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Tissue architecture: the ultimate regulator of epithelial function?

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The architecture of a tissue is defined by the nature and the integrity of its cellular and extracellular compartments, and is based on proper adhesive cell–cell and cell–extracellular matrix interactions. Cadherins and integrins are major adhesion-mediators that assemble epithelial cells together laterally and attach them basally to a subepithelial basement membrane, respectively. Because cell adhesion complexes are linked to the cytoskeleton and to the cellular signalling pathways, they represent checkpoints for regulation of cell shape and gene expression and thus are instructive for cell behaviour and function. This organization allows a reciprocal flow of mechanical and biochemical information between the cell and its microenvironment, and necessitates that cells actively maintain a state of homeostasis within a given tissue context. The loss of the ability of tumour cells to establish correct adhesive interactions with their microenvironment results in disruption of tissue architecture with often fatal consequences for the host organism. This review discusses the role of cell adhesion in the maintenance of tissue structure and analyses how tissue structure regulates epithelial function.

Keywords: mammary gland; intestine; epithelium; basement membrane; cell adhesion

1. INTRODUCTION

Evolution might be viewed as a game of trial and error generating various forms able to adapt to, and survive within, a given environment through functional specialization. This is true for the development of a complex multicellular organism as a whole as well as for the generation of the various types of tissues of which higher organisms are built. Each tissue is composed of many different cell types that assemble into organized patterns and cooperate with each other through division of labour, an indispensable and successful move towards the establishment of a higher organism. A tissue's form is highly optimized to its physiological role. In skeletal muscle, for instance, myoblasts fuse into large syncytial myofibres that are arranged in compact bundles to allow synchronized transmission of mechanical forces over a long distance, a task not feasible for individual cells. The relationship between form and function is also obvious in absorptive and secretory organs such as lung, kidney, intestine and mammary gland, where the epithelium acquires specific morphologies that serve to increase the surface area for absorption of nutrients and secretion of fluids.

While the question of how complex tissues are established has always been a central question for developmental biologists, it is of equal importance to understand how tissue integrity is maintained and how function is modulated. Most tissues reach the endpoint of their development during embryogenesis. Some tissues, however, undergo major developmental changes in the adult organism and, thus, represent excellent model systems to study tissue morphogenesis and homeostasis at the same time. In this article, we will focus on two tissues typically remodelled during adulthood: the mammary gland and the intestine.

During puberty, the mammary gland epithelium branches into numerous ducts with terminal endbuds. This morphological state is basically maintained in the postpubertal virgin gland, but undergoes further pronounced modification during pregnancy. Expansive branching and formation of large alveoli during pregnancy prime the mammary gland for milk secretion. After parturition, the lactating mammary gland represents the fully developed and fully functional state. The microanatomy of the mammary gland as it progresses from the virgin to the pregnant and lactating state is a result of the coordinated action of local and systemic signals. During the post-weaning involution phase the alveoli collapse, lose their ability to produce milk and the gland regresses to its resting, virgin-like state. Irrespective of the developmental state of the gland, the mammary epithelium is a bilayered structure consisting of an inner continuous layer of luminal epithelial cells and an outer layer of myoepithelial cells (figure 1*a*). The epithelial bilayer is polarized and in close contact with a laminin-rich basement membrane (BM) at its basal surface. The epithelium is embedded in the mammary stroma which consists mainly of fibroblasts and adipocytes (for details, see Sakakura (1991) and Ronnov-Jessen *et al.* (1996)).

In contrast to the mammary gland, which differentiates only in response to pregnancy, the adult small intestine matures and recycles constantly during adult life. Its epithelium is folded into an alternating pattern of villi that extend into the gut lumen and crypts that are

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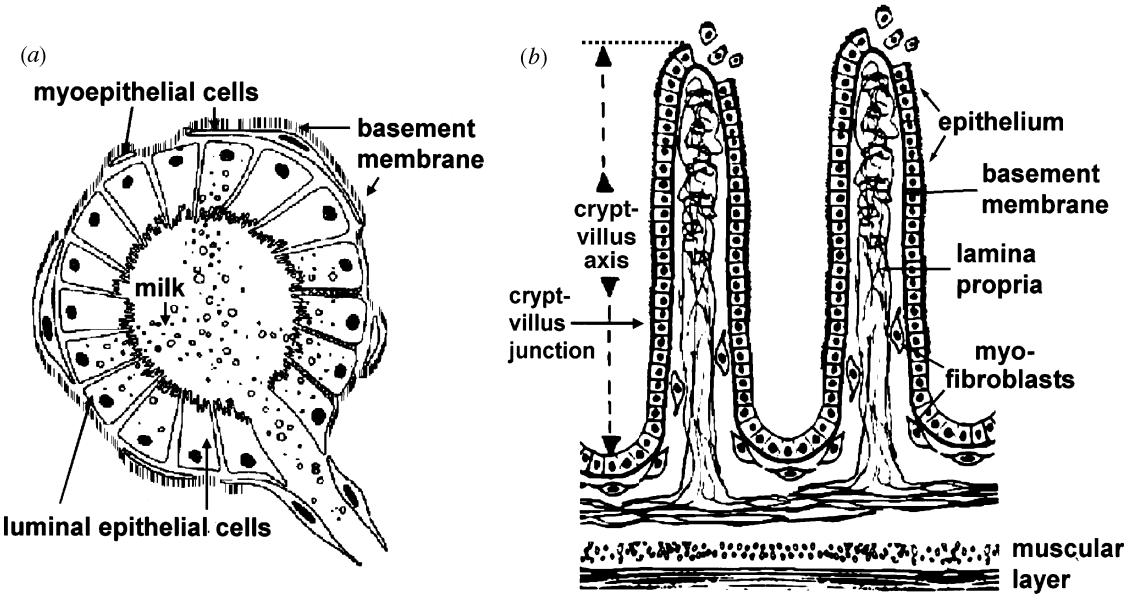

Figure 1. Schematic representation of a mammary gland alveolus and the intestinal wall. (*a*) Mammary gland alveoli are formed by luminal epithelial and myoepithelial cells that are surrounded by a BM. Milk proteins and fat are secreted into a central lumen and pumped through the mammary ductal system in response to suckling. Reproduced with minor modifications from Streuli (1993), with permission. (*b*) The epithelium of the small intestine is folded into an alternating pattern of crypts and villi. It is separated from the underlying stroma by a BM. The connective tissue is built of myofibroblasts and the lamina propria. Underneath the connective tissue, the muscular layer confers intestinal movements.

located at the bases of the villi (figure 1*b*). Throughout adult life, a proliferative compartment within the crypt epithelium is maintained from which epithelial cells continuously migrate to the villus tip. During this journey, the cells differentiate and mature into four epithelial cell types: enterocytes, endocrine cells, mucus-producing goblet cells and Paneth cells. Enterocytes, which comprise the majority of intestinal epithelial cells, are the actual absorptive cells and characterized by a chronologically defined expression of hydrolytic enzymes that function to digest absorbed nutrients. Functional differentiation in the small intestine is under hormonal control. When epithelial cells reach the villus tip, epithelial cells undergo apoptosis and are shed into the gut lumen. These events occur along the continuum of the subepithelial BM and are at least partly accompanied by co-migration of pericryptal fibroblasts within the mesenchyme. A mesenchyme-derived interstitial compartment, the lamina propria, demarcates the crypt–villus compartment from the underlying muscular layers and reaches into the tip of the villi (figure 1*b*) (for details, see Dauca *et al.* (1990) and Simon-Assmann & Kedinger (1993)).

