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REVIEW

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Cancer as a disease of epithelial–mesenchymal interactions and extracellular matrix regulation

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Abstract Carcinogenesis – the process of cancer formation – is commonly discussed in terms of genetic alterations that lead to deregulation of cell growth. Recently, there has been a resurgence of interest in epigenetic factors and, in particular, the role of the stromal microenvironment and angiogenesis in tumor formation. In this article, cancer is presented as a disease of the developmental processes that govern how cells organize into tissues and tissues into organs. This histogenetic perspective raises the possibility that epithelial–mesenchymal interactions and the extracellular matrix (basement membrane) that is deposited through these interactions may actively contribute to the carcinogenic process. Experimental work is reviewed that confirms that extracellular matrix plays a key role in normal histodifferentiation during both epitheliogenesis and angiogenesis, and that epigenetic deregulation of cell–matrix interactions may actively promote tumor initiation and progression. The contributions of integrins, cytoskeleton, tensegrity and local variations in extracellular matrix mechanics to these processes are discussed, as are the implications of this work for future studies on cancer formation.

Key words carcinogenesis · tumor formation · basement membrane · integrin · cytoskeleton · mechanics

Introduction

Cancer is commonly characterized as a disease that results from unrestricted cell proliferation. However, abnormal growth patterns can be observed in benign tumors, and certain normal tissues, such as bone marrow and intestine, exhibit higher cell turnover rates than seen in most cancers. The reality is that cancer is not just a disease of the cell. In addition to increased growth, classic hallmarks of malignancy include loss of normal tissue architecture, breakdown of tissue boundaries, stromal changes, angiogenesis, and compromise of distant organs through metastatic spread. Cancer therefore may be viewed to result from deregulation of the finely coordinated processes that normally govern how individual cells are integrated into tissues, tissues into organs, and organs into a functional living organism (Ingber and Jamieson, 1982). For this reason, we must go beyond current reductionist approaches that focus on analysis of the abnormal properties of individual tumor cells. Instead, we need to examine the process of carcinogenesis in context of normal tissue formation and developmental control. Only in this way will we gain full insight into how tissues undergo malignant transformation and hence, how this process may be controlled or even reversed.

In this article, I explore cancer as a disease of tissue development. First, older (perhaps even "lost") literature will be reviewed that suggest cancer may result from deregulation of the normal process of histodifferentiation by which multiple cells collectively generate functional tissue architecture. As the large majority of cancers are epithelial in origin, this perspective leads to a view of cancer as a breakdown of epithelial–mesenchymal interactions. In the embryo, active interactions between these neighboring tissues drive epitheliogenesis and determine the tissue's characteristic three-dimensional (3-D) form. Epithelial–stromal interactions also play a key role in control of angiogenesis in the surrounding stroma and thereby guide the formation of a functional vasculature that is required to feed the growing organ. As will be described, the extracellular matrix (ECM) that accumu-

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lates along the epithelial–mesenchymal interface as a result of these interactions is a critical control element in these developmental processes. More recent experimental results will be summarized that provide insight into how ECM acts locally to regulate individual cell responses to soluble growth factors and thereby control tissue patterning. A model of cancer formation will then be presented along with supporting experimental data that suggest deregulation of tissue growth and form during cancer formation may result from progressive breakdown of ECM-dependent developmental constraints.

Cancer as a disease of epithelial–mesenchymal interactions

Genesis of characteristic epithelial tissue form (e.g. acinar, tubular, branched, planar) is determined through complex interactions between the epithelium and its underlying mesenchyme in the embryo (Dodson and Hay, 1971; Banerjee et al., 1977). Studies in which embryonic epithelia and mesenchyme were isolated independently of different tissues and then recombined heterotypically revealed that the precise 3-D form that the tissue will express ("histodifferentiation") is determined by the source of the mesenchyme (Sakakura et al., 1976). In contrast, the epithelium governs what specialized products the cells will produce ("cytodifferentiation").

One of the key products of epithelial–mesenchymal interactions is the accumulation of a specialized ECM scaffold, called the "basement membrane" (BM) along the epithelial–mesenchymal interface (Grobstein, 1967). The BM functions as a extracellular complex of informative molecules that guides the differentiation, polarization, and growth of adjacent adherent cells, in addition to stabilizing the tissue's characteristic 3-D form (Grobstein, 1967; Dodson and Hay, 1971; Bernfield et al., 1972; Sakakura et al., 1976; Banerjee et al., 1977; Bernfield and Banerjee, 1978). Localized differentials in BM turnover also play a central role in tissue patterning. The epithelium physically stabilizes tissue morphology by producing BM; the mesenchyme actively induces histogenic changes in form by degrading BM at selective sites (Bernfield et al., 1972; Bernfield and Banerjee, 1978). The highest cell growth rates are observed in regions that exhibit the most rapid BM turnover, such as the tips of growing epithelial lobules during salivary morphogenesis (Bernfield and Banerjee, 1978) and in regions of capillary sprout formation during angiogenesis (Ausprunk and Folkman, 1977). At the same time, the mesenchyme slows matrix turnover and induces BM accumulation by depositing fibrillar collagen in slower growing regions of the same tissue (e.g. in clefts between growing lobules) (David and Bernfield, 1979). Branching morphogenesis in mammary gland also can be either stimulated or inhibited by increasing or decreasing ECM turnover, respectively, using modulators of stromal-derived matrix metalloproteinases (Simian et al., 2001). Thus, the stability of epithelial tissue form depends on the presence of an intact BM, whereas changes in tissue pattern are driven by spatial differentials in BM turnover. Most importantly, both activities are governed through active interactions between closely apposed epithelial and mesenchymal tissues.

