Modeling Morphogenesis and Oncogenesis in Three-Dimensional Breast Epithelial Cultures

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Key Words

ECM, lumen formation, apicobasal polarity, force

Abstract

Three-dimensional (3D) epithelial culture systems recreate the cardinal features of glandular epithelium in vivo and represent a valuable tool for modeling breast cancer initiation and progression in a structurally appropriate context. 3D models have emerged as a powerful method to interrogate the biological activities of cancer genes and oncogenic pathways, and recent studies have poignantly illustrated their utility in dissecting the emerging role of tensional force in regulating epithelial tissue homeostasis. We review how 3D models are being used to investigate fundamental cellular and biophysical mechanisms associated with breast cancer progression that have not been readily amenable to traditional genetic or biochemical analysis.

INTRODUCTION

DCIS: ductal carcinoma in situ

EGFR/ErbB: members of the epidermal growth factor receptor tyrosine kinase family

Apicobasal polarity: asymmetric organization of epithelial cells within glands where the apical pole borders the lumen, while the basal surface adheres to the extracellular matrix

Breast carcinoma is the most common female cancer in the Western world (1). For the practicing pathologist, epithelial cancers arising in the breast are remarkable for both their clinical significance as well as their diverse histopathological architecture. Breast carcinomas also represent a growing diagnostic challenge for pathologists because preinvasive lesions, such as atypical ductal hyperplasias and ductal carcinomas in situ (DCIS), are being diagnosed with increasing frequency (2, 3). The ability to effectively diagnose these early lesions, where the tumor does not invade the basement membrane or myoepithelial layer, and confidently predict future outcome for these patients has assumed great significance in breast cancer diagnosis and treatment (4).

For decades, pathologists have recognized that certain histological patterns harbor valuable prognostic information. For example, in the breast, tubular carcinomas, mucinous (colloid) carcinomas, and invasive lobular carcinomas are associated with better clinical outcome compared with the most commonly diagnosed pattern, invasive ductal carcinoma (5, 6). Furthermore, clear relationships between a specific genomic abnormality and histological phenotype have been defined. First, the comedo subtype of DCIS possesses amplifications in the *HER2/NEU* oncogene, which encodes the epidermal growth factor receptor (EGFR) tyrosine kinase ErbB2 (7). Second, basal-like breast cancers, an aggressive subtype of invasive ductal carcinoma remarkable for significant aneuploidy and a lack of hormone receptor expression, are strongly associated with mutations in the tumor suppressor gene *BRCA1* or with abnormalities in X chromosome inactivation (8). Third, invasive lobular carcinoma is commonly associated with genomic losses in *CDH1*, which codes for E-cadherin, an important epithelial cell-cell adhesion molecule (9). Moreover, high-throughput analytical approaches have provided a wealth of information about epithelial cancers and have raised new questions about how cancer genes and pathways influence both clinical prognosis and histological architecture (10–12). Despite these advances, much remains to be learned about the precise molecular and biophysical mechanisms that elicit the actual phenotypic changes observed under the microscope. A better understanding of the mechanisms and pathways involved in the disruption of normal tissue architecture will undoubtedly provide biological insight into breast carcinoma progression and aid in the discovery of new diagnostic markers and therapeutic strategies.

Over the past decade, three-dimensional (3D) organotypic culture systems have been increasingly utilized as powerful cell-based models to investigate the functions of cancer genes and pathways in a biologically relevant context and high-throughput manner (13, 14). These culture systems have provided unique insights into how basic cell biological and biophysical processes impact higher-order tissue architecture. In recent years, 3D models have significantly enhanced our understanding of carcinoma biology in the following four areas: (*a*) the formation and maintenance of a hollow glandular lumen and its disruption by cancer genes, (*b*) the regulation of apicobasal polarity in normal and cancerous epithelium, (*c*) the discovery that cell-cell and cell-matrix adhesion pathways can dominantly interfere with the phenotypic expression of the tumorigenic state, and (*d*) the emerging importance for tensional force in driving 3D tissue architecture and homeostasis. In this review, we focus on these four major areas of discovery and their potential implications for breast cancer pathogenesis. Importantly, we do not encyclopedically review findings in several important research areas—such as branching morphogenesis, invasion, epithelial-mesenchymal transition, and the role of the stroma in tumorigenesis—because these topics have been covered in recent reviews (13, 14). Finally, although most of the experiments discussed here employ normal and cancerous breast epithelial cells, we also

delineate salient experiments that use non-breast 3D culture systems, most notably cysts derived from Madin Darby Canine Kidney cells (MDCKs), because they provide valuable insight into the basic processes driving normal glandular architecture and its disruption in carcinomas.

MAMMARY GLAND MORPHOGENESIS IN VIVO

Cells in the human body organize into dynamic tissue complexes, orchestrated by numerous factors including cell-cell and cell–extracellular matrix (ECM) interactions, soluble ligands, and physical force. In epithelial tissues, these elements create an intricate architecture notable for tightly controlled cell growth, proliferation, differentiation, polarity, and survival. This phenotypic control is mediated not only by intracellular signaling pathways, but also by signals from the surrounding microenvironment. For instance, in the developing mouse, death signals initiated by the endoderm induce cells in the ectoderm to undergo apoptosis (15). In the developing kidney, tubule formation is mediated by soluble factors including hepatocyte growth factor and transforming growth factor-α, as well as by chemical and physical cues initiated by the adjacent mesenchyme (16, 17). Furthermore, intracellular signaling pathways and physical signals initiating from force and adhesion are elaborately linked; disruption of these interrelationships plays a major role in epithelial carcinogenesis (18). Indeed, this is beautifully illustrated in the human breast.

Mammary Gland Development

The breast is one of the few organs that develops and matures after birth, making it ideal for studying tissue development. The mammary gland forms via branching morphogenesis in which a two-dimensional (2D) layer forms a fluid-filled lumen via budding through a 3D mesenchymal mass (19). The mature breast is a complex organ consisting of a central lumen encapsulated by luminal epithelial cells, and further delimited by myoepithelial cells and the ECM. Hence, a unique microenvironment influences mammary tissue homeostasis through hormones, soluble factors, stroma, and physical stress and strain (20).

The mammary gland comprises an extensive network of branched ductal structures with epithelial-cell-lined hollow luminal spaces (19, 21). Two bilateral ridges of epidermal tissue known as milk lines form during the first trimester (22). Pairs of disk-shaped mammary placodes, which mark the site of each nipple, separate along these lines and form a bulb-shaped bud by proliferating into the adjacent mesenchyme. This structure forms the primary rudimentary mammary gland known as an anlage. Upon penetration of the mesenchyme, the epithelial bud branches into the mammary fat pad and forms several ductal trees. However, upon cessation of hormonal influences in the newborn, the mammary gland enters a quiescent state until puberty.

Upon reaching puberty, hormone-dependent development of the breast initiates with expansion, beginning at the ends of the ducts known as terminal end buds (TEBs) (**Figure 1**) (23). Two different cell types are contained within the TEBs: cap cells and body cells. The cap cells are ceded as a single layer at the edge of the TEBs in contact with the basal lamina, whereas the majority of the TEBs are composed of body cells structured in multicellular layers. The TEBs undergo ductal arborization via sprouting through the mammary fat pad. Although the resting gland is relatively static, during pregnancy the breast undergoes dynamic changes in which lobules form on the ends of the branched structures and secrete milk during lactation. With pregnancy, differentiation of the ducts occurs, resulting in the formation of luminal structures required for lactation. Lumen formation is intrinsic to milk production, and transport and is controlled by cell death as body cells bordering the TEBs exhibit high rates of apoptosis (24). Interestingly, in premalignant breast cancer lesions, either

Extracellular matrix (ECM): noncellular tissue components produced by cells such as collagen and basement membrane proteins

Apoptosis: programmed cell death marked by caspase activation, nuclear fragmentation, and subsequent phagocytic elimination of cellular remnants

TEB: terminal end bud

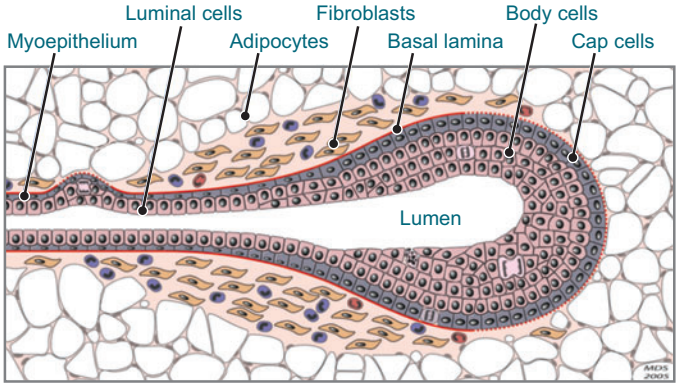

Figure 1

Terminal end bud morphology. Diagram depicting the terminal end bud, including the highly prevalent body cells found adjacent to the lumen as well as the surrounding single layer of cap cells. Large numbers of stromal fibroblasts are found around the collar of the duct. The dotted red line represents the thinning basal lamina that occurs at the edge of the invading ducts, although no evidence exists that ductal cells cross the basement membrane. Figure adapted with permission from References 19 and 21.

complete or partial filling of the lumen is observed, suggesting that normal apoptotic pathways may be altered in the early phases of mammary carcinogenesis.