The epithelium as a whole can only function correctly if its individual cells fulfil their defined roles. But how does an epithelial cell know what to do at any given time? In addition to genetic developmental programmes, a number of classical and modern studies show that the cellular microenvironment provides important information that is translated by the cell into specific skills. An essential part of the microenvironment of an epithelial cell is its neighbours. Epithelial cells are laterally interconnected through specialized adhesive elements such as adherens junctions, desmosomes and tight junctions (figure 2). Basally they are attached to a BM through hemidesmosomes and integrin- and non-integrin ECM receptors (figure 2) (Borradori & Sonnenberg 1996; Gumbiner 1996). We now know that extracellular matrix (ECM) in general, and BM in particular, constitute a critical part of the cellular microenvironment because they provide both a structural scaffold that defines cellular form and polarity as well as information encoding for specific biochemical signals. The cytoplasm and the nucleus of a cell are also organized into a structural scaffold, and the ECM, the cytoskeleton and the nuclear matrix are physically interconnected to provide an architectural framework for outside-in and inside-out signalling pathways, a scheme that was proposed in the model of dynamic reciprocity sixteen years ago (Bissell *et al.* 1982). It has also been argued that a cell and its surrounding ECM represent a structural and functional unit (Bissell & Barcellos-Hoff 1987). We now would like to propose that the final form of a tissue can determine cellular function and, furthermore, that the phenotype of a cell is dominant over its genotype (see Weaver *et al.* 1997).

This review will describe molecules involved in the maintenance of tissue structure and will discuss how they impinge upon cell function. Furthermore, we will illustrate the consequences of loss of appropriate structure for cell behaviour using cancer of the breast and the intestine as examples.

2. THE DEVELOPMENT AND MAINTENANCE OF TISSUE STRUCTURE

Epithelial cells depend on the formation of stable but nevertheless dynamic adhesive interactions with their neighbouring cells and their surrounding ECM to develop and maintain appropriate epithelial tissue architecture. Growth and differentiation of the mammary

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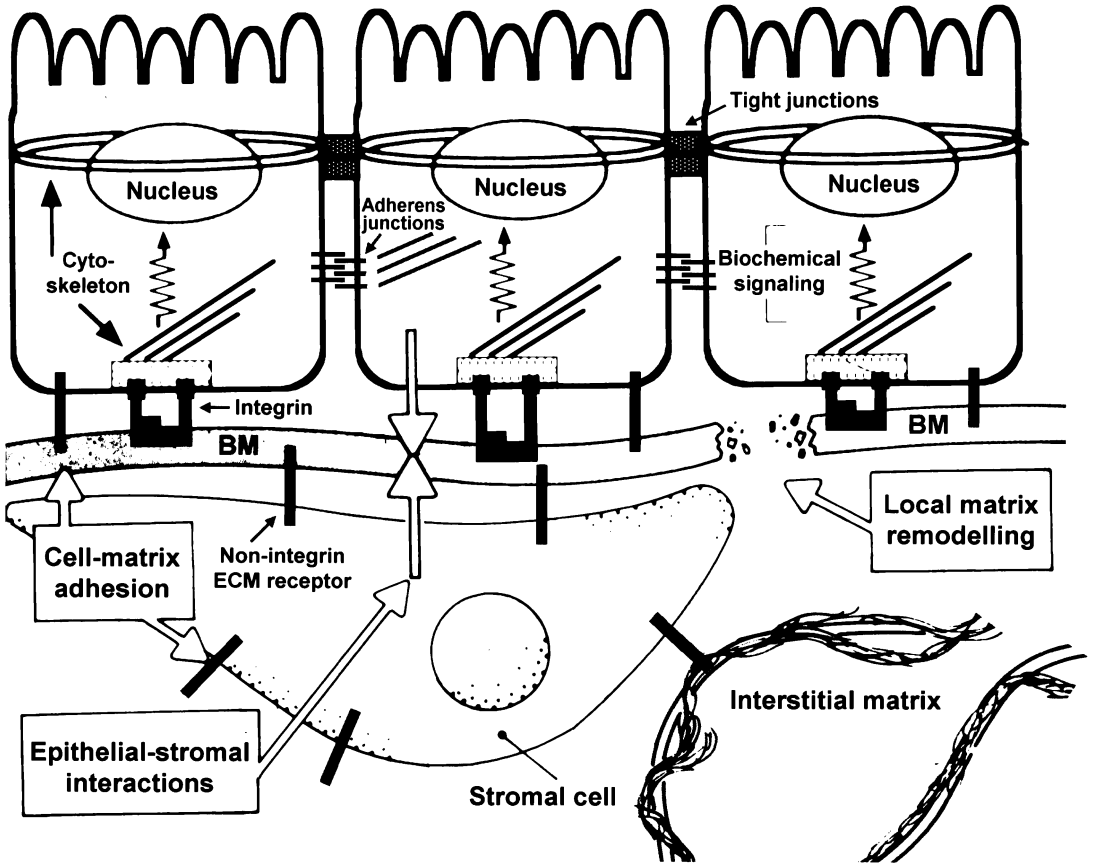

Figure 2. The structural and functional continuum of epithelial tissues. Epithelial cells are anchored to the BM by integrin and non-integrin ECM receptors. Mechanical and biochemical signals from the ECM and from the stromal cells, as well as cell–cell adhesion complexes, influence epithelial function. Local ECM remodelling by MMPs and other enzymes can promote or upset tissue homeostasis. Reproduced with minor modifications from Stoker *et al.* (1990), with permission.

epithelium and migration-associated differentiation within the crypt–villus unit are accompanied by modulation of cell–cell and cell–ECM interactions.

(a) *Cell–cell interactions*

Direct cell–cell contacts are established through homophilic and heterophilic cell adhesion molecules (Gumbiner 1996). Homophilic, calcium-dependent cadherins, such as E-, P- and N-cadherin, are found in epithelial adherens junctions that confer strong intercellular attachment (for details, see Ranscht (1994)). They interact directly with cytoplasmic β-catenin or γ-catenin, which in turn recruit α-catenin that binds to actin, thereby interconnecting the actin cytoskeleton of neighbouring epithelial cells (Kemler 1993). Besides its structural role as part of the E-cadherin/catenin complex, β-catenin is involved in transcriptional regulation. It shuttles to the nucleus where it forms a complex with Tcf/Lef transcription factors (Huber *et al.* 1996; Kuhl & Wedlich 1997). These Tcf/Lef-β-catenin complexes were shown to bind to the E-cadherin promoter *in vitro* indicating that they may regulate E-cadherin expression (Behrens *et al.* 1996; Huber *et al.* 1996). The tumour-suppressor protein adenomatous polyposis coli (APC) competes for the binding of β-catenin to Tcf/Lef and thus may indirectly modulate the function of E-cadherin (Alman *et al.* 1997; Barth *et al.* 1997). The role of cadherins in maintaining tissue structure has been analysed to some extent in the mammary gland and in the intestine.