Similar developmental controls are apparently sustained throughout adult life. Mature epithelial tissues retain the ability to undergo normal morphogenesis when mixed with embryonic mesenchyme (Sakakura et al., 1979; Cunha et al., 1983; Chung et al., 1984) and to switch histologic form when combined with different types of stromal tissue (e.g. epidermal grafts in different dermal sites; Tarin, 1972a). Studies of teratocarcinoma cells that exhibit multiple differentiated lineages suggest that tumors may mimic their tissue of origin in both appearance and mode of development (Pierce et al., 1978). Given that over 90% of tumors are carcinomas (epithelial in origin), then formation of most cancers may involve deregulated interactions between epithelial cells and underlying mesenchymally derived stromal tissue. In fact, examination of the microscopic anatomy of various cancers supports this hypothesis (Leighton, 1969). Examples include the finding that tumor architecture varies depending on the source of connective tissue (Foley et al., 1968), whereas production of stromal collagen by host fibroblasts depends on the epithelial tumor cell type (Gullino, 1966). The finding that an epithelial neoplasm must gain the ability to induce angiogenesis within its surrounding stroma to switch from hyperplasia to cancer (Folkman et al., 1989) and to progressively expand in size (Ingber et al., 1990; Folkman, 1996) is another clear example of how epithelial–stromal interactions are critical to cancer progression.

Epithelial–mesenchymal interactions also may contribute to tumor initiation. For instance, chemical carcinogenesis of epidermis requires the presence of closely apposed carcinogen-treated dermis (Orr and Spencer, 1972). Malignant transformation of embryonic mouse submandibular gland by polyoma virus also only occurs in intact or reconstituted glands; transformation cannot take place in isolated submandibular epithelium or mesenchyme, even though the resultant tumor is epithelial in origin (Dawe et al., 1966, 1971). Moreover, once transformed, these epithelial tumor cells can then substitute for mesenchyme in either normal epithelial morphogenesis or viral transformation of isolated embryonic epithelia (Dawe et al., 1968). Grafted human epithelial tumors also recruit normal murine stromal cells to become tumorigenic in nude mice (Goldenberg and Pavia, 1981). But most impressive is the finding that combination of various disorganized epithelial cancers with normal embryonic mesenchyme results in reversal of the malignant phenotype, as evidenced by restoration of normal epithelial organization and histodifferentiation (Ellison et al., 1969; Lakshmi and Sherbet, 1974;

DeCosse et al., 1973; Fujii et al., 1982; Cunha et al., 1991; Wong et al., 1992; Chung et al., 1990). Thus, continued epithelial–mesenchymal interactions appear to play an important role in the maintenance of normal tissue architecture in adults and deregulation of these interactions may contribute to both early and late stages of cancer formation.

ECM as a mediator of epithelial–mesenchymal interactions during tumor formation

The BM serves as an *in vivo* adhesive scaffold that ensures orderly tissue renewal and maintenance of normal tissue form in the adult (Vracko, 1974) as it does in the embryo (Grobstein, 1967; Dodson and Hay, 1971; Bernfield et al., 1972; Sakakura et al., 1976; Bernfield and Banerjee, 1978). Thus, changes in ECM metabolism could mediate the effects of deregulated epithelial–mesenchymal interactions during neoplastic transformation in mature tissues. In fact, local breakdown of the BM boundary is used as a histologic hallmark of malignant conversion. However, the more fundamental question is: do subtle changes in ECM structure that precede its complete breakdown actively contribute to cancer initiation and progression?

In support of this possibility, ultrastructural changes in the BM are commonly observed during early phases of cancer formation, prior to development of a palpable tumor (Tarin, 1972b; Li et al., 2001; Lu et al., 2000). Early stages of skin carcinogenesis are characterized by BM gaps, thickening and reduplication as well as loosening of basal cells from one another and from neighboring connective tissue (Tarin, 1972b), probably as a result of repeated stages of BM breakdown and attempted repair. Long-term treatment of prostate with steroids that leads to neoplastic transformation results in altered expression of ECM proteins and matrix metalloproteinases in the stroma of dysplastic lesions as well as newly formed tumors (Li et al., 2001). The epithelial BM of carcinogen-treated thyroid gland becomes discontinuous and is eventually completely lost during progression of lesions from prenodular to nodular forms and finally overt carcinomas (Lu et al., 2000). Studies of the invasive growth of normal epithelium (Vasilie, 1958) and endothelium (Ausprunk and Folkman, 1977) also show that local dissolution of BM and growth of underlying connective tissue occur *before* the onset of cell proliferation.

In later stages of cancer formation, a direct correlation exists between carcinoma cell invasion and local BM disruption, whereas cells in tumors that retain an intact BM do not penetrate into surrounding tissue (Ozzello, 1959; Luibel et al., 1960; Rubio and Biberfeld, 1979). This relationship may be confused at times, however. For example, BM discontinuities were rare in a virally induced murine mammary tumor that was spontaneously metastatic (Pitelka et al., 1980), but the smallest metastatic tumor in this study displayed multiple BM breaks. This observation emphasizes the dynamic nature of BM metabolism and epithelial architecture as well as the heterogeneity of tumor cell populations (Fidler, 1978; Pierce et al., 1978) within different microenvironments. In fact, it is likely that local environmental cues may cause intermittent changes in BM dissolution and resynthesis. For instance, while BM was absent from one primary squamous cell carcinoma, it was present surrounding metastatic tumor cells that grew at a distant site (Tarin, 1972b). The finding that the ability of a variety of tumors to enzymatically degrade BM collagen correlates closely with their metastatic potential (Liotta et al., 1980) and that tumor angiogenesis also involves local microenvironmental control of BM structure (Ausprunk and Folkman, 1977; Ingber, 1992) further emphasizes that dynamic changes in BM may play important roles at many points during the carcinogenic process.

Experimental confirmation of the active role that ECM plays in tumor formation

Many years ago we demonstrated the importance of BM for neoplastic disorganization of tissue architecture in studies using a rat pancreatic acinar cell carcinoma (Ingber et al., 1981, 1985, 1986b). The experiments revealed that these cancer cells failed to produce a complete BM within the tumor parenchyma and they grew in a disorganized array. Yet, the same cells spontaneously accumulated intact BM and reformed into a polarized epithelium wherever they contacted stromal tissue of the tumor vasculature and surrounding connective tissue capsule (Fig. 1). The cells were then mechanically isolated from the parenchyma of the pancreatic tumor and cultured *in vitro* on intact BM or collagenous stroma from human amnion. The exogenous acellular BM reversed the neoplastic disorganization process and restored normal epithelial organization *in vitro*, whereas tumor cells grew in a disorganized form on the amniotic stroma (Ingber et al., 1986b). Induction of pancreatic epithelial tumor cell reorganization by BM is also accompanied by restoration of normal cell proliferation rates within the adherent tumor cells (Watanabe et al., 1984). These findings led us to propose that BM normally functions as a spatial organizer of polarized epithelia and that progressive loss of BM may actively contribute to the neoplastic disorganization of epithelial cell–cell relations that is the hallmark of tumor formation (Ingber et al., 1981, 1985, 1986b).

