Published in final edited form as:

Int J Biochem Cell Biol. 2007 ; 39(11): 1987–1994. doi:10.1016/j.biocel.2007.06.025.

Mammary epithelial cell: Influence of extracellular matrix composition and organization during development and tumorigenesis

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Abstract

Stromal–epithelial interactions regulate mammary gland development and are critical for the maintenance of tissue homeostasis. The extracellular matrix, which is a proteinaceous component of the stroma, regulates mammary epithelial growth, survival, migration and differentiation through a repertoire of transmembrane receptors, of which integrins are the best characterized. Integrins modulate cell fate by reciprocally transducing biochemical and biophysical cues between the cell and the extracellular matrix, facilitating processes such as embryonic branching morphogenesis and lactation in the mammary gland. During breast development and cancer progression, the extracellular matrix is dynamically altered such that its composition, turnover, processing and orientation change dramatically. These modifications influence mammary epithelial cell shape, and modulate growth factor and hormonal responses to regulate processes including branching morphogenesis and alveolar differentiation. Malignant transformation of the breast is also associated with significant matrix remodeling and a progressive stiffening of the stroma that can enhance mammary epithelial cell growth, perturb breast tissue organization, and promote cell invasion and survival. In this review, we discuss the role of stromal–epithelial interactions in normal and malignant mammary epithelial cell behavior. We specifically focus on how dynamic modulation of the biochemical and biophysical properties of the extracellular matrix elicit a dialogue with the mammary epithelium through transmembrane integrin receptors to influence tissue morphogenesis, homeostasis and malignant transformation.

Keywords

Mammary epithelial cells; Extracellular matrix; Matrix stiffness; Integrins

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Cell facts

- Stromal–epithelial interactions regulate mammary epithelial cell growth and differentiation during embryonic and postnatal development.
- The spatial organization and composition of the extracellular matrix influence mammary epithelial cell behavior.
- Alterations in extracellular matrix receptor expression can enable malignant transformation of the mammary epithelial cells.
- Differences in the biophysical properties of the extracellular matrix due to an increase in deposit and/or cross-linking can induce the malignant transformation of mammary epithelial cells.

1. Introduction

The mammary gland is a dynamic tissue derived from the epidermis that achieves full maturity in the adult. The development of the mammary ductal tree depends on stromal–epithelial interactions, and these interactions are important in embryonic and postnatal evolution. The stroma not only modulates the normal development of the mammary gland but also actively participates in malignant transformation of the tissue. Mammary ducts consist of luminal cells associated with myoepithelial cells surrounded by the basement membrane (BM) that separates the epithelium from the stroma. The stromal compartment is composed of mesenchymal cells (fibroblasts, blood cells and leukocytes) and extra-cellular matrix (ECM) (laminin, fibronectin, collagen, proteoglycans, etc.), which influence mammary development. In this short review, we focus on how the ECM modulates mammary epithelial growth and differentiation in embryonic development, postnatal ductal growth, branching morphogenesis, and carcinogenesis. Of the myriad of ECM–mammary epithelial cell interactions, integrin signaling will be discussed in more detail. The biochemical and biophysical cues from the extracellular stroma that guide mammary epithelial morphogenesis, homeostasis and malignant transformation will also be described.

2. Cell origin and plasticity

The mammary gland is a modified sweat gland derived from the ectoderm. Mammary gland development begins with ectodermal cell migration induced by the mesenchyme, followed by the formation of five pairs of disk-shaped placodes (emerging epithelial formations) and invasion of the cells into the dermis (Veltmaat, Mailleux, Thiery & Bellusci, 2003). The mesenchyme is instructive and provides critical information to drive mammary differentiation. For example, mammary mesenchyme can induce embryonic epidermis from dorsal or midventral sites to form mammary buds that will undergo functional differentiation and milk synthesis (Cunha et al., 1995), whereas recombination of embryonic mammary epithelial cells with salivary mesenchyme produces epithelial salivary structures (Sakakura, Nishizuka, & Dawe, 1976).

The stroma is not a static compartment; the cellular and ECM compositions evolve over time adapting to changes in the development of the gland. Two different mesenchymal tissues are involved in mammary gland development during embryogenesis: the fibroblastic cells surrounding the epithelial rudiment (the fibroblastic mesenchyme), and the fat pad cells (the fat pad mesenchyme). These two mesenchymes have different developmental properties demonstrated through tissue recombination studies. The fibroblastic mammary mesenchyme induces embryonic or adult mammary epithelial cells to form atypical ductal branching with hyperplastic ducts, while the fat pad induces epithelial cell elongation and branching (Sakakura, Sakagami, & Nishizuka, 1982). These changes in mammary development can be explained, in

part, by differences in ECM composition between the fibroblastic mesenchyme and the fat pad. For example, expression of laminin and protoheparan sulphate induced by the fat pad occurs simultaneously with epithelial rudiment elongation and branching (Kimata, Sakakura, Inaguma, Kato, & Nishizuka, 1985). Similar changes in ECM composition have been found in the growing ductal structure during puberty. The BM at the tip of the duct (the invasive front) is rich in hyaluronic acid, whereas the BM surrounding the duct is composed of collagen type IV, laminin, and proteoglycans (Fata, Werb, & Bissell, 2004). These data suggest that the abundance and composition of the ECM can regulate epithelial cell behavior.