In the mammary gland, luminal epithelial cells express E-cadherin but are devoid of P-cadherin, whereas myoepithelial cells express P-cadherin but not E-cadherin (Rasbridge *et al.* 1993; Daniel *et al.* 1995; Glukhova *et al.* 1995; Palacios *et al.* 1995). Both molecules may therefore contribute to segregation and maintenance of the two epithelial subcompartments. Indeed, application of function-perturbing antibodies to E-cadherin to mammary glands *in vivo* caused disruption of luminal epithelial sheets and accumulation of single cells within the ductal lumen. Treatment of glands with antibodies against P-cadherin exclusively disrupted the organization of myoepithelial cells without affecting the luminal layer (Daniel *et al.* 1995).

E-cadherin is also the predominant cadherin in the epithelium of the small intestine. It is expressed first as the cells reach the crypt–villus junction, and continues to be present as they migrate to the villus tip. E-cadherin expression increases at sites of cell–cell contact near the apical extrusion zone of the villus where dying cells are shed into the intestinal lumen (Hermiston & Gordon 1995*a*). It has been suggested that increased E-cadherin prominence at this site maintains the physical and chemical permeation barrier, and thus tissue integrity, at the apical surface of the villus (Madara 1990). The function of cadherins in the

intestine was addressed in transgenic mice where either functional E-cadherin, or N-cadherin lacking the extracellular domain, was targeted to the crypt–villus unit (Hermiston & Gordon 1995*a*; Hermiston *et al.* 1996). Expression of the N-cadherin mutant, shown to display a dominant-negative effect on heterologous cadherins in other systems (Kintner 1992; Fujimori & Takeichi 1993; Dufour *et al.* 1994; Holt *et al.* 1994), leads to a reduction of endogenous E-cadherin levels in the intestine. Whereas expression of the truncated N-cadherin increases the rate of enterocyte-migration to the villus tip, overexpression of E-cadherin reduces the rate of cell migration. Enterocytes overexpressing E-cadherin did not express terminal differentiation markers, showing that differentiation depends on a correct balance of cell–cell adhesion molecules (Hermiston *et al.* 1996). Furthermore, both dominant-negative N-cadherin and functional E-cadherin overexpression led to precocious apoptosis of enterocytes: in the villus, in the case of dominant-negative N-cadherin, and in the crypt, in the case of E-cadherin overexpression. This clearly indicates that enterocytes respond to these manipulations according to their position within the crypt–villus axis, and that they differ in their requirements to adhere to their neighbouring cells for their survival. The two approaches show that cadherins play important roles in cellular migration, differentiation and survival in the adult intestine, and are required to maintain correct tissue structure and function.

Cell–cell adhesion through tight junctions involves homophilic binding of the transmembrane protein occludin and recruitment of cytoplasmic proteins zonula occludens-1 (ZO-1) and zonula occludens-2 (ZO-2). ZO-1 is the key protein mediating linkage of tight junctions to the actin cytoskeleton. Tight junctions provide a barrier against lateral diffusion of solutes through the epithelium. Hence, their appropriate permeability state is important for correct epithelial function (reviewed in Ballard *et al.* (1995)). The presence of glucose in the small intestine increases tight-junction permeability, which facilitates paracellular absorption.

Glucocorticoids were found to induce tight-junction formation and to decrease paracellular permeability in mouse mammary epithelial cells in culture (Zettl *et al.* 1992), suggesting that the regulation of tight-junction permeability prevents loss of milk into the interstitium and may be an important event in the glucocorticoid hormone-aided differentiation of the mammary gland during pregnancy. Interestingly, ZO-1 and ZO-2 share significant sequence homology with the Disks large (DLG) tumour suppressor and the *lin-2* gene, which are involved in both the organization of cell junctions and regulation of growth control in invertebrates (Jesaitis & Goodenough 1994; Kim 1995; Woods *et al.* 1996; Dimitratos *et al.* 1997). This may indicate that the state of permeability of tight junctions could participate also in cellular growth control in higher organisms (Kim 1995).

(b) *Cell–ECM interactions*

Virtually all epithelia interact with a BM that is composed mainly of laminin, type IV collagen, entactin/nidogen and heparan sulphate proteoglycans. This specialized type of ECM is an important regulator of cell polarity, differentiation, growth, apoptosis and gene expression (for a review, see Adams & Watt (1993)). The mechanisms by which ECM exerts such versatile functions have been partly deciphered by culturing cells in the presence of appropriate microenvironmental cues (Hahn *et al.* 1990; Streuli *et al.* 1991; Boudreau *et al.* 1995, 1996).

A reconstituted BM isolated from the Engelbreth–Holm–Swarm tumour (EHS) induces recapitulation of the alveolar phenotype of mammary epithelial cells in culture and results in resumption of functional differentiation as monitored by the expression of milk components (Li *et al.* 1987; Barcellos-Hoff *et al.* 1989). Similarly, induction of an endogenous BM in mammary epithelial cells plated on floating type I collagen gels is sufficient to bring about functional differentiation as exemplified by the expression of milk proteins (Streuli & Bissell 1990). The milk proteins lactoferrin, β-casein and whey acidic protein (WAP) can be used as markers for the differentiated state of mammary epithelial cells in culture. Studies on expression of these proteins have dissected the effects of mechanical versus biochemical signals on the differentiation state of mammary epithelial cells (Roskelley *et al.* 1994, 1995; Roskelley & Bissell 1995.). On plastic, in the absence of an exogenously added BM, mammary epithelial cells form a flat monolayer and do not produce milk proteins even in the presence of lactogenic hormones. Cell rounding, hence alteration in cytoskeletal organization, is sufficient to induce lactoferrin expression, but the presence of BM is required in addition for β-casein production (Roskelley & Bissell 1995). Low levels of β-casein are expressed in mammary epithelial cells embedded as single cells in EHS matrix, indicating that cell–cell interactions are not necessary for induction of β-casein *per se* (Streuli *et al.* 1991). However, β-casein expression is readily enhanced upon cell aggregation indicating that cell–cell contact formation and the establishment of epithelial polarity positively modulate β-casein expression (Roskelley *et al.* 1994). WAP production absolutely depends on the formation of alveolar structures in EHS matrix (Chen & Bissell 1989; Lin *et al.* 1995). These findings demonstrate that functional specifications in the mammary gland are achieved by hierarchical signalling cascades involving hormonal and growth factor control, cell-shape changes and cell–cell and cell–ECM interactions (figure 3) (Roskelley *et al.* 1995).