These early experimental studies were provocative in that they suggested that changes in ECM may play an active role during early stages of tumor formation *prior* to the onset of malignant invasion. However, this epigenetic hypothesis was recently confirmed in a series of elegant studies from the laboratories of Bissell and Werb

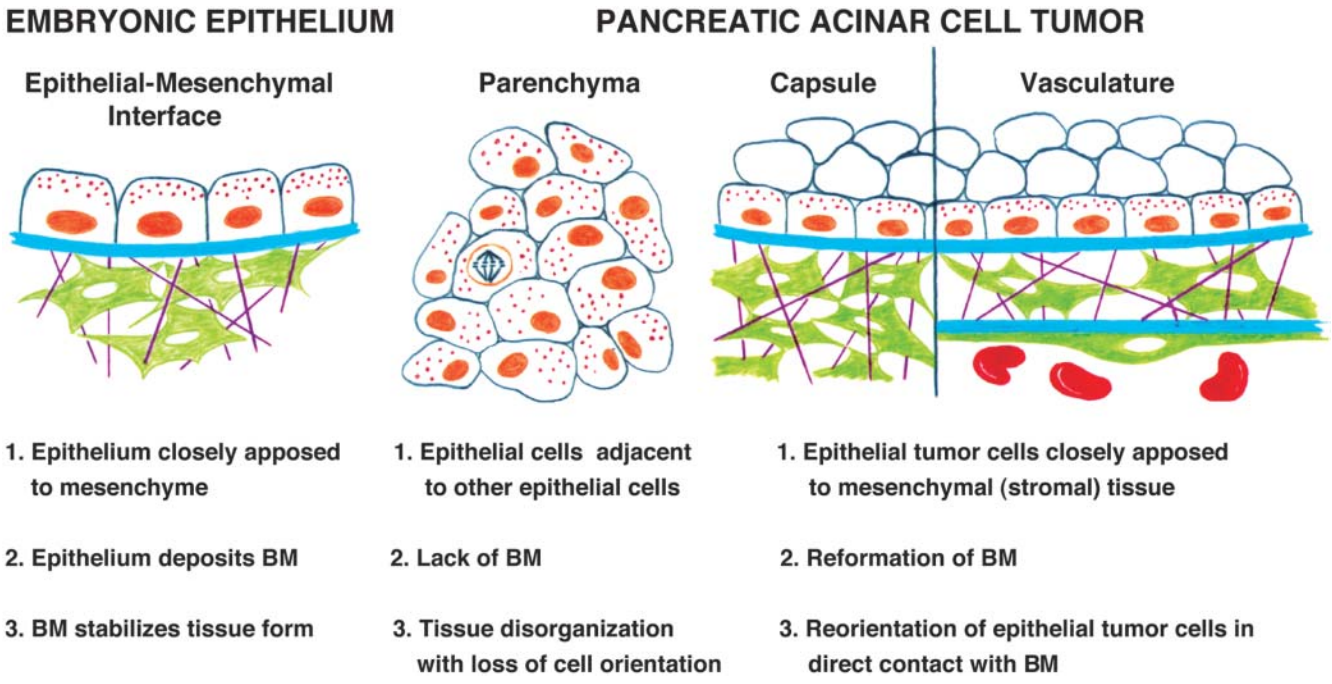

Fig. 1 Schematic summary of the epithelial organization process within embryonic tissue and in an adult, pancreatic acinar cell tumor. The *solid blue line* represents BM beneath the epithelium along its interface with underlying mesenchyme or stroma. See text for details.

that showed that deregulation of ECM metabolism can actively promote cancer development. Specifically, they demonstrated that targeting of autoactivating or tetracycline-inducible forms of the matrix metalloproteinase stromelysin-1 to the mammary epithelium leads to formation of a reactive stroma (Thomasset et al., 1998) and promotes both tumor initiation and malignant transformation in transgenic mice (Lochter et al., 1997; Sternlicht et al., 1999, 2000). Interestingly, stromelysin-1 is a natural product of the stromal cells and enhanced ECM degradation is again observed in early phases of this process prior to full expression of the invasive phenotype and BM disruption (Thomasset et al., 1998). Moreover, once transformed, mammary epithelial cells lose the ability to downregulate their production of stromelysin-1 in response to cell adhesion to BM (Lochter et al., 1999).

Analysis of transgenic mouse models of cancer also has revealed that early stages in cancer formation are accompanied by changes in the expression of cell surface ECM receptors, known as "integrins" (Tennenbaum et al., 1992). Moreover, cancer formation can be reversed *in vitro* and *in vivo* by changing integrin expression levels (Ruoslahti, 1996), modulating integrin binding (Weaver et al., 1997) or altering integrin signaling activities (McLean et al., 2001). Integrin antagonists also inhibit angiogenesis (Brooks et al., 1994) and tumor metastasis (Ruoslahti, 1996). Thus, ECM and its integrin receptors clearly play an active role in both carcinogenesis and tumor progression.

Architectural control of tissue development and tumor formation by ECM

The literature described above indicates that BM provides critical cues for growth and differentiation during embryogenesis, and its continued maintenance is mandatory for the normal functioning and growth regulation of adult tissues. But how could a macromolecular complex like the BM influence normal tissue morphogenesis or cancer formation? This is a question that has plagued the field of epithelial–stromal interactions for many years.