MODELING GLANDULAR EPITHELIUM USING THREE-DIMENSIONAL CULTURES

Cell culture systems are well suited for dissecting the biochemical and cell signaling pathways necessary for studying oncogenic transformation. Although significant information has been gleaned from studies of 2D monolayer cells, these systems do not lend themselves to the 3D architecture characteristic of epithelial cells in vivo. To address the limitations imposed by monolayer cell culture, 3D methods were developed to model the in vivo environment of epithelial cells. In contrast to mouse models or human tissue studies, 3D systems can be exploited to rapidly identify genes and deconstruct signaling pathways that regulate mammary morphogenesis and epithelial cancer development. In this review, we focus on the use of 3D models as a means to uncover the basic cellular mechanisms that contribute to normal breast morphogenesis and malignancy.

MEC: mammary epithelial cell

Acini: although anatomically imprecise, a widely used operational term for epithelial cyst-like spheroids grown in 3D culture

The terminal ductal lobular unit, which is composed of mammary epithelial cells (MECs), is the smallest functional unit of the breast (25). When MECs are placed in traditional 2D cultures, they grow as monolayers and fail to differentiate, even in the presence of prolactogenic hormones (26). However, upon culture within basement membrane proteins, these cells organize into spherical acini in which the lumen becomes hollow and milk is secreted. A breakdown in this formation is seen in the early stages of breast cancer, where loss of polarized organization, increased cellular proliferation, and filling of the luminal space are observed (1, 14). Therefore, understanding the basic mechanisms that regulate acini formation and luminal clearance provides key insights into the early events in carcinoma formation.

If one uses 3D culture conditions, epithelial cells initially proliferate to eventually form growth-arrested spherical acini characterized by polarized cells surrounding a hollow lumen (12, 27, 28). Two different methods are typically used to induce acini formation (**Figure 2*a***). The first involves completely embedding epithelial cells within a gelled ECM, which is grown in the presence of culture media that contain growth factors and hormones (28). The second method utilizes a thin gel bed (approximately 1 mm thick) of ECM molecules upon which epithelial cells are seeded as single cells and overlaid with culture media that contain diluted ECM (12). Both methods are highly advantageous compared with traditional 2D cultures because cells proliferate and develop into polarized growth-arrested structures that resemble normal glands in vivo (**Figure 2*b***).

Matrices for Three-Dimensional Culture

A salient feature of 3D organotypic methods is the utilization of an ECM to provide

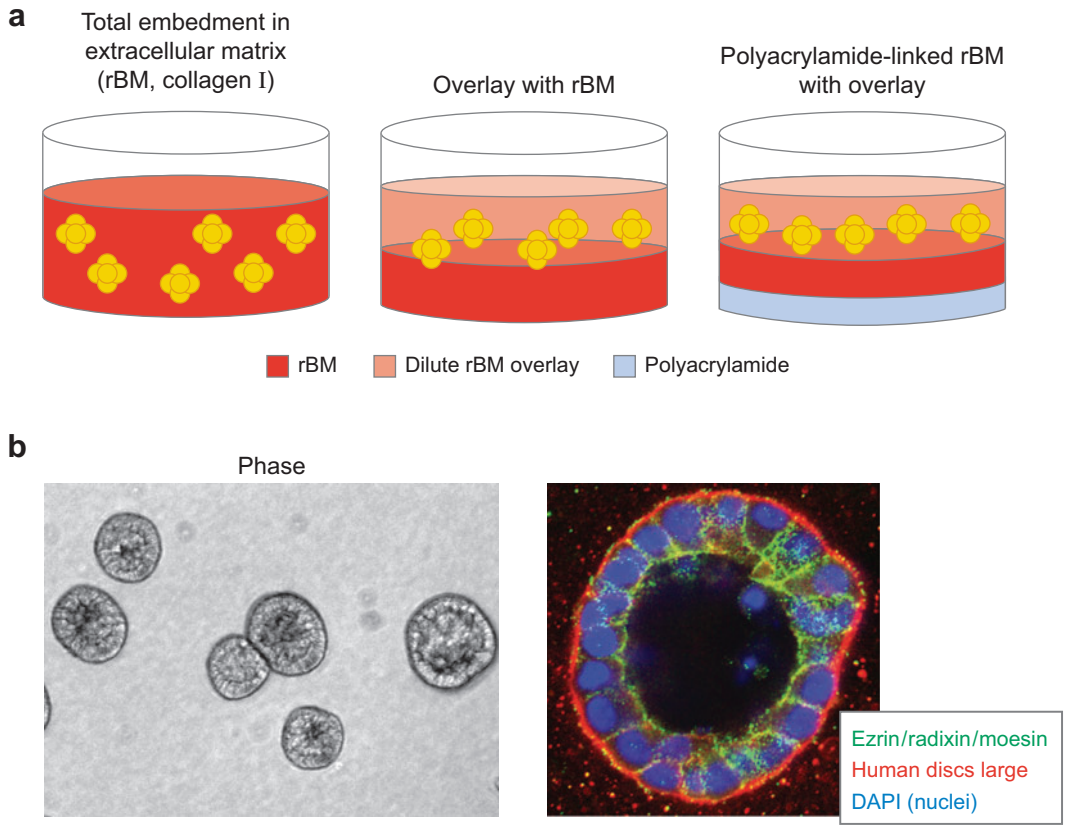

Figure 2

3D culture methods. (*a*) Schematic of commonly used techniques. (*Left*) Complete embedding of mammary epithelial cells (MECs) in reconstituted basement membrane (rBM). (*Center*) MECs seeded upon a thin layer of solidified rBM are overlaid with a dilute solution of rBM in culture media. (*Right*) Polyacrylamide gel of a given elastic modulus is cast upon a glass coverslip and rBM is allowed to cross-link to the polyacrylamide. MECs are seeded upon the rBM/polyacrylamide matrix and overlaid with dilute rBM in culture media. (*b*) Phase micrographs of day 18 MCF-10A acini grown in overlay culture. Equatorial confocal cross sections of a single acinus stained with ezrin/radixin/moesin (*green*), human discs large (*red*), and the nuclear dye 4′, 6-diamidino-2-phenylindole (DAPI, *blue*).

the necessary structural and biochemical cues for proper glandular differentiation and homeostasis. A wide variety of materials have been utilized, each with their own pros and cons (**Table 1**). Reconstituted basement membranes (rBMs) from Engelbreth-Holm-Swarm tumor-derived basement membrane matrix (EHS) mouse sarcomas have been routinely employed to study mammary morphogenesis as well as cellular transformation and carcinogenesis (12, 28). EHS is composed primarily of the basement membrane components laminin-1, collagen 4, and entactin (29). Recent studies have shown that the exogenously provided laminin in the EHS-derived matrix drives the morphogenetic process when MECs are cultured three-dimensionally (30). Various nontransformed MEC lines can be induced to undergo acinar morphogenesis, including S1 cells (from the HMT-3522 progression series) and MCF-10A cells, which are human in origin, as well as mouse MEC lines, such as Scp2 and Eph4 (28, 31–35). In addition, primary mouse and human MECs also

Reconstituted basement membrane (rBM): preparations of basement membrane–derived ECM proteins, most notably laminin, which can drive 3D epithelial acini morphogenesis

Table 1 Advantages and disadvantages of various matrices for 3D culture studies

Component	Advantages	Disadvantages
Reconstituted basement membrane	Successfully applied to many 3D systems	Poorly defined content; lot-to-lot variability
Fibrin	Successfully applied to many 3D systems	Easily proteolyzed by cellular proteases
Collagen I	More biologically defined; easy to manipulate	Lot-to-lot variability; limited range of elastic moduli
Polyacrylamide gels	Easy to manipulate; nonreactive; large range of elastic moduli	Acrylamide toxicity; not a true 3D system

form polarized structures with a hollow lumen when cultured three-dimensionally (30, 36).