The major BM component, laminin, was identified as the critical player in this signalling cascade because it is sufficient to induce β-casein production in mammary epithelial cells in the presence of lactogenic hormones (Streuli & Bissell 1990; Streuli *et al.* 1991, 1995; Roskelley *et al.* 1995). Laminin, but not other ECM proteins, was found to induce β-casein synthesis and to drive β-casein transcription through BCE-1, an ECM-responsive enhancer (figure 3) (Schmidhauser *et al.* 1992; Streuli *et al.* 1995). Within this 160 base-pair enhancer, site-specific binding of the transcription factors C/EBPβ and Stat-5 are essential for the laminin- and lactogenic hormone-dependent transcriptional activation of the β-casein gene (Schmidhauser *et al.* 1994; Myers *et al.* 1998). Furthermore, the requirement of stable integration of BCE-1 into the genome, and the fact that butyrate and trichostatin, which interfere with histone-deacetylation, can circumvent the laminin-dependence of BCE-1 activity, suggests a role for nuclear localization and chromatin/histone

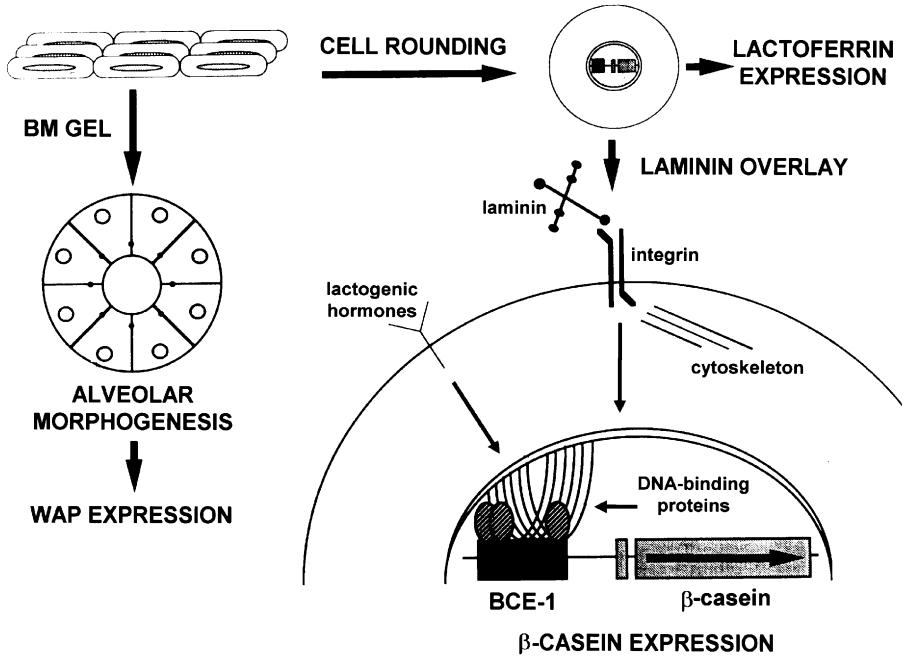

Figure 3. A hierarchy of ECM-dependent signals regulate milk protein expression. The first level of hierarchy is mediated by mechanical changes accompanying cell rounding. Cell rounding is sufficient to induce lactoferrin expression, which cannot be detected in flat cells. The second level of hierarchy is mediated by biochemical signals elicited by binding of laminin to integrin receptors. Signalling through laminin leads to β-casein transcription mediated by an ECM-responsive enhancer, BCE-1. The third level of hierarchy requires formation of alveolus-like structures and leads to induction of whey acidic protein (WAP) expression. Reproduced with modifications from Roskelley & Bissell (1995), with permission.

conformation (Schmidhauser *et al.* 1994; Myers *et al.* 1998). This leads to the hypothesis that the regulation of higher-order chromatin structure by ECM in general, and laminin in particular, may also be important for tissue-specific gene expression *in vivo*.

Changes in composition and organization of the BM, as well as ECM receptors, accompany tissue morphogenesis and development. For example, different laminins are present in the BMs of different tissues and in each tissue, expression of laminins is developmentally regulated (for details, see Wewer & Engvall (1994), Ekblom & Timpl (1996) and Ryan *et al.* (1996)). Different laminins are created by heterotrimeric combination of α, β and γ subunits. Several genes code for each α, β and γ chain, creating different structural isoforms that potentially differ in their binding abilities to integrin receptors (Burgeson *et al.* 1994; Mercurio 1995). The BM underlying the mammary epithelium contains mainly laminin-1 ($\alpha_1\beta_1\gamma_1$), but also laminin-5 ($\alpha_3\beta_3\gamma_2$). Both laminins are co-localized with the $\alpha_6\beta_4$ integrin in hemidesmosomal structures in mammary epithelial cell lines (Sonnenberg *et al.* 1993; Utani *et al.* 1995; Stahl *et al.* 1997), and may thus play a role in cell–BM anchorage. However, laminin-1 and laminin-5 do not function redundantly. The mammary epithelial cell line RAC-11P/SD spreads on laminin-5 but not on laminin-1, and only in the presence of an intact actin cytoskeleton. Furthermore, antibodies to the α_6-integrin subunit interfere only with adhesion of the cells to laminin-1 but not to laminin-5, indicating that a second receptor must be involved in the laminin-5-dependent adhesion and spreading (Sonnenberg *et al.* 1993). Integrin $\alpha_3\beta_1$ was proposed as a candidate receptor in this case, and has further been shown to be involved in laminin-5-mediated branching of mammary epithelial cells on EHS (Stahl *et al.* 1997). Interestingly, antibodies to either laminin-5 or to integrin-α_6 or integrin-α_3 subunits interfered with the assembly of hemidesmosomes (Stahl *et al.* 1997), raising the possibility that $\alpha_3\beta_1$ associates, at least transiently, with the hemidesmosomal complex, or is necessary for hemidesmosome establishment. While β_1 integrins are linked to the actin cytoskeleton, the $\alpha_6\beta_4$ integrin, as an essential component of the hemidesmosome, represents a link between the keratin cytoskeleton and ECM (for details, see Borradori & Sonnenberg (1996)). This suggests that hemidesmosomes can influence cell shape and behaviour by linking both cytokeratin and actin filaments to the ECM. In the mammary gland, $\alpha_3\beta_1$ integrin is present at the basolateral surface of both luminal epithelial and myoepithelial cells, whereas $\alpha_6\beta_4$ and $\alpha_1\beta_1$ are restricted to myoepithelial cells (Berdichevsky *et al.* 1994; Alford & Taylor-Papadimitriou 1996). Predominant or exclusive expression of integrin $\alpha_6\beta_4$ in myoepithelial cells may be in accordance with the fact that luminal cells are deprived of continuous BM contact because of the myoepithelial cell layer. This renders the myoepithelial cells the primary perceivers of positional and instructive input that is provided by the BM and is required for maintenance of mammary gland integrity. It is, therefore, questionable that luminal epithelial cells *in vivo* form hemidesmosomal structures, although mammary epithelial cells in culture do (Kemperman *et al.* 1994; Uematsu *et al.* 1994; Borradori & Sonnenberg 1996). We propose that epithelial cells in culture assume partial myoepithelial characteristics in order to maintain cellular polarity and, thus, function.