Remodeling of the ECM is a tightly regulated process where the action of metalloproteinases (MMPs), the principal matrix-degrading enzymes, is regulated by tissue inhibitors of metalloproteinase (TIMPs). Inappropriate expression of MMPs or TIMPs can drive aberrant mammary gland phenotypes (Fata et al., 2004). For example, MMP2-null mammary glands have an excessive lateral branching with reduced ductal length, MMP3-deficient glands can elongate the ducts but with reduced secondary branching, and TIMP1-overexpressing glands demonstrate reduced ductal length (Fata et al., 2004).

The epithelial cells sense modifications in ECM composition through transmembrane receptors. These receptors, which include integrins (collagen, laminin, and fibronectin receptors), dystroglycan (laminin-1 receptor), discoidin domain receptor 1 tyrosine kinase (collagen receptor), and syndecans (co-receptors for heparan sulfate proteoglycans and fibronectin, laminin, collagen and growth factors), can modulate branching morphogenesis (Fata et al., 2004). For example, gene knockout models showed that while $\alpha 2$ integrin is necessary for branching morphogenesis, $\alpha 3$, $\alpha 6$ or $\alpha 4$ subunits are dispensable (Chen, Diacovo, Grenache, Santoro, & Zutter, 2002; Klinowska et al., 2001). Similarly, mice deficient in discoidin domain receptor 1 tyrosine kinase display excessive collagen deposition, delayed ductal development, and incomplete lactational differentiation (Vogel, Aszodi, Alves, & Pawson, 2001).

Increased collagen deposition can also alter the biophysical properties of the ECM augmenting extracellular tension. This elevation in tension has been shown to perturb mammary epithelial cell differentiation. For example, the functional and morphological differentiation of primary mammary epithelial cells (MECs) in response to lactogenic hormones can proceed only when mixed mammary cell populations isolated from pre-lactating mice are plated on floating collagen gels with reduced tensional forces. Indeed, mechanically loaded collagen gels fail to support acinar morphogenesis and functional differentiation (β -casein expression), because these rigid gels (similar to two-dimensional rigid plastic) drive focal adhesion assembly to promote cell spreading, increase MMP activity, and thereby interfere with endogenous BM maturation (reviewed in Paszek & Weaver, 2004). Our laboratory has been investigating how the biophysical properties of the ECM can regulate cell shape and BM-dependent MECs morphogenesis (acini formation). Using two- and three-dimensional (2D, 3D) natural and synthetic laminin-rich matrices of precisely calibrated stiffness, we have demonstrated that substrate compliance regulates cell shape (rounding), mammary tissue morphogenesis, and endogenous BM assembly (Paszek et al., 2005) (Fig. 1). Recently, matrix compliance has also been implicated in modulating functional differentiation of MECs, as determined by β -casein expression (Alcaraz et al., personal communication).

3. Functions

During pregnancy, epithelial cells within the alveoli proliferate and differentiate in response to lactogenic hormones and growth factors, achieving full milk production capacity during lactation. The pregnancy-induced alveolar morphogenesis of the mammary gland is dependent upon ECM cues that are interpreted by the mammary epithelial cells through $\beta 1$ integrin

signaling. The association of $\beta 1$ integrins with their heterodimeric α integrin subunit partners anchor the cell to the BM (through laminin and collagen IV binding) and to the surrounding stroma (such as through binding to collagen I or fibronectin). In the mammary gland, both $\alpha 5\beta 1$ (fibronectin receptor) and $\alpha 2\beta 1$ (collagen and laminin receptor) integrin expression levels are regulated by ovarian hormones and, as such, have been implicated as key transducers of hormonal cues that drive growth and differentiation of the gland during pregnancy (Woodward, Mienaltowski, Modi, Bennett, & Haslam, 2001).

Conditional deletion of $\beta 1$ integrin in luminal mammary cells demonstrated an essential role for $\beta 1$ integrin in alveolar development and differentiation during pregnancy and lactation (Naylor et al., 2005). The ablation of $\beta 1$ integrin not only resulted in malformed alveoli, but also in failure of prolactin (PRL)-induced mammary epithelial cell differentiation and milk synthesis (Naylor et al., 2005).

How does $\beta 1$ integrin regulate mammary epithelial cell differentiation? In response to ECM cues such as changes in matrix composition, integrins assemble into intracellular signaling complexes that are connected to the actin cytoskeleton and that activate growth and survival pathways, thereby transmitting cues from the matrix to influence cell morphology and fate. For example, cell anchorage to laminin-1 through $\beta 1$ integrin permits PRL-dependent activation of Janus Kinase-2 and signal transducer and activator of transcription-5 (Stat5) signaling pathways, and the transcription of PRL- and Stat5-regulated milk proteins (Naylor et al., 2005). The small Rho GTPase Rac1 has been shown to be a critical downstream effector of $\beta 1$ integrin signaling for the activation of PRL/Stat5 signaling cascade (Akhtar & Streuli, 2006). However, the precise cross-talk between integrin and PRL signaling has not been fully elucidated.