However, because EHS is isolated from a mouse tumor xenograft, it has a complex and ill-defined composition, which is subject to inherent lot-to-lot variability. In contrast, employing an ECM scaffold in which the stoichiometry of critical components can be easily manipulated and controlled provides certain experimental advantages. Accordingly, collagen I has been utilized as a 3D matrix. Certain epithelial cells, most notably MDCK epithelial cells, develop into polarized cysts with a hollow lumen when embedded within matrices composed of collagen I (37). Collagen I is biologically better defined than EHS and can be easily manipulated through changes in concentration, orientation, and biochemical modification (30, 38–41). Unfortunately, numerous epithelial cell types fail to form polarized acini when cultured in collagen I gels, whereas in laminin-rich rBM they do polarize. Growing evidence indicates that the mechanical properties of the 3D matrix are also critical for the development of polarized, differentiated acinar structures. This was revealed originally in a series of studies involving normal breast epithelium grown on top of floating or attached collagen gels; mammary architecture, metabolic function, and differentiation could be maintained only in the malleable, floating collagen gel (42, 43). Nonetheless, the range in elastic modulus achieved through the use of collagen I remains limited owing to biochemical constraints. This becomes especially problematic with the increasing prominence of studies deciphering the effects of mechanotransduction in tissues. Finally, like rBM, collagen I is a biologically derived material and thus can present with experimental variability between preparations. Thus, newer techniques incorporating synthetic materials are now being utilized in 3D organotypic culture systems.

One system currently in use involves the deployment of polyacrylamide gels functionally cross-linked to ECM components upon which cells can be seeded (44). Polyacrylamide is a nonreactive material that can be easily manipulated through altering the concentrations of acrylamide and bisacrylamide cross-linker, thereby allowing for precisely controlled biochemical and mechanical properties. However, because acrylamide is cytotoxic in monomeric form, cells cannot be embedded directly in this material for 3D studies. To preclude this limitation, cells seeded upon polyacrylamide gels are overlaid with a blanket of rBM, resulting in a pseudo-3D system (**Figure 2*a***) (45). As this system is neither 2D nor 3D, cell behavior can be different than that observed in more traditional 3D culture systems, which can lead to difficulty in interpreting results. Alternatively, conjugated self-assembling peptide polymer gels and protein-conjugated methylcellulose and peptide-immobilized polyethylene glycol gels that are biocompatible for 3D and in vivo studies are now available for study, yet await rigorous experimental validation (46, 47).

Overall, a variety of matrix substrates and scaffolds can be utilized for 3D organotypic culture systems. As a result, interpretations of data from such experiments need to be weighed against limitations of the particular system in use. Below, we highlight some of the applications of these techniques and recent findings regarding mammary development and carcinogenesis.

EHS: Engelbreth-Holm-Swarm tumor-derived basement membrane matrix

APOPTOSIS AND LUMEN FORMATION

The 3D culture of MEC lines has been commonly used as an in vitro model of epithelial development. When cultured in rBM, MECs form growth-arrested acini in an ordered sequence of events (**Figure 3**). In the early stages of culture, cells form clusters surrounded by the ECM. As cultures progress, two distinct cell populations emerge within each acinus: polarized cells around the outer layer in contact with the ECM that secrete basement membrane proteins, and nonpolarized cells comprising the inner region. With further culturing, these inner cells undergo cell death and clearance, leading to lumen formation.

Luminal Apoptosis

Lumen formation appears to be due in part to apoptosis. Inhibition of apoptosis with caspase inhibitors in 3D cultures of primary mouse MECs results in delayed lumen formation (36). This is consistent with in vivo data in which mice overexpressing the antiapoptotic protein Bcl-2 in a mammary-specific fashion exhibit a delay in mammary lumen development (24). Furthermore, studies utilizing the cultured nonmalignant mammary cell

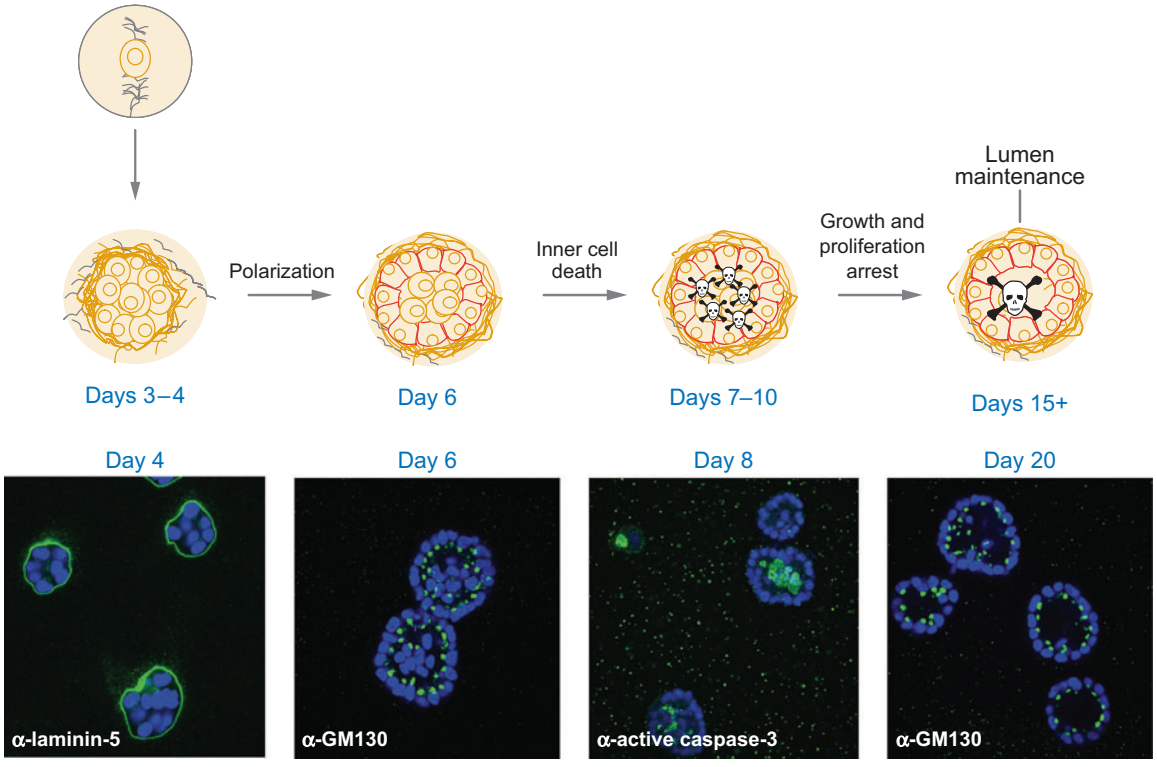

Figure 3

Lumen formation in MCF-10A acini. MCF-10A cells undergo an ordered series of morphogenetic events when grown in 3D culture. In early stages, developing cell clusters exhibit apicobasal polarization; thereafter, two distinct cell populations become discernable within each acinus: a well-polarized outer layer of cells in direct contact with extracellular matrix, and an inner subset of cells that is poorly polarized and lacking contact with the matrix. These inner cells undergo apoptosis, characterized by the expression of processed caspase-3, resulting in a hollow lumen. The hollow acinar structure remains stable thereafter. Figure adapted with permission from Reference 48.

line MCF-10A have shown similar effects. MCF-10A cells undergo polarized formation of acini and lumen formation in 3D culture (**Figure 3**). In experiments in which Bcl-2 or Bcl-xL was overexpressed, luminal clearance was delayed in acini, again implicating apoptosis in lumen development (48).

Epithelial cells depend critically upon integrin-mediated cell adhesion to ECM for proper growth and survival; *anoikis*, a form of apoptosis that epithelial cells undergo upon detachment from ECM, may contribute to lumen formation (49–51). Protection from *anoikis* may constitute a fundamental mechanism for tumor cell survival in vivo and may be responsible for luminal filling in glandular structures in vitro and in vivo (52, 53). As centrally located cells do not contact ECM, these cells may undergo death through *anoikis*. Consistent with this hypothesis, both MCF-10A *anoikis* and 3D lumen formation coincide with increased levels of the BH3-only proapoptotic protein Bim; RNA-interference-mediated Bim depletion prevents both MCF-10A *anoikis* as well as luminal apoptosis in 3D culture (53, 54). Moreover, disrupting Bim in the mouse mammary gland prevents apoptosis and luminal clearance in TEBs during puberty (55). Overall, these studies highlight Bim as an important regulator of apoptosis resulting from the matrix detachment of cells occupying the 3D lumen.