In the subepithelial BM of the intestine, the three laminins, laminin-1, laminin-2 ($\alpha_2\beta_1\gamma_1$) and laminin-5, are present in a temporally and spatially defined pattern along the crypt–villus axis (reviewed in Perreault *et al.* (1995), Simon-Assmann *et al.* (1995) and Leivo *et al.* (1996)). In the mature human intestine, laminin-1 expression gradually decreases from the villus tip to the crypt–villus interface, laminin-2 is restricted to the basis of the crypt and laminin-5 exhibits the inverse pattern of laminin-1 (Beaulieu & Vachon 1994; Simon-Assmann *et al.* 1994, 1995). The laminin-specific integrins, $\alpha_6\beta_1$ and $\alpha_6\beta_4$, both of which are receptors for laminin-1, laminin-2 and laminin-5, are present throughout the crypt–villus epithelium at cell–BM

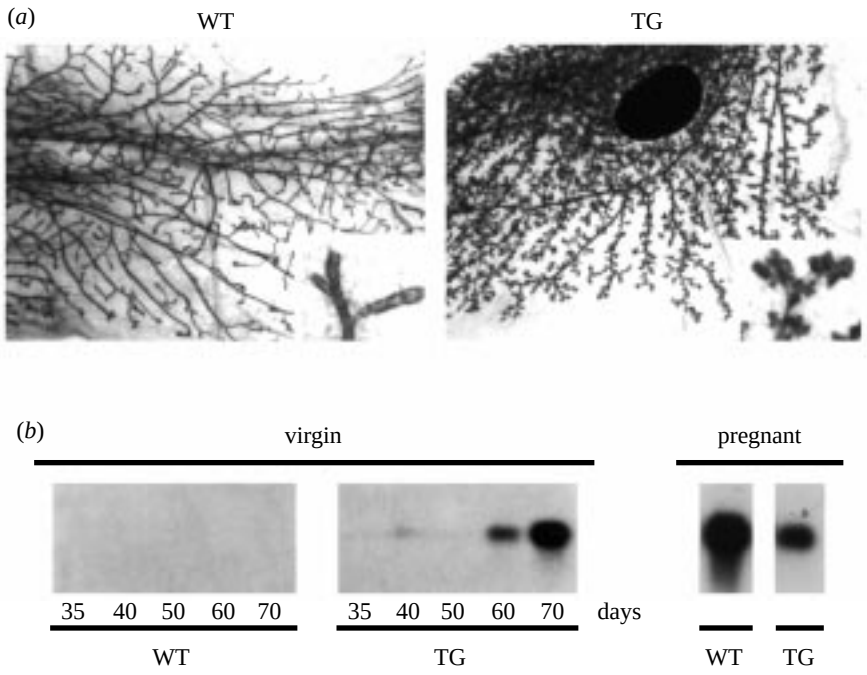

Figure 4. Consequences of SL-1 overexpression in mammary epithelium of SL-1 transgenic mice. (*a*) Mammary glands from virgin wild-type (WT) mice display normal branching morphogenesis, whereas mammary glands from virgin transgenic (TG) mice show an increase in lateral branching and alveolar differentiation. Inset shows terminal structures of mammary ducts and alveoli in wild-type and transgenic mice, respectively. (*b*) Northern blot analysis of β-casein expression in mammary glands from virgin and pregnant wild-type and transgenic mice. Note that β-casein is induced in glands from virgin transgenic mice compared with glands from virgin wild-type mice, beginning at 60 days of age. In contrast, β-casein expression is repressed during pregnancy in mammary glands from transgenic mice. Reproduced with modifications from Sympson *et al.* (1994), with permission.

contacts (Koretz *et al.* 1991; Simon-Assmann *et al.* 1994). In contrast, the α_3-integrin subunit, which also binds to all three laminins, was excluded from the basis of the crypt, and the α_2-integrin subunit, which binds to laminin-1 but not to laminin-2, was predominantly expressed by the crypt epithelium (Perreault *et al.* 1995). This expression pattern of laminins and integrins suggests that each laminin has distinct effects on intestinal epithelial cell behaviour and functions by triggering different cellular responses through interaction with different cell surface receptors. Furthermore, integrins containing α_2 and α_3 not only bind to laminin but also to other BM components including type IV collagen and fibronectin (Hynes 1992). Depending on functional state or affinity of particular integrins to certain laminins, distinct signals that are crucial for enterocyte differentiation may be created. In classical hemidesmosomes, as they are found in proliferating, but not in terminally differentiated epidermal cells, laminin-5 bridges the anchoring type VII collagen fibrils to the integrin $\alpha_6\beta_4$ (Jones & Watt 1993; Rousselle *et al.* 1997). Lack of clear co-distribution of laminin-5 and type VII collagen, and the presence of hemidesmosome-like structures in terminally differentiating cells of the intestine, suggest that these structures do not confer rigid adhesion, and indicate that the mechanisms of cell anchorage to ECM is distinct in different tissues (Simon-Assmann *et al.* 1995; Leivo *et al.* 1996). However, establishment of laminin-5/integrin $\alpha_6\beta_4$ anchorage in both keratinocytes and intestinal cells correlates with specific dephosphorylation of an 80 kDa membrane-associated protein (Xia *et al.* 1996), suggesting that epithelial cells of different origin are able to elicit similar responses to hemidesmosomal occupancy.

(c) *Modulation of epithelial organization by the stroma*

The epithelium and its surrounding mesenchyme cooperate to bring about the functional specifications of a tissue. During branching morphogenesis of the mammary epithelium, mesenchymal cells secrete matrix-metalloproteinases (MMPs) that degrade the subepithelial BM and interstitial ECM. The action of MMPs is controlled by tissue inhibitors of MMPs (TIMPs). A delicate balance of MMPs and TIMPs is required for tissue architecture and function (Boudreau *et al.* 1995, 1996; Alexander *et al.* 1996; Werb *et al.* 1996). In transgenic mice, where the ratio of MMPs to TIMPs was altered by overexpression of the MMP stromelysin-1 (SL-1) in the mammary epithelium, increased branching and precocious alveoli formation was observed in virgin animals (figure 4*a*) (Sympson *et al.* 1994). Remarkably, β-casein expression is detectable in glands of virgin transgenic mice despite the absence of a pregnancy-specific hormonal environment (figure 4*b*). In contrast to this gain-of-function phenotype, strong expression of the SL-1 transgene during pregnancy results in inhibition of alveolar differentiation and milk protein production (figure 4*b*). This is consistent with high levels of MMP expression during mammary gland involution, where proteolytic degradation of BM induces apoptosis within the epithelium and leads to regression to a virgin-like state (Talhouk *et al.* 1992; Lund *et al.* 1996; Werb *et al.* 1996).

Epithelium and stroma can cooperate in the assembly of a BM by contributing different ECM molecules or subunits. Although Keely *et al.* (1995) found no expression of ECM molecules in the mammary epithelium *in vivo*, observations by Reichmann *et al.* (1989), and in our own laboratory, show that mammary epithelial cells in culture deposit BM components such as type IV collagen and laminin (Streuli & Bissell 1990; Petersen *et al.* 1992; Weaver *et al.* 1997). However, on a flat tissue culture substratum, mammary epithelial cells cannot correctly assemble these molecules into a BM. In contrast, culturing mammary epithelial cells together with mesenchymal cells, or contact of epithelial cells with EHS or floating collagen gels, leads to deposition of an endogenous, immunohistochemically defined BM, indicating that polarity and micoenvironmental cues are required for appropriate BM architecture (Streuli & Bissell 1990; Petersen *et al.* 1992; Reichmann *et al.* 1989).