BM may influence cell behavior in many ways. For example, BM serves as the filtration barrier in the kidney glomerulus (Farquhar, 1978) and thus it may provide a similar ability to selectively control epithelial access to soluble molecules in other organs. Physical breakdown of this differentially permeable barrier, as is observed in regions of most rapid growth in embryonic tissues or during early tumor formation, could result in increased flow of chemical factors between the stromal and epithelial microcompartments. Certain cytokines, such as basic fibroblast growth factor, are stored within the basement membrane (Folkman et al., 1988) and thus increased BM turnover may also release growth factors locally. However, normal cells will not grow in response to growth factor stimulation when they are free of adhesion to ECM (Guadagno and Assoian, 1991) or when they are physically crowded (compressed) within a tight

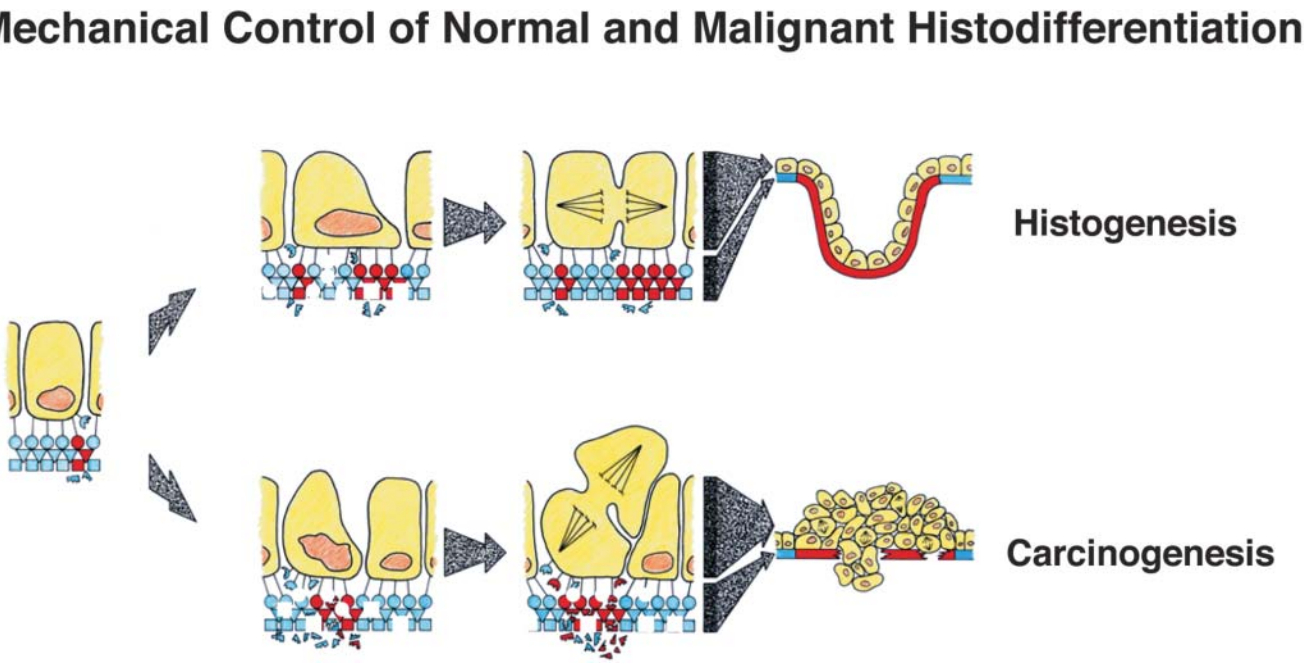

Fig. 2 Schematic diagram of a mechanical model of normal and malignant tissue differentiation based on BM remodeling and associated tension-driven restructuring of tissue form. *Circles*, *triangles*, and *squares* indicate different BM components; *broken figures*, molecules removed by degradation; *closed red figures*, newly added molecules. **Top** In normal histogenesis, increased BM turnover results in thinning of the BM and an associated increase in mechanical compliance (flexibility), which promotes cell distortion and growth locally. Because increased cell division is accompanied by net BM expansion, the tissue branches outward and initiates pattern formation. **Bottom** During carcinogenesis, an increase in BM turnover may lead to similar thinning of the BM, cell distortion, and associated growth. However, because net BM accumulation does *not* occur, piling up of cells and tissue disorganization result. If the growth stimulus (e.g. altered BM turnover) were to cease at this point, cells that lack contact with the BM would undergo apoptosis or stop growing and thus this hyperplastic process would be reversible. Continued stimulation of cell proliferation may eventually lead to selection of a subpopulation of cells that gain the ability to survive and grow free of adhesion to BM; at this point the process of tumor formation would become irreversible. If progressive deregulation of BM metabolism leads to physical compromise of the BM barrier, tumor cell invasion may occur and hence the tumor would become malignant.

epithelial monolayer (Stoker and Rubin, 1967; Folkman and Moscona, 1978). Thus, release of growth factors alone is not sufficient to explain local cell growth induction or why proliferating cells are observed in suprabasal regions of preneoplastic epithelium. Instead, these observations suggest that ECM-dependent growth regulation and normal cell "crowd controls" also must be deregulated in order for tumor formation to proceed (Schwartz and Ingber, 1994). To explain how this process may be involved in cancer formation, we must first understand how ECM acts to regulate cell growth and function during normal morphogenesis.

Normal histogenesis

Twenty years ago, we proposed that ECM may contribute to control of both morphogenesis and tumor formation based on its *mechanical* properties; that is, its ability to physically resist cell tractional forces (Ingber et al., 1981; Ingber and Jamieson, 1982, 1985). The concept was that the local variations in ECM remodeling that are observed during morphogenesis would change ECM structure and mechanics (Fig. 2, top). In this model, the local regions of BM at tips of growing epithelial glands and new capillary sprouts that become thinner due to high ECM turnover (Bernfield et al., 1972; Ausprunk and Folkman, 1977) would be expected to become more compliant. All soft tissues experience isometric tension or a tensile "pre-stress" based on the generation of tractional forces by their constituent cells. Because it is under tension, a weak spot in the BM would stretch out more than the neighboring tissue, just like a "run" in a woman's stocking. This alteration in ECM mechanics would change the balance of forces that are transferred across cell surface integrin receptors that physically connect the ECM to the internal cytoskeleton (CSK) within adjacent adherent cells. Increased tension on these adhesion receptors would, in turn, promote cell and CSK distortion in these distended regions and thereby alter cell sensitivity to soluble cytokines, resulting in the localized growth that drives tissue patterning (Ingber and Jamieson, 1982, 1985). If this increase in cell division were paralleled by a commensurate local increase in BM expansion (due to net BM accumulation), then coordinated budding and branching would result (Fig. 2, top).