In addition to cell-matrix interactions, cell-cell interactions may also regulate cell death, affecting lumen formation as well. The molecule CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) is a cell-cell adhesion molecule that has been identified as a regulator of lumen development. In MCF-10A cells, inhibition of CEACAM1 function results in the inhibition of lumen formation (56). Conversely, expression of CEACAM1 in MCF-7 cells, a mammary carcinoma cell line that does not normally express CEACAM1 or form a lumen in 3D culture, results in luminal development, showing that extracellular cues contribute to lumen formation as well (57).

Luminal Filling Induced by Cancer Genes

Although apoptosis plays an important role in the formation of the mammary lumen, inhibition of apoptotic pathways delays, but does not completely inhibit, luminal clearing. The tight control of proliferation also plays a role because overexpression of cyclin D1 or inhibition of the retinoblastoma proteins through expression of the HPVE7 results in a similar delay in lumen clearance (48). However, as is true of apoptosis inhibition, lumen formation is postponed, not ablated, with enhanced cellular proliferation. Filling of the lumen results from a combination of both apoptotic inhibition and enhanced proliferation, as activation of ErbB2—the gene product of *HER2/NEU*, which can induce both proliferation and inhibit apoptosis—results in luminal filling (34, 48). Notably, in some early human breast precancerous lesions, such as atypical ductal hyperplasias, a hollow architecture is maintained in the context of hyperproliferation, whereas advanced lesions such as DCIS exhibit varying degrees of luminal filling. Hence, one may speculate that in early breast lesions, architectural changes induced by proliferative signals are limited owing to compensatory increases in apoptosis. In contrast, more advanced phenotypes may express antiapoptotic signals that allow survival of these excess proliferating cells.

ErbB2 is a receptor tyrosine kinase from a group of four kinases (ErbB1–4) that bind growth factors from the EGF family. Binding of the ErbB receptors to EGF ligands can occur via homo- or heterodimerization, thereby leading to a complex array of signaling (58–60). ErbB2 is often overexpressed or amplified in breast tumors and correlates with a poor clinical prognosis (61, 62). Activation of the ErbB2 receptor during MCF-10A morphogenesis elicits a complex multi-acinar phenotype. Similar hyperplastic lesions, notable for the presence of multi-acinar clusters, have been observed in vivo in tumors upon transgenic expression of activated *Neu*

(NeuT) in the mouse mammary gland, as well as when primary tumor cells isolated from these mice are cultured on EHS (63–65). These altered structures exhibit the cardinal features of early-stage cancers, including high levels of proliferation, filling of the lumen, and the disruption of cell polarity. However, activation of ErbB2 in MCF-10A cells does not cause anchorage-independent growth or an invasive phenotype in 3D culture. These results suggest that ErbB2 can play a role in the initial stages of transformation, but ErbB2 alone is not sufficient for progression to invasive breast cancer (34).

Hence, other factors may synergize with ErbB2 to promote invasion and metastasis. Recent work utilizing a mutant version of β4 integrin, in which the signaling capacity but not the ability for adhesion was ablated, demonstrates that β4 integrin can amplify the signaling capability of ErbB2, thereby contributing to mammary tumor progression. β4 integrin can form a complex with ErbB2, thus enabling it to activate c-Jun and STAT3 and induce two hallmarks of oncogenesis, proliferation, and loss of cell adhesion. This study suggests that β4 integrin can function as an essential component of ErbB2-induced oncogenesis and serve as a potential therapeutic target (66).

Following the groundbreaking studies of ErbB2 in 3D culture, the activation of numerous other growth factor receptors and oncogenes have subsequently been interrogated in the MCF-10A acini model; some examples include colony-stimulating factor 1 receptor (67), insulin growth factor receptor (68), Akt/PKB (69), and phosphotidylinositol 3-kinase (70). As predicted, activation of these pathways elicits varying degrees of luminal filling, which results from increased proliferation combined with protection from apoptosis in the 3D lumen. Nonetheless, each of these molecules mediates additional distinctive biological activities in 3D culture, which ultimately influences the morphogenetic phenotype in unexpected ways. These phenotypes, in addition to the corresponding in vivo histological correlates, have already been the subject of a recent review (14).

Autophagy

Autophagy: tightly regulated lysosomal process where a cell self-digests cytoplasmic contents; proposed to mediate nonapoptotic (type 2) programmed cell death

Although much work has been done analyzing the effects of the classical pathways of cell death in lumen formation, recent work suggests that another process, autophagy, may play a role in lumen clearance. Autophagy is an evolutionarily conserved lysosomal degradation process in which a cell degrades its own cytoplasmic contents (i.e., eats itself). In eukaryotic cells, autophagy is a key mechanism for long-lived protein degradation and organelle turnover and serves as a critical prosurvival mechanism during nutrient deprivation or stress (71, 72). It has been proposed that when excessive autophagy occurs within a cell, a distinct form of programmed cell death ensues (termed type 2 death) (73). Remarkably, in experiments investigating the ultrastructure of cells during postlactational involution of the mouse mammary gland, autophagic vacuoles were seen in the early stages of involution (74). Similarly, 3D culture studies suggest the involvement of autophagy in lumen formation (48). Furthermore, TRAIL (tumor necrosis factor–related apoptosis-inducing ligand) may be involved in this process, as treatment with exogenous TRAIL induces autophagy in MCF-10A cells and expression of a dominant negative TRAIL receptor cooperates with Bcl-2 overexpression to increase luminal filling (75). Although these results implicate the potential for TRAIL-induced autophagy during luminal clearance, they are nonetheless correlative and ignore that the outer cells of developing acini exhibit direct ECM contact on their basal surface, whereas the central cells do not (48). Thus, one can alternatively predict that autophagy is actually induced during *anoikis* as a protective mechanism to mitigate the stresses of ECM detachment. Loss-of-function studies of autophagy genes (known as *ATG*s) are required to precisely validate the role of autophagy in cell survival versus

type 2 death during 3D lumen formation (73).

MAMMARY EPITHELIAL POLARITY

In addition to luminal filling, mammary carcinoma can also be characterized by a loss in epithelial polarity. Glandular epithelial tissues consist of cells exhibiting a characteristic polarity in which the apical poles face inward toward the central lumen, the lateral face mediates cell-cell contacts, and the basal face contacts the ECM (**Figure 4**). This polarity is often disrupted in early carcinomas, which is generally considered a poor prognostic sign. 3D culture models are beginning to address if intrinsic polarity regulators directly regulate cancer-promoting functions. The best-characterized 3D model to study epithelial polarization utilizes MDCK cells grown in a collagen I matrix. MDCK cells undergo cystogenesis in 3D culture characterized by polarized cells surrounding a cleared central lumen; moreover, the cells comprising these cysts form tight junctions, making this model advantageous for the study of mechanisms that govern epithelial polarity.