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Because both mesenchymal and epithelial cells of the intestine produce different BM components (Pujuguet *et al.* 1994; Orian-Rousseau *et al.* 1996), both cell types are needed for deposition of a subepithelial BM (Simon-Assmann *et al.* 1995). Co-culture of epithelial and mesenchymal cells from the intestine not only leads to BM assembly but also to a high degree of intestinal epithelial differentiation as evidenced by the development of an apical brush border membrane and expression of digestive enzymes as differentiation markers (Kedinger *et al.* 1988; Hahn *et al.* 1990; Simon-Assmann & Kedinger 1993). In contrast, an exogenously added BM alone results in only partial differentiation, indicating that the fully functional epithelial phenotype requires additional factors derived from the mesenchyme (Simon-Assmann *et al.* 1995; Sanderson *et al.* 1996). Furthermore, the hormonal influence on the functional differentiation of intestinal epithelium is mediated by the mesenchyme (Kedinger *et al.* 1983). It is obvious that imitating the presence of a BM by culturing epithelial cells on EHS matrix is not necessarily sufficient to reconstitute the function of all epithelial cells, but that higher-order tissue structures, such as epithelial–mesenchymal interactions, are undoubtedly unique for each tissue and have to be considered in order to understand tissue function.

(d) *Mechanical forces and the establishment of tissue integrity*

ECM molecules, by binding to integrins, contribute to organization of both the cytoskeleton and the nuclear skeleton (nuclear matrix) (Jones *et al.* 1993; Lelievre *et al.* 1996). This structural set-up allows dynamic and reciprocal communication between the three compartments and affects ECM-directed gene expression. Because cell–cell and cell–ECM adhesion systems are intimately linked to intracellular signal transduction pathways, it is difficult to distinguish between cell-shape changes occurring in direct response to cell adhesion *per se* and those occurring indirectly through second messenger and protein kinase pathways. In HeLa cells, expression of the 'foreign', Schwann cell-specific adhesion molecule protein zero (P_0) initiates a cascade of adhesion events, which include recruitment of cadherins and desmosomal proteins to sites of cell–cell contact and reorganization of the cytoskeleton. This finally results in the conversion of a spindle-shaped morphology to a polygonal, epithelial phenotype (Doyle *et al.* 1995). The fact that P_0 mediates homophilic cell adhesion without having obvious signalling properties (Doyle & Colman 1993; Doyle *et al.* 1995; Fannon *et al.* 1995) may indicate that a cell can perceive adhesion as a physical event *per se*. This 'awareness' of the adhesive state subsequently triggers a cascade of biochemical responses that lead to reorganization of the cytoskeleton and establishment of cellular adhesion devices and thus to the adjustment of the cell's behaviour according to its microenvironment. Data have now accumulated which show that cells are indeed able to perceive physical forces such as tension, compression or shear stress and to convert them into biological responses which in turn determine cellular form and behaviour (Resnick & Gimbrone 1995; for a review, see Ingber (1997)). Such a mechanical signal could potentially play a role in the initiation of mammary gland involution. When milk was allowed to accumulate in alveoli by sealing one gland of a lactating mouse, the alveoli of this gland subsequently collapsed and apoptosis was initiated while the non-sealed glands were functional (Marti *et al.* 1997). This suggests that physical deformation of milk-burdened alveoli could trigger the apoptotic response, despite the presence of lactogenic hormones. This finding is not surprising in the light of our own results where we illustrated that sealing of a lactating gland in mice leads to changes in expression of MMPs and their inhibitors (Talhouk *et al.* 1992); these are known to be involved in the regulation of apoptosis in mammary epithelial cells (Boudreau *et al.* 1996), presumably by regulation of BM integrity.

A plausible mechanism by which living cells perceive mechanical forces is presented in the working model of cellular 'tensegrity' (Ingber 1993). According to this model, an internal active tension ('prestress') is maintained analogous to the basal tonus in muscle cells. Cell–cell and cell–ECM adhesion molecules act as mechanoreceptors and mechanotransducers mediating immediate biochemical responses to physical stress (Ingber 1997). In the model of 'tensegrity', similar to the model of 'dynamic reciprocity' (Bissell *et al.* 1982; in addition, see Bissell & Barcellos-Hoff (1987)), nuclear matrix, cytoplasmic filaments, ECM and the connections between neighbouring cells represent a structural continuum that stabilizes cell shape. The identification of 'stress-response elements' in the promoter region of genes that encode growth factors (Resnick *et al.* 1993; Resnick & Gimbrone 1995) and ECM molecules (Chiquet-Ehrismann *et al.* 1994; Chiquet *et al.* 1996) proves the impact of mechanical forces on the regulation of gene expression.

3. DISRUPTION OF TISSUE STRUCTURE IN CANCER

Normal cell behaviour only results if the information a cell is receiving is temporally and spatially correct, and if the cells are able to appropriately sense, process and respond to it. Normal cell–cell and cell–ECM interactions are impaired in many pathological conditions, including cancer, and result in a loss of normal tissue morphology *in vivo*. In the course of the development of invasive carcinomas, which are tumours of epithelial origin, cells proliferate in an uncontrolled fashion, break away from the primary tumour, invade the underlying stroma and the endothelium of blood vessels and extravasate to a distant site to establish metastasis. Besides reduced intercellular contacts that facilitate the initial detachment, tumour cells exhibit various alterations in integrin expression that enable them to interact with the different ECM components they encounter during their migration (for details, see Mareel *et al.* (1993), Stetler-Stevenson *et al.* (1993), Howlett *et al.* (1995) and Alford & Taylor-Papadimitriou (1996)).

(a) *Disruption of cell–cell interactions*

The expression of E-cadherin and catenins is decreased in a number of tumours, including breast and colorectal carcinomas (Gamallo *et al.* 1993; Sommers *et al.* 1994; Palacios *et al.* 1995). This results in weakening of cell–cell contacts and promotes the migration of cells away from the primary tumour site, thus facilitating invasion and metastasis. Loss of E-cadherin is a marker for poorly differentiated and aggressive carcinomas, and correlates

with the invasive phenotype of carcinoma cells *in vivo* and in culture (Behrens *et al.* 1989; Mareel *et al.* 1990; Frixen *et al.* 1991; Takeichi 1993). Thus, E-cadherin has been proposed to function as a tumour-suppressor protein (Behrens *et al.* 1989; Vleminckx *et al.* 1991). Accordingly, E-cadherin expression is downregulated by oncogenes such as *fos*, *ras* and *src* (Vleminckx *et al.* 1991; Matsuyoshi *et al.* 1992; Reichmann *et al.* 1992; Behrens *et al.* 1993; Thompson *et al.* 1994). Rescue of E-cadherin expression in dedifferentiated carcinoma cells renders them non-invasive (Frixen *et al.* 1991). Conversely, blocking of E-cadherin-mediated adhesion in non-invasive carcinoma cells by anti-E-cadherin antibodies induced invasion (Behrens *et al.* 1989; Mareel *et al.* 1990). Furthermore, overexpression of mucins or large proteoglycans, which are highly charged glycoproteins on the cell surface, can compromise E-cadherin-mediated cell–cell adhesion presumably through steric hindrance (Ligtenberg *et al.* 1992; Kemperman *et al.* 1994; Vleminckx *et al.* 1994; Wesseling *et al.* 1996). In cases where carcinoma cells were found to be invasive despite abundant cell-surface E-cadherin, a defect in post-translational modification of E-cadherin or in catenin expression was noted (Sommers *et al.* 1994).

