated with vehicle only.

cells drive malignant invasion of genetically abnormal tissues; and experiments showing that tempering the desmoplastic response significantly reduces malignant transformation of HPV16 transgenic keratinocytes in mice (reviewed in Unger and Weaver, 2003). Interestingly, evidence also suggests that genetic mutations need not pre-exist in the target cells that give rise to the tumors for malignant transformation of a tissue to ensue. Instead, it is possible that tumors could arise through altered stromal-epithelial interactions because of pre-existing genetic mutations (familial) or acquired alterations in stromal cells. For example, Moinfar and colleagues found that distinct genetic alterations and loss of heterozygosity is present in a high proportion of DNA analyzed from excised stromal tissue

adjacent to primary breast tumors in patients (Moinfar et al., 2000). This finding is consistent with studies conducted several years ago by Schor and colleagues, who found that fibroblasts isolated from the healthy 'normal' relatives of patients with familial breast disease exhibited a tumor-like phenotype (Haggie et al., 1987). This perspective is also in accord with a recent report that Nf1 heterozygosity in resident stromal fibroblasts, mast cells and perineurial cells is probably essential for neurofibroma formation (Zhu et al., 2002). Such provocative observations therefore raise the possibility that genetic alterations present in stromal cells may contribute to or even drive malignant transformation of epithelial cells by perturbing the normal stromal-epithelial dialogue. As such, the 'microenvironmental' concept of tumorigenesis would benefit from being expanded to incorporate the possibility that genetic alterations in either the epithelial or the stromal cells could lead to altered stromal-epithelial interactions and thereby promote tumor formation (Fig. 1B).

It has been proposed that carcinogenesis results not from acquisition of key mutations in genes in epithelial or stromal cells, but rather is the consequence of a loss or breakdown of the biological organization of the tissue induced by perturbed stromal-epithelial interactions or an aberrant tissue microenvironment. The 'tissue organization field theory' asserts that the normal 'default' behavior of a cell is not quiescence but rather is proliferation, and that to sustain tissue homeostasis and promote differentiation this behavior must be restricted through cell-adhesion-dependent tissue organization. The concept further predicts that the molecules and pathways critical for maintaining tissue architecture, such as cell adhesion molecules, constitute tumor suppressors. Based upon this paradigm it follows that loss of tissue architecture or dysfunction of cell adhesion, which could be induced by perturbing stromal-epithelial interactions, will drive malignant behavior of cells within a tissue, even in the absence of primary genetic mutations. The theory further maintains that restoration of tissue organization should be able to repress the malignant phenotype of genetically aberrant cells (Sonnenschein and Soto, 2000). Support for the latter prediction comes from early experiments by Mintz and colleagues, who showed that tissue architecture can repress the malignant phenotype of undifferentiated embryonal carcinoma (EC) cells, and by Dolberg and Bissell, who reported that Rous sarcoma virus does not induce sarcomas in differentiated tissues derived from an avian embryo (reviewed in Kenny and Bissell, 2003). Additional evidence is provided by studies employing immortalized malignant MECs and Ras-transformed keratinocytes which demonstrated that reformation of a cell-adhesion-dependent 'differentiated' tissue structure was sufficient to repress expression of the malignant phenotype of transformed cells both in culture and in vivo, despite the presence of multiple genetic alterations (reviewed in Unger and Weaver, 2003). However, it should be noted that although these observations arguably support the tissue organization field theory they do not refute a role for genetic mutations in tumorigenesis.

To test the validity of the 'tissue organization field theory', a number of years ago Werb and colleagues engineered the luminal mammary epithelium of mice to overexpress stromelysin-1 (a metalloproteinase that has been shown to degrade ECM protein), which predictably disrupted normal stromal-epithelial interactions and perturbed tissue organization and differentiation. The experiments clearly showed that a desmoplastic stroma can drive malignant transformation of an epithelium; however, because the tumor latency in the studies was long the possibility that the mice developed tumors by acquiring the genetic 'hits' deemed necessary for malignant transformation could not be excluded. Indeed comparative genomic hybridization (CGH) analysis of tumor DNA from these mice revealed the presence of multiple genetic abnormalities, which suggest that either loss of tissue organization promotes genetic instability, or alternatively permits expression of pre-existing or acquired genetic abnormalities (reviewed in Kenny and Bissell, 2003).

To test the tissue organization field theory directly, Maffini and co-workers have now used an acute chemical carcinogen treatment to rapidly induce tumor formation, and a mammary gland epithelial reconstitution approach to distinguish between the contribution of stromal-epithelial interactions and genetic mutations to malignancy (Maffini et al., 2004) (see pp. 1495-1502 in this issue). To explore the role of genomic alterations in tumorigenesis the investigators monitored both the stroma and epithelial tissue for evidence of oncogenic Ras mutations. Using the tumor susceptible strain of Wistar-Furth rats and the well characterized carcinogen *N*-nitroso-methyl urea (NMU; which is a direct carcinogen that does not require metabolic conversion for DNA adduct formation and has a very short half-life) they surgically cleared the epithelium from the mammary fat pads of test rats. After recovering from surgery the animals were divided into four treatment groups, including two groups that received a single NMU treatment to their mammary fat pad stroma, and two other groups that received vehicle only. Mammary gland reconstitution was then performed on all four treatment groups using MECs from primary cultures of explanted tissue of older mature littermates that had been acutely treated either with vehicle or NMU. The animals were thereafter monitored for development of a normal mammary ductal tree or tumors (Fig. 1C). Control animals included one group of 'negative', vehicle-treated animals and another group of 'positive', NMU-treated, non-surgically manipulated animals (Fig. 1Ce,f). Interestingly, all of the animals that received NMU treatment developed tumors, regardless of whether or not the mammary epithelium used to reconstitute their mammary fat pad was treated with carcinogen. Even more intriguing was the investigators observation that none of the animals that received MECs treated with NMU in culture developed neoplastic lesions, unless their stromal fat pad had also received a prior bolus of NMU. Because NMU is a direct carcinogen such data suggest that the

stroma might itself constitute an important mutagenic target (Fig. 1B). Such an observation would accord with an expanded tissue microenvironment view of malignancy.