Cell-Intrinsic Regulators of Polarity

Invertebrate models have provided a wealth of genetic information about the regulation of polarity in epithelial tissues (76, 77). Cell polarity is believed to be controlled by the interplay of three major complexes, broadly

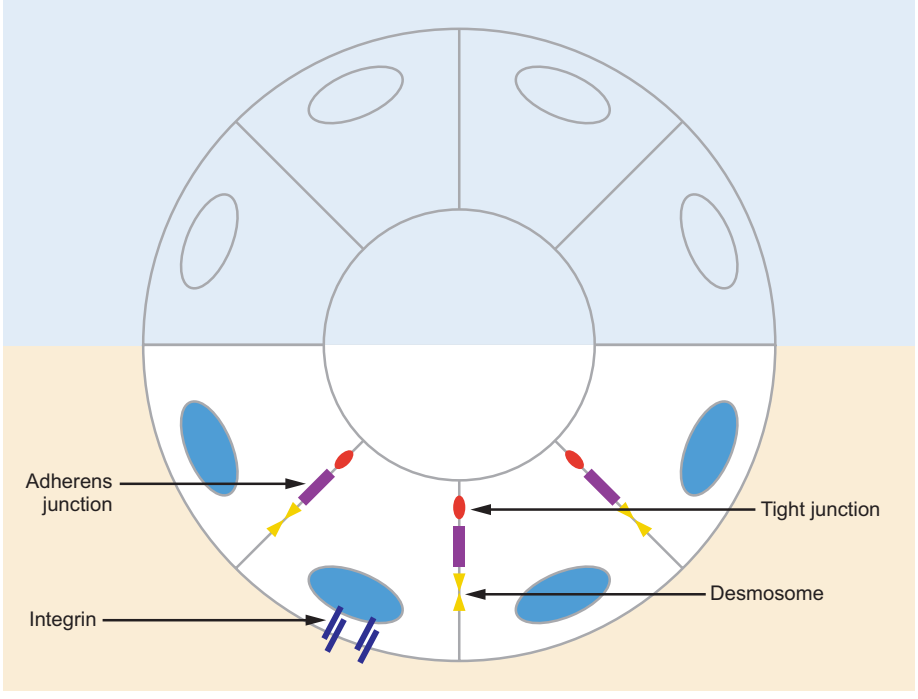

Figure 4

Cross section of a polarized gland architecture. Each individual epithelial cell within an intact gland has a microvilli-rich apical membrane facing the lumen, a lateral membrane contacting adjacent cells, and a basal surface contacting the basement membrane (extracellular matrix). The extracellular matrix attachment is mediated through integrin receptors, and cell-cell junctions consist of tight junctions, adherens junctions, and desmosomes. Tight junctions demarcate the boundary between apical and basolateral surfaces.

defined as the Par complex, the Scribble complex, and the Crumbs complex (78, 79). The concurrent activities of these three complexes combine to formulate apical and basal polarity in epithelial cells (80–82). Nonetheless, much remains to be learned about these fundamental regulators during cancer initiation and progression.

Recent studies of MDCK cyst formation have illustrated that specific proteins in these polarity regulator complexes are critical for the development of polarity and lumen formation. The mammalian orthologs of three genes involved in epithelial polarity in *Drosophila*—CRB3, PALS1, and PATJ—exist in a macromolecular complex that localizes to the tight junction in MDCK cells (83, 84). The ablation of PALS1 by RNA interference elicits a concomitant loss of PATJ in MDCK cells and completely disrupts apicobasal polarity when these cells are grown in 3D culture. The resulting structures do not form a central lumen; instead, they contain multiple small and incomplete lumens, or completely lack a lumen. In addition, well-established markers of apical polarity in MDCK cells, such as GP135, are completely mislocalized in these structures (85). Notably, the formation of multiple small lumens in these MDCK structures highly resembles the cribriform patterns observed in breast DCIS and in certain prostate hyperplasias (14).

Two key recent reports have focused specifically on the convergence of signaling pathways upon the apical protein complex Par-3/Par6/atypical PKC (aPKC), resulting in the establishment of epithelial polarity. The first analyzes a molecular mechanism connecting phosphatase and tensin homolog on chromosome 10 (PTEN), which signals to form the apical surface and lumen. siRNA-mediated PTEN depletion in MDCK cells grown in rBM results in the formation of multiple small lumens upon cystogenesis versus the single central lumen seen in controls, suggesting that PTEN signaling regulates epithelial polarity and central lumen formation (86). Furthermore, markers segregating with the PTEN phospholipid product $PI(4,5)P_2$, which is normally confined to the apical border, and $PI(3,4,5)P_3$, its basolateral precursor, show altered localization. Furthermore, addition of exogenous $PI(4,5)P_2$ basolaterally to cysts resulted in the relocation of the apical marker gp135 and of the tight junction component zona occludins 1 to basolateral membranes, thus highlighting a role for this PTEN-regulated lipid product in establishing polarity. Moreover, $PI(4,5)P_2$ exogenously applied to the basolateral membrane of MDCK cysts also targets activated Cdc42 to the basolateral membrane. Finally, both PTEN and Cdc42 regulate the apical location of Par6/aPKC because depletion of either molecule results in the aberrant intracellular localization of aPKC. Importantly, aPKC inhibition disrupts central lumen formation in cysts. Overall, these results elegantly delineate interconnections between PTEN, Par6/aPKC, Cdc42, and the establishment of epithelial polarity and central lumen formation.

A second report establishes a link between Par6/aPKC and the effects of the oncogene ErbB2 on polarity (87). In this report, ErbB2 activation disrupts apicobasal polarity as characterized by changes in zona occludins 1, a tight junction protein, and gp135 localization in 2D cultures of MDCK cells. This coincides with loss of Par6 localization at the apical-lateral border. Interestingly, activation of ErbB2 leads to a decrease in the association of Par3 with Par6 and aPKC, as seen in contact-naïve cells, thereby suggesting that ErbB2 inhibits appropriate cell-cell junction assembly through inhibition of Par3/Par6/aPKC complex formation. Furthermore, ErbB2 associates directly with Par6 and aPKC, suggesting that the formation of this trimeric complex inhibits Par3 association. In addition, this interaction requires ErbB2 dimerization, thereby suggesting a mechanism by which ErbB2 leads to a breakdown in cell-cell contacts in *HER2/NEU*-positive cancers. Similar results are observed in ErbB2-expressing MCF-10A cells. Also,

in 3D MCF-10A culture, multi-acinar structures are observed in cells expressing wild-type Par6, but not in cells expressing an aPKC binding-deficient mutant of Par6, suggesting that the ErbB2/Par6/aPKC complex is responsible for the multi-acinar phenotype induced by ErbB2. Interestingly, cellular proliferation is similar in wild-type and mutant Par6 cells, demonstrating that the signaling pathways through which ErbB2 induces proliferation are completely distinct from those that alter cell polarity.

Extracellular Matrix and Polarity

In addition to the cell-intrinsic regulators and pathways, the ECM itself plays a critical instructive role in the generation of apicobasal polarity. Classical 3D studies investigating polarity reversal in MDCK cysts and thyroid follicles point to the importance of ECM as a polarity cue (37, 88). When grown in liquid suspension culture, these cells form structures in which the free apical surface points away from the central axis of the sphere and toward the surrounding culture medium on the outside; these structures still generate a basal surface but it is in the interior of the structure, created by depositing basement membrane into an internal cavity. When the inverted MDCK cysts are embedded in collagen I, thus providing a strong ECM cue on the outside of these structures, extensive cellular remodeling occurs, resulting in cysts with a central hollow lumen (37). The plasticity of cyst organization in response to varying external cues indicates that epithelial cells possess hard-wired mechanisms for generating polarity within the context of a 3D tissue structure and, furthermore, that ECM plays a fundamental instructive role in directing these mechanisms.

Interestingly, intracellular signaling pathways regulate the polarity of a glandular structure by actively modifying the surrounding basement membrane. In MDCK cysts, the orientation of apical poles requires the small GTPase Rac1, which mediates the proper assembly of laminin at the cyst-ECM interface. Expression of a dominant negative Rac1 (N17Rac) in MDCK cysts inverts the apical pole toward the cyst periphery; remarkably, this polarity reversion is observed only in 3D, but not 2D, cultures. By providing an exogenous source of laminin to the cyst periphery, one can rescue the phenotypic effects of Rac inhibition on MDCK cyst formation (89). Once again, these results illustrate how cell-intrinsic pathways can influence tissue morphogenesis.

Overall, several questions on the relationship between ECM and the generation of apicobasal polarity remain unanswered, all of which are fundamental for understanding the role of polarity in early carcinoma pathogenesis. First, what are the signaling pathways downstream of ECM, tensional force, and adhesion (integrin) receptors responsible for generating the proper apicobasal axis in normally formed glands? Second, what is the cross talk between cell-matrix adhesion pathways and the intrinsic polarity-regulating complexes? Finally, do specific components exist in any of the aforementioned pathways that can be used as biomarkers for early detection or targeted for therapeutic intervention in premalignant breast lesions? 3D culture models may prove useful for answering these important questions.

HMT-3522 SERIES: A REVERSIBLE MODEL OF TUMOR PROGRESSION

Early studies revealed that human breast tumor cell lines do not form acini when grown in 3D culture; rather, they develop into nonpolarized clusters with limited differentiation (28). These landmark experiments demonstrated the stark contrast between the behavior of normal and tumor cells in 3D culture, even though only subtle phenotypic differences were evident when the same cells were grown as 2D monolayers. They also broached a fundamental biological and clinically relevant question regarding cancerous epithelium: Could the disorganized, nonpolarized

phenotype of tumorigenic cells be reorganized into a well-ordered normal architecture? Subsequently, numerous studies in the HMT-3522 model of human breast cancer progression demonstrated that modulating aberrantly expressed cell adhesion proteins in tumor cells can dominantly interfere with the phenotypic expression of the transformed state, even in the context of multiple oncogenic mutations (90).