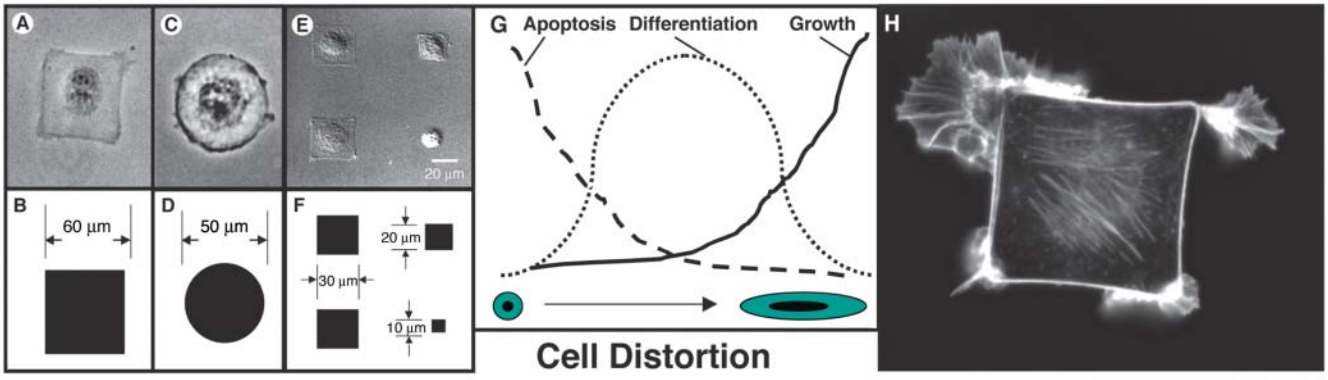

Fig. 3 Microscopic images of capillary endothelial cells (**A, C, E**) whose shape, size and position were controlled using the corresponding micropatterned adhesive substrate designs shown in (**B**), (**D**), and (**F**), respectively. Note that when cells are cultured on different-sized islands in the same dish that are coated with a constant fibronectin density and in the same growth factor-containing medium, cell distortion can be varied independently, as shown in (**E, F**). **G** A schematic summary of cell distortion-dependent switching between growth, differentiation, and apoptosis that has been demonstrated using micropatterned substrates of different size, as described in the text. **H** A fluorescent micrograph of a fibroblast cultured on a square, fibronectin-coated adhesive island (40×40 μm) and stimulated with the motility factor, PDGF, after staining for F-actin with rhodamine-phalloidin. Note that the cell preferentially extends actin-containing lamellipodia and filopodia from its corners.

Experimental results from many laboratories have confirmed that ECM exerts this form of mechanical control over epithelial and endothelial cell behavior both *in vitro* and *in vivo* (Folkman and Moscona, 1978; Ben Ze'ev et al., 1980, 1988; Ingber et al., 1986a, 1987; Li et al., 1987; Ingber and Folkman, 1988, 1989; Opas, 1989; Ingber, 1990; Mochitate et al., 1991; Mooney et al., 1992; Singhvi et al., 1994; Chen et al., 1997; Huang et al., 1998; Dike et al., 1999; Flusberg et al., 2001; Huang and Ingber, 2002; Parker et al., 2002). For example, in our studies, we used a microfabrication method to vary cell distortion independently of growth factor stimulation and ECM binding. This was accomplished by adapting a micropatterning technology that was developed by George Whitesides' Laboratory (Dept. of Chemistry and Chemical Biology, Harvard U.) as a method for fabricating microchips for the computer industry (Singhvi et al., 1994; Chen et al., 1997, 2000). This technique was then used to create adhesive islands of predefined size, shape and position on the micrometer scale coated with a saturating density of immobilized ECM molecules; the surrounding barrier regions were made nonadhesive by exposing polyethlylene glycol moieties on their surface that prevented protein adsorption (Fig. 3).

When primary liver epithelial cells were cultured on rectangular islands coated with laminin that ranged in diameter from 10 to 50 μm, they spread to the limits of the island and thus took on rectangular shapes (Sinhvi et al., 1994). Similar shape control was observed when capillary endothelial cells were plated on square or circular islands of similar size coated with fibronectin or other ECM ligands (Chen et al., 1997) (Fig. 3A–F). When both types of cells were cultured on different-sized adhesive islands coated with a high ECM density in chemically defined medium containing a saturating amount of growth factor, cell growth increased in parallel as island size was raised and cell spreading was promoted (Fig. 3G). We also confirmed that both small and large ECM islands induce similar levels of integrin signaling (Yan et al., 2000), and that when cells spread over multiple focal adhesion-sized islands (3 to 5 μm in diameter), growth scaled with cell spreading and not the total area of ECM contact formation (Chen et al., 1997). When the island area was decreased further and cell growth was shut off, apoptosis – the cellular "suicide" program – was switched on in capillary cells (Fig. 3G), even though the cells still remained adherent to the ECM substrate (Chen et al., 1997; Flusberg et al., 2001). Moreover, treatment of growing embryonic tissues with drugs that produce BM breakdown and associated cell retraction (rounding) resulted in inhibition of endothelial cell growth and induction of capillary regression (i.e. angiogenesis inhibition) *in vivo* (Ingber et al., 1986a; Ingber and Folkman, 1988). Similar treatments that result in complete dissolution of BM also induce regression of growing mammary epithelium (Wicha et al., 1980) and müllerian duct (Trelstad et al., 1982).

Interestingly, when cells were cultured on intermediate-sized ECM islands that supported a moderate degree of spreading and promoted neither growth nor apoptosis, a differentiation program was induced (Fig. 3G). Moderate-sized hepatocytes secrete higher levels of liver-specific blood proteins, including albumin and fibrinogen (Mooney et al., 1992; Singhvi et al., 1994), and endothelial cells form hollow capillary tubes (Ingber and Folkman, 1989; Dike et al., 1999). More recently, we also found that if a motility factor, such as PDGF or FGF, is added to a cell adherent to a square ECM island, the cell will extend out lamellipodia, filopodia, and

microspikes that drive cell migration. However, formation of these new cell processes is physically constrained in that they preferentially extend from the corners of the square cells, where cells form focal adhesions and exert their tractional forces on ECM (Fig. 3H) (Parker et al., 2002; Wang et al., 2002a). In other words, the geometry of the substrate and orientation in which the cell stretches dictates the direction of cell movement.