Provocatively, however, in the studies reported by Maffini and colleagues (Maffini et al., 2004) no correlation could be established between NMU-induced Ras mutations in either the mammary epithelium or stroma and tumor formation. Although it is possible that NMU treatment of the stroma promoted malignant behavior of the tissue by inducing novel, as-yet-unidentified mutations in the DNA of the stromal cells, it is also reasonable to suggest that chemical treatment of the stroma per se induced the 'malignant behavior' of the tissue. With regards to the former possibility, exploitation of a genetic screening approach such as CGH array analysis should help to identify additional candidate genetic changes. Addressing the latter question, however, will necessitate determining just what type of stromal change is induced by the chemical treatment, exploring whether other chemical mutagens act similarly on the stroma, and most importantly delineating just how such chemical modification of the stroma might operate to incite malignant behavior of a tissue.

The studies reported by Maffini and colleagues provide tantalizing evidence in support of the 'tissue organization field theory' of malignancy (Maffini et al., 2004). However, these studies also raise several important questions not easily resolved, not the least of which is what constitutes malignancy? From a morphological perspective a malignant lesion is defined by subjective macroscopic and microscopic criteria that include histological evidence of a loss of normal tissue architecture, cell proliferation, invasion of the epithelium into the interstitial stroma, the presence of well-defined nuclear changes in the cells (such as anaplasia, large and multiple nucleoli, and chromatin asymmetry), as well as evidence of tumor metastasis. Thus from a strictly gross morphological perspective the neoplastic lesions obtained in the studies reported by Maffini and colleagues do appear to qualify as bona fide tumors (Maffini et al., 2004). However, further analyses are needed to clarify just how malignant these lesions really are, including an assessment of their nuclear morphology and an assay of their metastatic potential. Moreover, given that loss of tissue architecture could predispose cells to genomic instability (Sternlicht et al., 1999), it will be important to characterize these tumors genetically, and to determine whether or not they can be phenotypically reverted by transplantation into a normal mammary gland stromal microenvironment. In this regard, all tissues are chronically exposed to environmental mutagens (radiation, chemical) and therefore will probably harbor some genetically mutant but 'dormant' cells eventually. Indeed, the very definition of genetically 'normal' tissue is fast becoming dubious at best. Therefore, it is possible and probable that the cells comprising the malignant lesions detected by Maffini and colleagues are genetically abnormal (Maffini et al., 2004). The question then becomes what contribution would these mutations make to the malignant behavior of their tissue and how important is the desmoplastic stroma to their phenotype? In fact, defining malignancy in strictly non-genetic terms may be difficult if not impossible, and from a practical perspective the tissue organization field theory of carcinogenesis might just converge or collide with the somatic mutation theory. Regardless, based upon the ever expanding body of evidence supporting the importance of stromal-epithelial interactions and cell adhesion in tumor pathogenesis, it seems advisable if not imperative to study tumorigenesis as a disease that occurs within the context of a dynamic microenvironment that is regulated by the spatial organization of the tissue.

References

Bissell, M. J. and Radisky, D. (2001). Putting tumours in context. *Nat. Rev. Cancer* **1**, 46-54.

Deng, G., Lu, Y., Zlotnikov, G., Thor, A. D. and Smith, H. S. (1996). Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* **274**, 2057-2059.

Haggie, J. A., Sellwood, R. A., Howell, A., Birch, J. M. and Schor, S. L. (1987). Fibroblasts from relatives of patients with hereditary breast cancer show fetal-like behaviour in vitro. *Lancet* **1**, 1455-1457.

Holst, C. R., Nuovo, G. J., Esteller, M., Chew, K., Baylin, S. B., Herman, J. G. and Tlsty, T. D. (2003). Methylation of p16(INK4a) promoters occurs in vivo in histologically normal human mammary epithelia. *Cancer Res.* **63**, 1596-1601.

Kenny, P. A. and Bissell, M. J. (2003). Tumor reversion: correction of malignant behavior by microenvironmental cues. *Int. J. Cancer* **107**, 688-695.

Kinzler, K. W. and Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. *Cell* **87**, 159-170.

Maffini, M. V., Soto, A. M., Calabro, J. M., Ucci, A. A. and Sonnenschein, C. (2004) The stroma as a crucial target in rat mammary gland carcinogenesis. *J. Cell Sci.* **117**, 1495-1502.

Moinfar, F., Man, Y. G., Arnould, L., Bratthauer, G. L., Ratschek, M. and Tavassoli, F. A. (2000). Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res.* **60**, 2562-2566.

Sonnenschein, C. and Soto, A. M. (2000). Somatic mutation theory of carcinogenesis: why it should be dropped and replaced. *Mol. Carcinog.* **29**, 205-211.

Sternlicht, M. D., Lochter, A., Sympson, C. J., Huey, B., Rougier, J. P., Gray, J. W., Pinkel, D., Bissell, M. J. and Werb, Z. (1999). The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell* **98**, 137-146.

Unger, M. and Weaver, V. M. (2003). The tissue microenvironment as an epigenetic tumor modifier. *Methods Mol. Biol.* **223**, 315-347.

Zhu, Y., Ghosh, P., Charnay, P., Burns, D. K. and Parada, L. F. (2002). Neurofibromas in NF1: Schwann cell origin and role of tumor environment. *Science* **296**, 920-922.