Phenotypic Reversion in Three-Dimensional Culture

The HMT-3522 progression series is a model of human breast cancer that progressively spans the continuum of morphological phenotype from normal cells to tumorigenesis (**Figure 5**). In the series, a nonmalignant S1 cell population forms growth-arrested normal acinar structures in 3D culture; premalignant S2 cells form noninvasive proliferating nonpolarized colonies; and malignant T4-2 cells exhibit a highly disorganized, rapidly proliferating invasive structure in culture (31, 91, 92; A. Rizki & V.M. Weaver, unpublished results).

In investigating the differences in these cell lines at the molecular level, T4-2 cells were found to display dramatically higher levels of β1 integrin and EGFR as compared with nonmalignant S1 cells. The T4-2 phenotype could be reversed by blocking β1 integrin signaling via a function-blocking antibody, leading to the formation of growth-arrested acini similar to those observed in S1 cells (31). Moreover, this phenotypic reversion was functionally linked to the downregulation of both β1 integrin and the EGFR and their associated signaling networks, highlighting the intricate connection between β1 integrin and the EGFR signaling pathways. Conversely, neutralizing EGFR signaling results in normalized levels of β1 integrin signaling. Provocatively, these observed effects on tumor behavior depend completely upon a 3D context because such reciprocal cross talk between β1 integrin and EGFR is not observed in 2D monolayer cultures (93). Overall, these studies highlight the interplay of cell adhesion and growth factor receptor signaling pathways essential for maintaining the complex phenotypes during breast cancer progression.

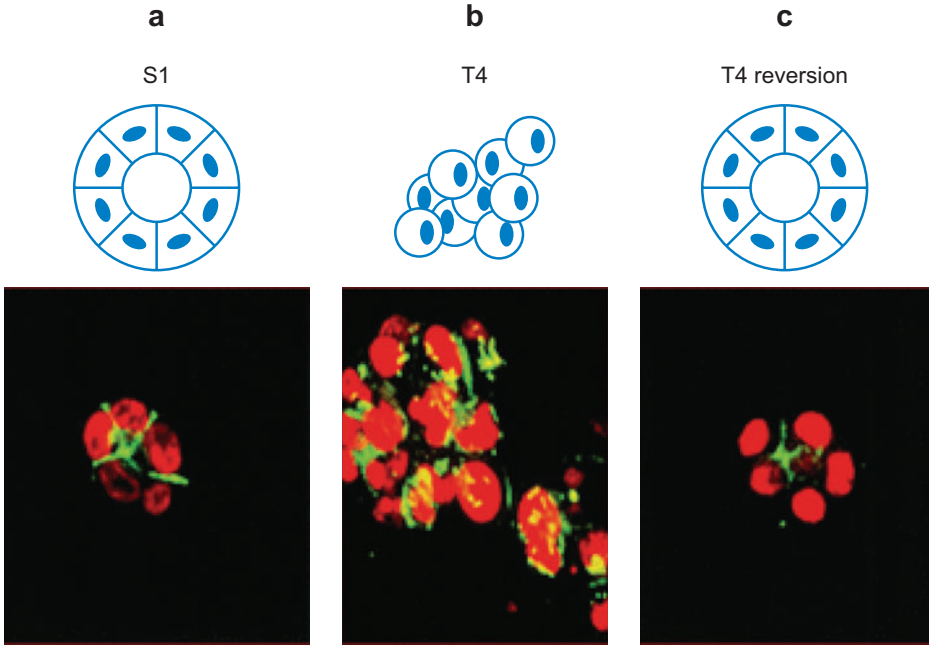

Figure 5

(*a*) HMT-3522 progression series. S1 cells form normal polarized acini in 3D culture. (*b*) T4 cells show a disruption in ductal morphology and uncontrolled cellular proliferation. (*c*) Following treatment with an antibody that blocks β1 integrin function, T4 cells undergo a morphogenetic reversion and form normal-appearing acini similar to the phenotype observed in S1 cells. Figure adapted with permission from Reference 31.

The HMT-3522 series has also facilitated the identification of novel molecules with potential tumor suppressor function. T4-2 cells possess reduced levels of α-dystroglycan (α-DG), a basement membrane receptor. Upon restoration of DG in T4-2 cells, polarized structures that are growth arrested form in 3D cultures; these cells also exhibit reduced tumorigenicity when engrafted into nude mice (94). Finally, analysis of multiple carcinoma cell lines indicates that higher levels of α-DG correlated with the increased ability of these cells to form polarized structures in 3D culture, and examination of human breast and prostate cancers revealed losses in DG expression (94, 95). Although the exact role of DG in breast cancer remains unclear, these initial results obtained from 3D culture assays strongly suggest that it functions as a tumor suppressor and serves as a critical mediator of polarization effects induced by the basement membrane (96).

Studies in other models have further substantiated the importance of cell adhesion proteins in modulating the tumor phenotype. α2 integrin is expressed in normal differentiated MECs and frequently lost in undifferentiated carcinomas. Re-expression of α2 in the Mm5MT mouse adenocarcinoma cell line causes a dramatic reversion from disorganized clusters of spindle-shaped cells to organized gland-like structures in 3D culture (97). In addition, re-expression of the cell adhesion molecule CEACAM1 in the MCF-7 human breast cancer cell line and the restoration of gap junctions in MDA-435 breast tumor cells have both been shown to promote the formation of organized spheroid structures (57, 98).

Polarity and Cell Survival

The importance of polarity in tumor cell survival has also been demonstrated through 3D culture studies of the HMT-3522 progression series and MCF-10A cells. The formation of polarized 3D structures confers protection from apoptosis to a variety of chemotherapeutic insults in both normal and malignant MECs from the HMT-3522 progression series and MCF-10A cells (99; J. Friedland & V. Weaver, manuscript submitted). In contrast, nonpolarized cells are uniformly sensitive to apoptosis (99). Accordingly, disrupting the activation of the laminin receptor, α6β4 integrin, perturbs hemidesmosome organization, disrupts polarity, and promotes apoptosis (99). Interestingly, resistance to apoptosis can be acquired in nonpolar cells by the autocrine secretion of laminin-5, which results in the ligation of $\alpha_6\beta_4$ integrin, as well as the activation of Rac and NFκB, a known positive modulator of cell survival (100). The excess secretion of laminin-5 in a nonpolarized manner upon hyperactivation of growth factor receptors, such as ErbB2, has also been observed during MCF-10A morphogenesis and may contribute to the ability of cells to survive in the lumen (34). Overall, these results indicate that the prosurvival effects associated with the polarized state in 3D culture can be co-opted by the activation of specific survival signals provided by nonpolarized laminin-5 secretion.

Predicting Patient Outcome Using Three-Dimensional Models

More recently, a microarray analysis was conducted in S1 cells to search for transcripts that changed upon transition to an organized, growth-arrested state in 3D culture. These expression profiles were compared against a breast cancer–specific gene expression signature derived from patients of known outcomes to identify potential prognostic markers (101). In comparing transcripts that were downregulated in growth-arrested acini to previously published human breast cancer microarray data, a group of 19 genes was selected to test as markers for cancer prognosis. Of these 19 genes, 14 correlated significantly with disease-free survival. In addition, by using a second less-restrictive strategy, 287 genes that were downregulated with the formation of growth-arrested 3D acini were tested for their ability to predict cancer prognosis and showed

significant correlation with poor prognosis patient outcomes. This study thus represents an innovative method for identifying genome-wide prognostic markers in breast cancer (101).

FORCE AS A REGULATOR OF ADHESION FORMATION AND SIGNALING

In addition to intracellular signaling pathways mediating effects on tissue morphogenesis, the impact of force upon cells is quickly emerging as a mediator of cell growth, differentiation, and 3D tissue architecture. Within a given tissue, individual cells are subject to dynamic macroforces mediated by the tissue as a whole, as well as microphysical forces resulting from local cell-cell and cell-ECM adhesion (see What is Force?). These forces dictate tissue architecture and have been demonstrated to be critical for normal embryogenesis. Venous embryo explants require mechanical stress and strain for normal development and tissue differentiation (102). In *Drosophila*, formation of the dorsal-ventral axis in the fly embryo via the Armadillo-Twist signaling pathway requires cell compression, which occurs through normal movement during morphogenesis; remarkably, this compression can be simulated by externally applying force with a micropipette (103). In addition, cells transduce force onto their surrounding milieu, which is required for development. For example, *Xenopus* gastrulation requires the specific patterning of the small GTP-binding proteins Rho and Rac, main mediators of cell adhesion and contractility, for orientation of the trunk and head mesoderm (104, 105).