These results, taken together with findings from other experiments (Folkman and Moscona, 1978; Ben Ze'ev et al., 1980, 1988; Li et al., 1987; Opas, 1989; Mochitate et al., 1991; Dembo and Wang, 1999; Wang et al., 2002a), demonstrate that cell distortion *per se* governs whether individual cells will grow, differentiate or die (Fig. 3G) when stimulated by soluble mitogens as well as the direction in which they move (Fig. 3H). These data support our model in which mechanical changes in ECM compliance that affect cell shape and structure play a central role during normal histogenesis (Fig. 2, top). This tension-driven remodeling hypothesis for morphogenetic regulation (Ingber et al., 1981; Ingber and Jamieson, 1982, 1985; Huang and Ingber, 1999) is also consistent with the finding that variations in the pattern-directing behavior of different mesenchyme correlate with their ability to generate mechanical tension (Nogawa and Nakanishi, 1987). This is important because, as described above, the mesenchyme determines tissue-specific pattern formation in epithelium (Sakakura et al., 1976). Moreover, pharmacologic inhibition of tension generation inhibits morphogenesis in developing salivary gland (Ash et al., 1973) and lung (Moore et al., 2002), while increasing cytoskeletal tension through activation of the rho/ROCK pathway actually accelerates branching morphogenesis (Moore et al., 2002).

Reliance on local distortion for spatial control of cell growth may explain why expressing high stromal levels of growth factors in transgenic mice increases branching of mammary epithelium, rather than producing proliferation in all cells and amorphous tissue growth (Joseph et al., 1999). While soluble growth factors act to regulate overall tissue and organ size, their effect may be spatially restricted in a tissue-specific manner through this tension-molding mechanism to generate distinct histologic patterns. The key point here is that this spatial restriction may involve localized changes in ECM mechanics and cell structure as well as localized production of soluble growth modulators (Metzger and Krasnow, 1999).

Carcinogenesis

If cancer results from a breakdown of the rules that guide normal histogenesis, then loss of this form of tension-driven structural remodeling could contribute to neoplastic disorganization of tissue architecture (Ingber et al., 1981; Ingber and Jamieson, 1982, 1985; Huang and Ingber, 1999). As described above, the BM commonly remains physically intact during early stages of tumor formation (i.e. prior to malignant transformation and invasion), but a reduction in BM thickness or subtle decreases in the levels of certain ECM constituents often can be detected. In contrast to changes observed during normal tissue development, these neoplastic changes are not restricted in space or time and hence tissue disorganization results (Fig. 2, bottom). In fact, it is this loss of tissue pattern that usually catches the eye of the pathologist who recognizes it as abnormal (Clark, 1995). As in the embryo, continued changes in BM structure that lead to increased mechanical compliance (e.g. thinning) may promote cell distortion or increase CSK tension locally and thereby increase the sensitivity of adjacent cells to mitogenic stimuli. But in this case, a piling up of cells would result because the BM does not expand in parallel to match increases in cell number (Fig. 2, bottom), as occurs in normal development (Fig. 2, top).

If these changes are maintained over many years and the growth stimulus is sustained, cells that grow free of anchorage *in vivo* may spontaneously emerge just as continued culturing of normal cells may lead to spontaneous transformation *in vitro*. This transformation process would require that the cells gain the ability to grow independent of both ECM adhesion and cell distortion to fully overcome normal crowd controls. Natural selection and expansion of this autonomous cell would result in the "clonal" origin of proliferating tumor cells, yet the evolutionary process that led to creation of this cancer cell would have taken place at the tissue level. Cell growth and survival free of contact with the BM is then sufficient to explain the disorganization of normal cell–cell relations that is observed during subsequent stages of neoplastic transformation (Ingber et al., 1981, 1985).

In support of this concept, the ability of cells to form 3-D organotypic structures and to maintain normal cell shapes suppresses expression of the malignant phenotype, including production of stromelysin-1 (a promoter of tumor progression), as shown in studies involving culture of normal mammary epithelial cells and their transformed counterparts on BM gels (Peterson et al., 1992; Wang et al., 1998; Sternlicht et al., 1999, 2000). Some studies attribute the ability of BM gels (e.g. Matrigel) to modulate epithelial cell behavior to be due to the presence of specific ECM molecules (e.g. laminin) within these gels (Grant et al., 1989; Streuli et al., 1995). However, similar differentiation can be induced by culturing the cells on rigid 2-D substrates coated with various ECM molecules (e.g. laminin, fibronectin, types I and IV collagens) that restrain cell spreading to an intermediate degree similar to that observed on the flexible ECM gels (Ingber and Folkman, 1989; Mooney et al., 1992). In this latter case, it is the ability of the ECM to resist cell tension and promote cell distortion, and not the precise 3-D arrangement of the ECM ligand, that controls cell behavior. Moreover, this control can be exerted in the

complete absence of cell–cell contact formation (Mooney et al., 1992; Singhvi et al., 1994; Chen et al., 1997).

In fact, BM gels inhibit stromelysin-1 production and promote normal differentiation in normal mammary epithelium based on their mechanics: similar inhibition can be obtained by preventing cell spreading through other means or culturing cells in suspension (round) (Roskelley et al., 1994; Lochter et al., 1999). Mammary epithelial cells also progressively lose sensitivity to this shape-dependent control of metalloproteinase production as they become more highly transformed and malignant (Roskelley et al., 1994), just as many cell types progressively become more resistant to shape-dependent growth control (Wittelsberger et al., 1981; Tucker et al., 1981; Schwartz et al., 1990). Thus, the local structural and mechanical context of the cell may represent a critical epigenetic safeguard against neoplasia *in vivo*, in addition to guiding normal developmental patterning. Loss of this mechanical form of growth control at the level of tissue architecture may therefore represent a key step in the multistep process of cancer formation.