Loss of cell–cell junctions can also be a result of disturbed cell–ECM interactions (for reviews, see Hilkens *et al.* (1995) and Sommers (1996)). When epithelial cells, which are normally in contact with a BM, are placed on a substratum of stromal type I collagen they downregulate E-cadherin, they internalize desmosomes and acquire motile and invasive properties (Boyer *et al.* 1993; Hay & Zuk 1995). Likewise, when the ECM receptor syndecan-1 is depleted from normal mammary epithelial cells, E-cadherin expression decreases and cells adapt a fusiform morphology (Kato *et al.* 1995). This suggests that cell adhesion through syndecan-1 and E-cadherin may be coordinated events in the maintenance of normal mammary epithelial morphology.

E-cadherin may also be subject to removal from the cell surface through proteolytic cleavage by proteinases released from the stroma (Damsky *et al.* 1983; Kato *et al.* 1995). We have found that inducibly expressed SL-1 in a normal mammary epithelial cell line triggered cleavage of cell-surface E-cadherin and loss of E-cadherin/catenin complexes from cell–cell contacts (Lochter *et al.* 1997*b*). The cells gradually replace cytokeratin intermediate filaments by vimentin, change from an originally polygonal to a fibroblastoid morphology, and readily migrate through EHS matrix. The SL-1-dependent reduction in β-catenin levels coincides with the time it takes the cells to become invasive. Thus, the stepwise, SL-1-mediated transition of functionally normal, non-transformed mammary epithelial cells to an invasive phenotype appears to be a result of destruction of tissue structure by disruption of cell–cell adhesion. This is in accordance with the finding that invasion through EHS is dependent on SL-1 expression in mouse mammary tumour cell lines (Lochter *et al.* 1997*a*). Remarkably, targeted expression of an SL-1 transgene in the mouse mammary gland *in vivo* results in a high incidence of breast tumours, suggesting that SL-1 mediates transformation and tumorigenesis through similar mechanisms *in vivo* and in culture (Sympson *et al.* 1995; Lochter *et al.* 1997*b*). Therefore, SL-1 plays a dual role in that it influences morphogenesis on the one hand, and acts as a multipotent, dominant proto-oncogene on the other hand.

Nevertheless, examples accumulate where carcinoma cells with E-cadherin and catenin deficiencies are non-invasive, indicating that loss of E-cadherin-mediated adhesion alone is not sufficient to establish an invasive phenotype (Sommers 1996). In highly invasive breast tumours, N-cadherin was shown to replace E-cadherin at cell–cell contacts and appears to mediate carcinoma cell interaction with mammary stromal cells (Hazan *et al.* 1997). This suggests that N-cadherin is involved in the promotion of breast cancer metastasis by facilitating carcinoma cell migration through the mammary stroma as well as in reestablishment of homophilic cell–cell adhesion in metastasis.

In many hereditary and sporadic forms of colorectal cancer, signalling through cadherin/catenin complexes is also perturbed (Inomata *et al.* 1996; Kinzler & Vogelstein 1996; Gould & Dove 1997). Here, mutations in the tumour-suppressor protein APC prevent binding of APC to β-catenin (Inomata *et al.* 1996; Gumbiner 1997). This could in turn lead to predominant complex formation of β-catenin with Tcf/Lef-transcription factors (see above), and increased signalling through this complex may be a potential mechanism in intestinal tumorigenesis (Korinek *et al.* 1997; Rubinfeld *et al.* 1997). Furthermore, overexpression of functional APC in the intestinal epithelium leads to a marked decrease in cell adhesion and to disorganized cell migration along the crypt–villus axis, possibly through competing with E-cadherin for β-catenin-binding (Wong *et al.* 1996). Perturbing signalling through cadherin/catenin complexes by either expression of a dominant-negative N-cadherin in the intestine (Hermiston & Gordon 1995*b*) or of truncated β-catenin in cultured epithelial cells (Barth *et al.* 1997) leads to hyperplastic and neoplastic lesions and affects epithelial cell morphology, respectively. The dynamic control of β-catenin distribution through binding-competition by APC, Tcf/Lef and cadherins therefore appears to be essential in the regulation of cell adhesion, migration and tumorigenesis.

(b) *Disruption of cell–ECM interactions*

Malignant cells interact differently with their microenvironment as compared with their normal or benign counterparts (for details, see Mareel *et al.* (1993), Stetler-Stevenson *et al.* (1993) and Lochter & Bissell (1995)). Accordingly, tumour cells exhibit various alterations in expression profiles of cell surface receptors, including integrins (Zutter *et al.* 1990, 1993; Weaver *et al.* 1997). In breast and colorectal carcinomas, altered integrin expression, loss of integrin subunits from the cell surface, disorganized integrin distribution and a shift in integrin subunit-ratios have been reported (Zutter *et al.* 1990, 1993; Koukoulis *et al.* 1991; Natali *et al.* 1992; Berdichevsky *et al.* 1994; Fujita *et al.* 1995; Howlett *et al.* 1995; Weaver *et al.* 1997). In particular, deregulated expression of the laminin-specific, integrin receptors $\alpha_6\beta_1$ and $\alpha_6\beta_4$ have been implicated in breast cancer progression, and appear to play a key role in tumour cell invasion (Cress *et al.* 1995; Friedrichs *et al.* 1995; Shaw *et al.* 1996). The direct correlation found between high levels of α_6-integrin subunits and

the increased death rate of breast cancer patients may thus involve both, $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins (Friedrichs *et al.* 1995). That integrins are indeed involved in tumour progression *in vivo* has been shown by using function-blocking antibodies against β_1-containing integrins, which significantly interfere with metastasis formation in a mouse model (Fujita *et al.* 1992).

To study cell–ECM interactions in cultured cells, appropriate culture models that closely recapitulate the physiological microenvironment of cells *in vivo* are indispensable. A three-dimensional culture assay using EHS matrix has proven a powerful tool to analyse the structural and functional specifications of mammary epithelial cells (Barcellos-Hoff *et al.* 1989), and to discriminate between normal and malignant cell behaviour (Petersen *et al.* 1992; for a review, see Weaver *et al.* 1995).

We have studied the human mammary epithelial cell line HMT-3522 that originated from a biopsy of a non-malignant breast lesion (Briand *et al.* 1987). Continued passage of HMT-3522 cells gave rise to a unique tumour progression model representing the functionally normal phenotype (denoted S1) and various stages of premalignancy (S2) and malignancy (T4) (Nielsen & Briand 1989; Briand *et al.* 1996; Weaver *et al.* 1997). The 'normal' S1 and the tumorigenic T4 cell lines represent the start and end points, respectively, of this tumour progression series (Weaver *et al.* 1995, 1996). Upon plating inside EHS matrix, the S1 cells form well-organized and growth-arrested acini after several days in culture, whereas the T4 cells grow as large, disorganized colonies that do not growth arrest (Weaver *et al.* 1997). Correct cell–cell and cell–ECM interactions are compromised in T4 cells: besides the loss of functional E-cadherin/catenin complexes from the lateral cell surface and the random distribution of integrins, there is a shift in the ratio of β_1- to β_4-integrin expression at the cell surface (Weaver *et al.* 1997). Most strikingly, treatment of the disorganized T4 cell colonies with different inhibitory β_1-antibodies independently restores a normal acinar architecture in EHS matrix with correct rearrangement of E-cadherin/catenin complexes. Thus, T4 cells retain, and are able to correctly use, their molecular equipment to appropriately respond to a laminin-rich microenvironment when the normal balance of integrins is restored. In contrast to malignant T4 cells, the reverted T4 cells (referred to as T4-β_1) are markedly less tumorigenic in nude mice despite genetic defects such as chromosome trisomy, c-myc amplifications and a p53 mutation (Madsen *et al.* 1992; Moyret *et al.* 1994; Nielsen *et al.* 1994; Briand *et al.* 1996). Interestingly, the tumour cell phenotype of T4 cells can also be reverted when growth factor receptor and other signal transduction pathways are manipulated (Wang *et al.* 1998). Thus, signals elicited by growth factors and ECM appear to be coupled and integrated through common signal transduction pathways. These findings unravel important feedback mechanisms between E-cadherin-mediated cell–cell adhesion and integrin-mediated cell–ECM interactions as well as between integrins and growth factor receptors. These pathways act cooperatively to maintain tissue integrity and function in mammary epithelial cells and play a key role in the transition from normal tissue to malignant tumours. It is important to note that the effect of β_1-blocking antibodies on T4 cells was reversible (figure 5)