Focal Adhesions and Force

A variety of distinct adhesions form between cells and the ECM, each with their own size, shape, and molecular composition (106, 107). These include focal adhesions (FAs), focal complexes, fibrillar adhesions, and 3D matrix adhesions; the best characterized of these is the FA (108–110). FAs are relatively stable complexes that form through the coordinated interactions of various signaling, adaptor, and structural molecules, thereby linking the ECM and integrin receptors to the cytoskeleton (111). Binding of the ECM by adherent cells occurs via integrins, making them a central component of microenvironmental sensing. Interestingly, force appears to play a role in the development of FAs, as maturation of this complex has been shown to require mechanical tension (112, 113). Within minutes of placement of cells upon an ECM-coated surface, cells form focal complexes, small precursor adhesions to the larger FAs (114). By utilizing flexible substrates that deform upon cellular contraction, investigators have found that FA assembly and individual FA size correlate positively with the local distribution of force at adhesions (113, 115, 116). Similarly, in experiments in which external forces are applied directly to adhesions, the strength of the FA increases with the amount of force applied (117–119). These results suggest that external stress and strain promote the formation and strength of adhesion complexes.

Many signaling proteins are located at cell-ECM contacts, including focal adhesion kinase (FAK) and Src family kinases (120–122).

WHAT IS FORCE?

Cells within tissues are exposed to a variety of mechanical stresses that differ in magnitude and duration. These stresses are defined as force per unit area in Newtons per meters squared. They can be macroforces, occurring at the level of the tissue, or microforces, owing to the interaction of the cell with other cells or with the ECM. The intrinsic resistance of a given tissue to a stress is measured by the elastic modulus of that tissue, *E*, which is defined in Pascals and can be obtained by applying a force to a section of tissue and measuring the relative change in length or strain. Generally, cells sense mechanical stress as either a parallel applied force, also known as shear stress, or a perpendicularly applied force that includes both compressive stresses and tensile stresses.

Focal adhesions (FAs): dynamic complexes of signaling, adaptor, and structural proteins that transmit extracellular matrix/integrin-based biochemical and mechanical signals to the cytoskeleton

As these proteins function in growth-factor-mediated signaling cascades, their presence strongly suggests that signaling through adhesions coordinates integrin and growth factor signaling; moreover, they may translate ECM-associated mechanical stimuli to elicit changes in cellular morphology and function (123). FAK is an essential regulator of mechanotransduction; increased mechanical stress at FAs through increased intracellular tension or externally applied forces results in signaling changes at FAs through FAK activation of downstream signaling effectors (**Figure 6**) (124, 125). When mechanical stretch is applied to cells, FAK is activated, resulting in both increased magnitude and duration of activation of the mitogen-activated protein kinase ERK, and increased cellular proliferation (126, 127). FAK activation is required for ERK activation and subsequent progression of cells through G1 (**Figure 6**) (128). Interestingly, use of a kinase-dead mutant of FAK results in the abrogation of ERK activation and leads to a block in mechanically induced cellular proliferation (129, 130). Conversely, expression of a constitutively activated FAK leads to cellular transformation and adherence-independent growth (131). Taken together, these results suggest that FAK serves as a major mediator of

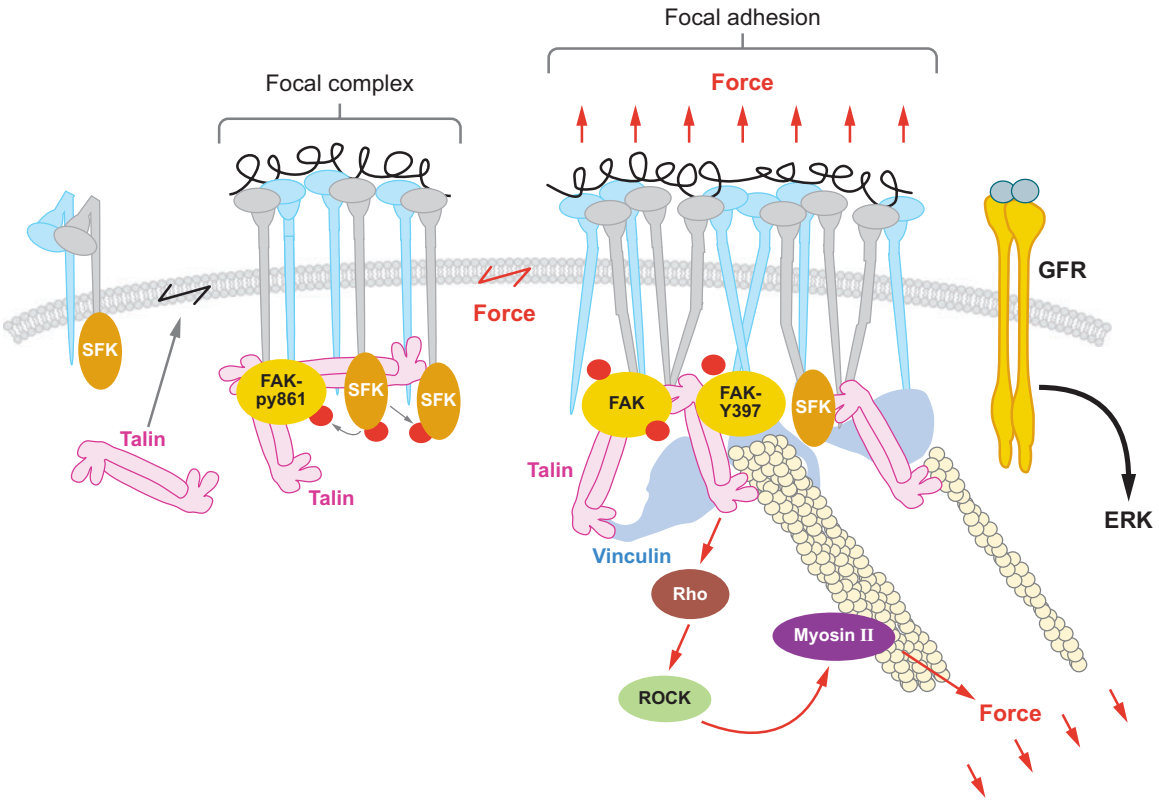

Figure 6

Force-dependent focal adhesion assembly. Application of force implemented by the presence of a stiff matrix results in activation-specific phosphorylation of focal adhesion kinase (FAK) at tyrosine 397 and tethering of the mature adhesion complex, including Src family kinases (SFK), to the cytoskeleton. Integrin clustering and subsequent focal adhesion maturation is accompanied by subsequent growth factor receptor (GFR)-induced activation of ERK signaling and potential activation of cell growth. Figure adapted with permission from Reference 45.

mechanotransduction and acts to translate extracellular forces sensed by integrins into proliferative cellular cues.

Force and Mammary Tissue Homeostasis

During mammary morphogenesis, physical force, including clefting force and surface tension resulting from the interplay between the epithelial and mesenchymal tissues, tremendously impacts the shaping of the ductal branches (132). In similarly branched tissues, such as the lung and kidney, the activity of the small protein GTPase Rho, a major signaling molecule in intracellular tension generation, plays a regulatory role in branching morphogenesis (133, 134), suggesting a similar role for Rho in the developing mammary gland. Indeed, rigid 3D collagen gels influence branching behavior of malignantly transformed MECs in culture by regulating RhoA GTPase activity (135). Furthermore, the mechanical properties of the basement membrane are crucial for normal 3D mammary differentiation in vitro because expression of milk proteins, such as β casein expression, occurs only on a highly compliant basement membrane or on floating collagen gels (26, 42). Conversely, the morphogenesis and functional differentiation of mammary epithelial acini cannot occur when MECs are placed on rigid collagen gels because collagen I does not facilitate proper cell rounding and, thus, hinders the assembly of an endogenous basement membrane at the basal cell surface. Therefore, the mechanical environment in which MECs exist can dictate their behavior.