Mechanochemical transduction

This path of investigation leads us to yet another question: how can changes in ECM mechanics and cell distortion alter cell behavior? In essence, our hypothesis is that the architectural form of a tissue may itself regulate the shape, orientation, and growth of its cells through transmission of the physical forces of tension and compression characteristic for a given 3-D configuration (Ingber et al., 1981; Ingber and Jamieson, 1982, 1985; Huang and Ingber, 1999). This system may function in a manner analogous to the way in which other tissues, such as bone, cartilage, blood vessels, and skin, remodel themselves in response to physical stress (Ingber, 1997).

To link mechanics and biochemistry, this model also assumed that the epithelial tissue is organized and mechanically stabilized through use of "tensegrity" (tensional integrity) architecture (Ingber et al., 1981; Ingber and Jamieson, 1985; Ingber, 1993a, 1997, 1998). A tensegrity structure is comprised of a series of compression-resistant members that resist the inward pull exerted by a surrounding network of tensile elements and thereby create an internal pre-stress (isometric tension) that mechanically stabilizes the entire system. The human body with its compression-resistant bones resisting the pull of a continuous series of tensile muscle, tendons and ligaments is a simple example. In tissues, CSK microtubules, cross-linked bundles of microfilaments, and extracellular cross-linked collagen bundles are assumed to function as compression struts; the tension elements are represented by contractile microfilaments, intermediate filaments, individual epithelial and mesenchymal cells, elastin fibrils and the BM (Ingber and Jamieson, 1985; Ingber, 1993a; Chen and Ingber, 1999). Living cells organized within this type of tensegrity array would be expected to respond to physical alterations in their environment as an integrated unit due to distribution of forces across interconnected load-bearing elements and resulting stress-dependent restructuring of the lattice. Changes in the CSK may then produce changes in cellular biochemistry through modulation of any one of the many signaling molecules, enzymes, and biochemical intermediates that physically associate with these insoluble load-bearing scaffolds (Ingber and Jamieson, 1982; 1985; Bissell et al., 1982; Ingber, 1993b, 1997).

Recent experimental and computational modeling studies confirm that individual cells use tensegrity to stabilize their shape (Wang et al., 1993, 2001, 2002b; Stamenovic et al., 1996, 2002; Maniotis et al., 1997; Pourati et al., 1998; Coughlin and Stamenovic, 1998; Stamenovic and Coughlin, 1999, 2000; Wang and Stamenovic, 2000). The possibility that living tissues also use tensegrity remains to be demonstrated directly; however, whole tissues exhibit mechanical behaviors that are characteristic of both individual living cells and tensegrity structures (Wang et al., 1993). The important point here is that the tensegrity model explains why the importance of normal tissue architecture for cell regulation may not be based on the presentation of adhesive ligands in a precise 3-D orientation. Rather, tensegrity predicts that ECM gels may promote different cell behaviors based on their mechanics (gels are softer and more compliant than rigid dishes). As discussed above, many studies confirm this point: flexible ECM gels promote cell differentiation and suppress growth, whereas the same gels stimulate cell proliferation if they are chemically fixed with cross-linking reagents or immobilized on a plastic substrate and hence mechanically stiffened (Li et al., 1987; Opas, 1989; Mochitate et al., 1991).

The importance of the tensegrity model is that it suggested that cell surface receptors that physically link ECM to the CSK may function as mechanoreceptors and mediate mechanochemical transduction (Ingber and Jamieson, 1985; Ingber, 1991, 1997). For example, the model predicted that integrins would provide preferred paths for mechanical signal transfer across the cell surface, whereas other transmembrane receptors that do not link to the internal CSK should dissipate stress locally and thus fail to transmit the same signals. In fact, there is now a large body of literature that clearly shows that alterations in the level of mechanical stress transmitted across cell surface integrin receptors can directly alter signal transduction inside the cell in a specific manner (Ingber, 1991, 1997; Wang et al., 1993; Davies, 1995; Shyy and Chien, 1997; Chicurel et al., 1998a, 1998b; Meyer et al., 2000; Alenghat and Ingber, 2002). This appears to be mediated through restructuring of molecular linkages between integrins and the CSK in the focal adhesion (Wang et al., 1993; Chicurel et al., 1998b; Meyer et al., 2000; Alenghat and Ingber, 2002). In this manner,

local changes in ECM compliance may directly alter cellular biochemistry and thereby modulate cell responses to soluble cytokines and hormones during morphogenesis. Deregulation of ECM mechanics, integrin linkages to the CSK, integrin signaling, or any other element of this mechanochemical regulation scheme also could lead to abnormal tissue growth and remodeling, and hence contribute directly to tumor formation.

Mechanical control of normal and malignant tissue differentiation: an overview

The most important point of this discussion is that cancer represents more than uncontrolled cell growth; it is a disease of tissue structure that results from a breakdown of normal epithelial–mesenchymal interactions. The structural coordination, homogeneity of cell form, and intercellular communication required for successful tissue function are maintained by normally constant architectural relationships. In the tension-driven remodeling hypothesis for developmental control presented here, local thinning of the BM scaffold (which resists cell tractional forces and stabilizes tissue form) locally increases CSK tension within the adjacent epithelial cells. Due to the use of tensegrity for control of shape stability, this local change in the mechanical forces balanced across integrins would produce cell and CSK distortion that, in turn, would alter cellular biochemistry and increase cell growth. As long as accelerated cell division was matched by a commensurate increase in BM expansion (net BM accumulation), then orderly tissue expansion and morphogenetic remodeling would proceed (Fig. 2, top).

In certain situations in which epithelial–mesenchymal interactions become deregulated, accelerated BM remodeling may lead to a continued release of mechanical constraints and an associated increase in cell growth without commensurate BM extension (i.e. no net BM accumulation) (Fig. 2, bottom). Cancer formation would be prevented as along as cell viability and proliferative capacity remained dependent upon continued anchorage to ECM. Thus, this hyperplastic state would be reversible; if the stimulus ceased, cells no longer in contact with the BM would undergo apoptosis and normal tissue form would return. On the other hand, if the conditions that led to release of tensile constraints within the tissue were sustained over an extended period of time (years), then this continued stimulus for cell division may lead to selection of an anchorage-independent population that, by definition, could proliferate autonomously. As the tumor grew in size, autonomous epithelial cells would become separated from the stroma by large distances and so would become less susceptible to the normal regulatory influences of the mesenchymally derived connective tissue. Loss of normal cues from the deregulated epithelium also may alter stromal cell behavior and further compromise ECM regulation.