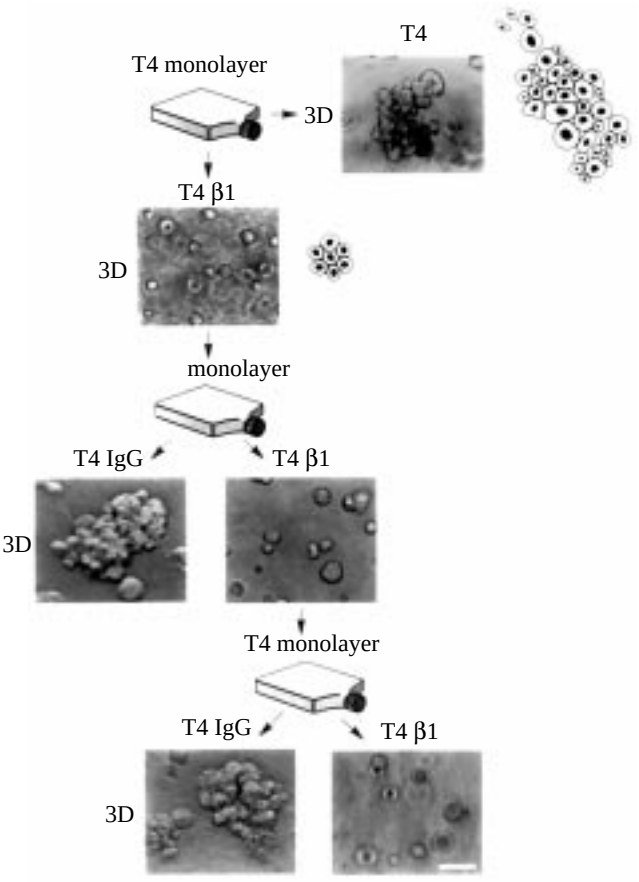

Figure 5. Phenotypic reversion of human mammary epithelial tumour cells (T4) by integrin-blocking antibodies. Phase contrast micrographs and schematic representations of T4 cells cultured in three-dimensional EHS matrix in the absence of antibodies (T4), or presence of anti-β1-integrin antibodies (T4 β_1) or mock antibodies (T4 IgG). T4 β_1 spheres stop growing and resemble normal S1-acini in EHS matrix (see Weaver *et al.* 1997). Despite two rounds of treatment, cells reverted by antibodies against β_1 integrins still formed large, disorganized colonies when cultured in the absence of antibodies. All cultures were analysed after 10–12 days in EHS matrix. Bar, 50 μm. Reproduced from Weaver *et al.* (1997), with permission.

indicating that a simple manipulation of the cell–microenvironment interactions was sufficient to switch cells from tumorigenic to 'normal' phenotypes and vice versa. Although the specifics may vary, these findings have a broader application: rescue of integrin $\alpha_2\beta_1$ in α_2-deficient, spindle-shaped, invasive mammary carcinoma cells resulted in phenotypical and behavioural reversion. Reverted cells readily differentiated into alveolar structures on EHS matrix, and were significantly less tumorigenic *in vivo* (Zutter *et al.* 1995). This supports a putative role of integrin $\alpha_2\beta_1$ in alveolar formation *in vivo* (Keely *et al.* 1995) and correlates with the fact that $\alpha_2\beta_1$-expression is markedly reduced in a number of breast carcinomas.

4. CONCLUSION

In this review, we have cited examples of how both cellular microenvironment and tissue structure correlate with a given behaviour or function. Many of the resulting

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concepts are not new and developmental biologists have alluded to most of them in the course of the past 50 years. The examples provided in this article further indicate that the dynamic reciprocity concept (Bissell *et al.* 1982) indeed operates in maintenance of tissue specificity. Both mutational and biochemical inside-out signalling, and biochemical and mechanical outside-in signalling, generate a unity of form and function that goes above and beyond the question of whether form follows function or vice versa. The examples discussed here for cancer cells, indeed, can be echoed in other diseases. When mechanical integrity of muscle cells or skin is impaired by genetic aberration of proteins involved in the formation of structural complexes such as dystrophin in muscular dystrophies (for details, see Campbell (1995)), or proteins of the BM or type VII collagen in skin blistering diseases (for reviews, see Bruckner-Tuderman (1994, 1996)), tissue architecture and function is abolished resulting in premature death of the patients. If generalizations were to be made, it should be pointed out that experimental gene knockouts of ECM molecules that are predominantly expressed during embryonic and adult tissue formation, such as tenascin-C, SPARC or thrombospondin-1, lack obvious phenotypes, whereas knockouts of ECM molecules that are ubiquitously present throughout life, such as fibronectin, laminin, collagen and entactin, show severe phenotypes (for a review, see Hynes (1996)). Furthermore, null mutations for receptors that establish cell adhesion, such as integrins and cadherins, also result in drastic developmental defects with only few exceptions (see Hynes 1996). Because cell adhesion systems and the appropriate extracellular ligands represent the basic tools to bring about correct tissue structure, ECM and cell adhesion molecules represent nodal points in the transmission of mechanical and biochemical information between cells and their microenvironment. Thus, both classes of molecules act together to teach the cell 'social behaviour' by functioning as 'social advisors'. Examples that show the importance of tissue structure for function are still rare. However, the example discussed above for breast cancer cells is striking because a simple switch in the 'social advisor set-up' reverts tumour cells with pronounced genetic defects to assemble into correct tissue architecture with the reestablishment of normal regulatory arrangements (Weaver *et al.* 1997; Zutter *et al.* 1995; see above). This is consistent with a finding *in vivo*, where breast acinar cells surrounding mammary tumours appear phenotypically normal despite genetic insufficiencies (Deng *et al.* 1996). These examples provoke the striking conclusion that cellular microarchitecture and the form of the tissue dictates cell behaviour and function and overrides mutations and aberrations encoded in the genome. Furthermore, tumour cells in an advanced state create their own tumour microenvironment, thereby changing normal tissue architecture and creating a stable state of malignancy. We therefore propose that correct architecture of a tissue governs tissue function, and thus suggest the title 'tissue architecture: the ultimate regulator of epithelial function' to be affirmative.

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