Breast malignancy is characterized by a dramatic increase in mammary gland tension due to increased compression force and tensile stress resulting from altered vasculature and tumor growth as well as stiffening of the ECM due to fibrosis (18, 136). Such increased rigidity has been postulated to both impede treatment effectiveness and enhance metastasis (137, 138). Because force is required for FA maturation and FAK-induced signaling, one can hypothesize that increased tensional force in the mammary gland contributes to tumorigenesis via activation of integrin-linked proliferative signaling pathways in individual MECs. Moreover, because cells grown as 3D cultures behave differently with regard to cell morphology, integrin signaling, and actin cytoskeletal structures (108, 139), it is important to analyze the contribution of force to mammary malignancy within a 3D context.

Recently, matrix stiffness was linked to malignancy in the context of 3D architecture (**Figure 7**) (45). When 3D tissue culture methods utilizing basement membrane/collagen I gels of increasing stiffness were employed, MCF-10A cells and S1 cells from the HMT-3522 series were grown and allowed to form acini. Interestingly, acinar morphology was increasingly perturbed with increases in matrix stiffness, resulting in nonspherical structures devoid of a central lumen and increased cell spreading, suggesting that the architectural tension surrounding MECs can alter normal 3D morphogenesis (**Figure 3**). Furthermore, increased FAK activation was observed in cells grown in a stiff environment, implying that integrin adhesions may be altered in cells grown on a stiff matrix and that maturation of focal complexes into FAs may take place more readily in a stiff environment. Interestingly, expression of mutant integrin subunits that promote integrin self-association and clustering led to a stiff-matrix cell phenotype with altered acini formation and increased cell spreading in a soft-matrix environment. In addition, repressing ERK activity elicited phenotypic reversion in these cells, implicating ERK signaling as a critical mediator of integrin-associated sensing of ECM compliance. Finally, matrix rigidity increased Rho activity, and a constitutively active Rho mutant increased the number and size of FAs as well as promoted cell spreading in a soft environment (45). Overall, these 3D studies broached the concept that changes in mechanical properties may regulate cell behavior relevant for malignant transformation.

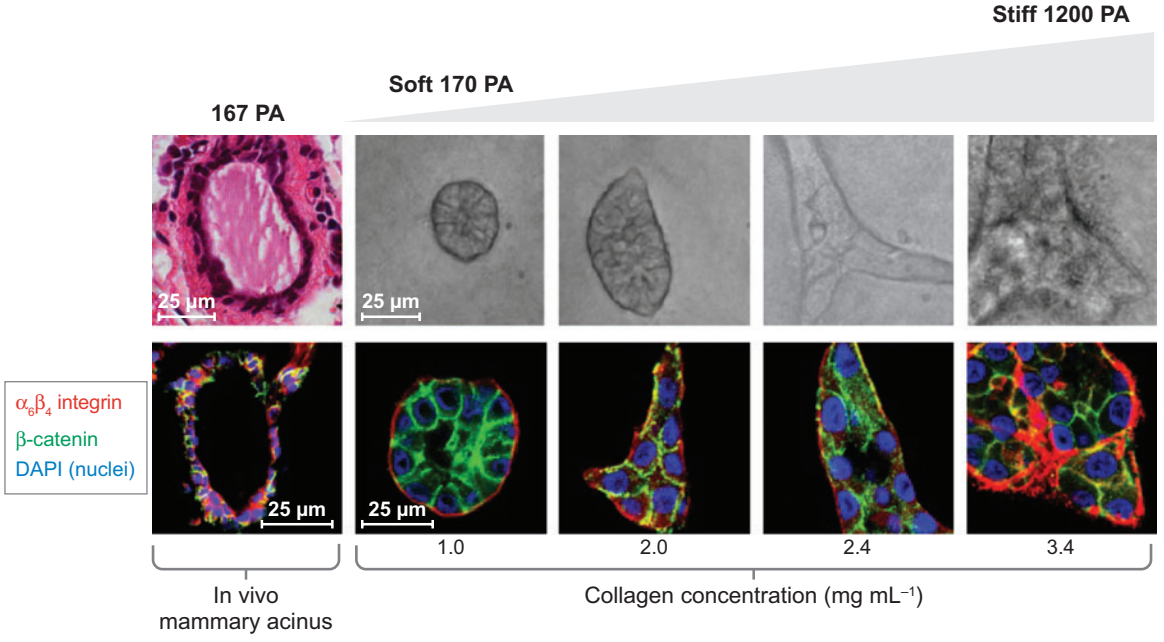

Figure 7

Altered 3D growth and morphogenesis of mammary epithelial cells with increased matrix rigidity. (*Top*) Phase contrast images and images of H&E-stained tissue comparing the morphogenesis of a mammary gland duct in a normal compliant environment (167 Pa) to mammary epithelial cells grown in reconstituted basement membrane/collagen I gels of increasing stiffness (170–1200 Pa). (*Bottom*) Confocal microscopic cross sections of a normal mammary duct versus mammary epithelial cell colonies cultured in increasingly stiff environments. Figure adapted with permission from Reference 45.

CONCLUSION

The studies overviewed here illustrate the power of 3D epithelial culture models as a tractable in vitro approach to model the early events in breast carcinoma formation. In recent years, these models have greatly illuminated our understanding of the fundamental biological processes that direct the histological abnormalities observed microscopically during the earliest stages of breast cancer. They also have provided a unique platform to discover previously unappreciated mechanical influences on glandular epithelial architecture and homeostasis. Nevertheless, current 3D culture models do have inherent limitations in modeling in vivo tissue behavior. The further improvement of 3D culture systems, particularly the development of innovative heterotypic coculture strategies and tunable biomaterial scaffolds, will be invaluable in modeling cancer progression and testing novel therapeutic strategies in a biologically relevant context.

SUMMARY POINTS

1. Epithelial cells grown in 3D recreate several cardinal features of glandular epithelium in vivo, including the formation of cyst-like acini with a hollow lumen, the apicobasal polarization of cells surrounding this luminal space, and the tight regulation of cell growth and proliferation. Cancer genes produce diverse morphogenetic phenotypes

in 3D cultures that resemble important histopathological features observed in carcinomas in vivo.

2. Cells occupying the hollow lumen of acini normally undergo apoptosis owing to the lack of ECM attachment (*anoikis*). However, numerous oncogenes and pathways aberrantly promote cell survival in the luminal space of 3D structures; the resultant phenotypes recapitulate the luminal filling observed in premalignant breast lesions.
3. Apicobasal polarity is a fundamental characteristic of glandular epithelium in vivo and in vitro. Recent studies using 3D culture systems are beginning to delineate how cell polarity regulates normal gland architecture and how the disruption of this polarity may contribute to carcinoma formation.
4. Studies of epithelial tumor cells in 3D culture demonstrate that modulating cell adhesion and growth factor receptor pathways can repress the phenotypic expression of their transformed state, even in the context of multiple oncogenic mutations.
5. Breast cancers are characterized by a dramatic increase in tissue stiffness due in part to bulk tumor growth, altered vasculature, and the expansion of a rigid (desmoplastic) stroma. Recent studies reveal that increasing matrix stiffness disrupts normal 3D morphogenesis and promotes several architectural features associated with malignancy.

FUTURE ISSUES

1. Immortalized human MEC lines commonly utilized for 3D culture assays express markers of both luminal and myoepithelial cell lineages. The hybrid nature of such lines limits 3D culture models in terms of their ability to simulate events in breast carcinoma progression in vivo. Improving cell culture conditions to propagate primary human mammary cells is critical in developing faithful 3D models for studying breast cancer initiation and progression.
2. A large fraction of human breast cancers are hormonally regulated; indeed, estrogen receptor and progesterone receptor status are a major prognostic determinant in breast tumors. However, owing to the lack of availability of human MECs (both primary cells and cell lines) responsive to these principal mammary hormones, the impact of hormone receptor activation on 3D breast morphogenesis remains completely unknown. 3D culture systems incorporating the intricate hormonal signaling unique to the mammary gland should be developed.
3. The studies discussed here utilize monotypic culture systems, in which epithelial cells are grown in isolation within a 3D scaffold. Because both normal epithelial tissues and tumors represent a community of heterotypic cell types, the use of such monotypic 3D cultures has inherent limitations. Hence, both current and future research efforts demand heterotypic culture systems that recreate the histological complexity of epithelial tissue in vivo.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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45. Increasing matrix stiffness disrupts normal 3D morphogenesis, demonstrating that mechanotransduction may directly regulate malignant transformation.

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Errata

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