In this manner, a positive feedback system may develop that would move the tissue along a spectrum of progressive deregulation and eventually result in invasion of epithelial cells through the BM (i.e. malignant conversion) (Fig. 2, bottom). This may be manifested either through increased BM degradation relative to synthesis, or through acquisition of some new transformed cell product that in some way further compromises ECM-dependent developmental control. While progressive BM dissolution and altered CSK structure may be directly involved in early carcinogenesis in certain tumors, other cancers may enter this positive feedback loop at a later stage after gaining the ability to proliferate independently of anchorage by chemical, genetic or viral means (Wirth et al., 1992; Boyd et al., 1995; Wang et al., 1996; Jung et al., 2000). In this manner, gene mutations for growth signaling, adhesive (integrin, cadherin) signaling, and mechanical (cell shape) signaling may all need to occur for full malignant conversion of a benign neoplasm (Schwartz and Ingber, 1994).

It is important to note that the changes in the epithelial BM that are observed during neoplastic transformation of the epithelium are quite similar to those induced within the BM of nearby capillaries when they are induced to grow by epithelial tumor-derived angiogenic factors (Ausprunk and Folkman, 1977). Tumor angiogenesis is required for progressive growth and expansion of the tumor mass (Folkman et al., 1989; Folkman, 1976; Ingber et al., 1990). Thus, altered epithelial–stromal interactions that compromise ECM regulation in the local tissue microenvironment may actively contribute to all stages of cancer development, including early tumor initiation, the onset of malignant invasion, and the final switch to the angiogenic phenotype that represents the end of tumor dormancy.

Implications for the future

Where do we go from here? If structure is as important for cancer formation as individual oncogenes, suppressor genes and signaling molecules, as suggested by our group and others (Ingber et al., 1981; Ingber and Jamieson, 1982, 1985; Pienta et al., 1989; Huang and Ingber, 1999; Bissell et al., 1999), then how can we use this information to advance cancer therapy? First, by advancing our understanding of the role of tissue structure in the carcinogenic process, we may be able to better develop a scientific basis for approaches that are currently used in pathology laboratories to diagnose and stage cancer. These approaches commonly rely on histologic alterations in BM, cell shape, nuclear size, and chromatin organization. Yet, almost all accepted explanations of cancer formation focus exclusively on

changes in the expression of individual genes. Given that work on ECM-dependent control of cell shape and function provide a link between changes in ECM and resultant alterations in cell, CSK, nuclear, and chromatin structure, this seems like an exciting path for future investigation (Ingber et al., 1986a, 1987; Pienta et al., 1989; Pienta and Coffey, 1992; Sims et al., 1992; Maniotis et al., 1997; LeLievre et al., 1998; Hagios et al., 1998; Chicurel et al., 1998a, 1998b; Bissell et al., 1999; Wang and Stamenovic, 2000; Wang et al., 2001, 2002b).

Second, the microengineering methods we developed to analyze the structural basis of cell growth control by ECM (Chen et al., 2000) may prove valuable in future efforts focused on development of *in vitro* models of cancer development. We have used these methods to create "minimal" systems that retain all the information necessary to exert various forms of physiologic control *in vitro*. However, we also have demonstrated that these systems may be modified and combined with microfluidic systems and other microengineered substrates (Chiu et al., 2000; Takayama et al., 2001; Ostuni et al., 2001; Wang et al., 2002a) to create cellular microchips that permit analysis of cell–cell interactions, cell tractional forces, directional migration, and histodifferentiation. Thus, this microengineering approach may prove to be useful for analysis of the cellular and molecular basis of epithelial–stromal interactions in tumor development.

Third, the finding that changes in ECM remodeling, integrin signaling, and CSK structure appear to be critical for tumor formation and progression suggests that the molecules that contribute to these processes may represent potential targets for anticancer therapy. Tissue inhibitors of matrix metalloproteinases and integrin antagonists that interfere with cancer metastasis as well as angiogenesis have already entered human clinical trials. It is possible that more focused and effective therapies may be created by developing inhibitors of integrin-signaling molecules or cell type-specific CSK proteins that mediate ECM-dependent and mechanical stress-dependent developmental controls.

Finally, our work on mechanochemical control of cell behavior and tissue development by ECM raises an even more fundamental question. How can a stimulus as nonspecific as cell distortion produce identical cell fates (growth, differentiation, apoptosis) as specific growth factors and hormones that bind to distinct high affinity receptors? A related question is how can the same "critical" growth factor (e.g. FGF, PDGF, EGF), signaling molecule (e.g. Ras, MAPK, rho, etc.), or environmental stress (e.g. UV light, heat, carcinogens) produce entirely different functional outputs (growth or differentiation or death) in different microenvironments. The answer to these questions will require us to confront the ultimate challenge in cell biology: how complex behaviors *emerge* through collective interactions among thousands of different molecular elements. This is the beginning of "Complex Systems Biology" in which we strive to understand the behavior of the biological network as a whole, rather than focusing on the properties of the individual elements.

Our work on tensegrity represents a first step in this direction. We can now predict mechanical behaviors of living mammalian cells by viewing the CSK as a prestressed tensegrity network comprised of compressed microtubules and ECM adhesions interconnected by tensed microfilaments and intermediate filaments (Wang et al., 1993, 2001, 2002b; Maniotis et al., 1997). The properties of the whole cell cannot be predicted by analysis of any individual element; mechanical behaviors emerge from a combination of network architecture and pre-stress as well as the material properties of the individual components. Similarly, recent insights from Complex Systems Science suggest that cell phenotypes represent mathematical "attractors" (Huang and Ingber, 2000). This observation may provide a handle with which to approach the question of how these structural networks impact on information processing networks in normal cells and how cell behavior and mechanochemistry become deregulated through progressive loss of structure during cancer formation.

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