4. Associated pathologies

Modifying ECM-integrin interactions can profoundly influence expression of the malignant phenotype in culture and *in vivo* (Park et al., 2006). However, the molecular mechanisms whereby altered stromal-epithelial interactions regulate tumorigenesis are not well defined. The cellular component of the stroma has been implicated in promoting breast cancer development. Infiltrating leukocytes, which are recruited to the tumor as a result of tumor cell expression of chemotactic cytokines, provide cytokines, proteases and growth factors to stimulate tumor growth and promote neo-angiogenesis (Pollard, 2004). Moreover, the overexpression of transforming growth factor- β or hepatocyte growth factor by tumor associated-fibroblasts has also been implicated in the initiation of breast cancer (Kuperwasser et al., 2004). Besides secreting growth factors, activated fibroblasts are a vast source for ECM proteins. Changes in ECM composition can induce changes in epithelial cell integrin expression. For example, altered expression of $\beta 1$ -, $\beta 4$ -, $\alpha 2$ -, $\alpha 3$ - and $\alpha 6$ -integrins has been observed in mammary cancer cells (Taddei et al., 2004).

Integrin-mediated adhesion to the ECM is essential for cell growth and survival through activation of focal adhesion kinase (FAK) signaling cascades that promote cell viability (White et al., 2004). Moreover, reciprocal interactions between epidermal growth factor receptor (EGFR) and integrin signaling pathways control proliferation and survival of MECs, such that inhibiting either $\beta 4$ integrin, $\beta 1$ integrin or EGFR represses the malignant phenotype of tumor MECs growing in 3D BM matrix (Weaver et al., 1997). The functional integrity of $\beta 1$ integrin signaling is also essential for the induction of mammary tumors, as the ablation of $\beta 1$ integrin expression in tumor cells *in vivo* inhibits proliferation and expansion of the tumors cells (White et al., 2004). Recently, it was shown that $\beta 4$ integrin promotes tumor progression through amplified ErbB2 signaling, and that the loss of $\beta 4$ integrin signaling suppresses mammary

tumor onset and invasive growth *in vivo* (Guo et al., 2006). These data demonstrate a key role for integrins in malignant progression.

The development of breast cancer is characterized by the loss of tissue organization (Fig. 2A). Although breast tumor cells originate from epithelial cells, the stroma is an active participant of the epithelial malignant transformation. In the past decade, the stroma surrounding developing lesions has increasingly been appreciated as a critical regulator of malignant transformation and as a vital modifier of tumor behavior including metastasis and treatment responsiveness. The stromal desmoplastic response is characterized by the activation of fibroblasts, increased deposition, cross-linking and remodeling of the ECM, angiogenesis, and invasion of leukocytes. Up-regulated ECM gene expression and elevated MMP activities are not only found in tumors but can also correlate with poor patient prognosis (Jinga et al., 2006). For example, expression levels of the ECM protein lysyl oxidase (LOX), which is responsible for collagen cross-linking, is elevated in cancer patients and associated with metastasis and reduced patient survival (Erler et al., 2006). Furthermore, the increased ratios of MMP2:TIMP2 and MMP9:TIMP1 found in malignant breast tissues have been suggested to play a role in the aggressiveness of invasive breast carcinomas (Jinga et al., 2006).

Metastasis of breast cancer cells is associated with the majority of the disease fatalities, such that restricting disease progression and preventing metastasis represents the primary objective of many cancer prevention programs. In order for a tumor cell to metastasize, it must first degrade the BM and migrate into the surrounding stroma. Cleavage of laminin-5 by MMPs (2 and 14) exposes cryptic sites of the proteins that induce epithelial migration (Giannelli, Falk-Marzillier, Schiraldi, Stetler-Stevenson, & Quaranta, 1997). Another consequence of MMP activity is the release of growth factors trapped in the ECM that in turn can stimulate further epithelial cell invasion. During invasion, cells extend pseudopodia at the leading edge that attach to collagen fibers in the ECM at the migration front, allowing the cells to “crawl” linearly along the fibers toward the blood vessels (Condeelis & Segall, 2003).

Cell migration is also tightly regulated by cell adhesion, cell-generated contractility and cell haptotaxis to maximize their ligand binding and even duro-taxis towards a stiffer matrix to increase cell tension (Sheetz, Felsenfeld, & Galbraith, 1998; Wong, Velasco, Rajagopalan, & Pham, 2003). This suggests that elevated matrix deposition and tension likely couple with degradation to modulate tumor invasion and metastasis. In this respect, small Rho GTPases are functionally linked to cell migration and MMP-mediated invasion. Rac and Rho expression and activity are elevated in tumors and RhoC is implicated in the metastatic, aggressive phenotype of inflammatory breast tumors (Fritz, Just, & Kaina, 1999; Kleer et al., 2004). Consistently, inhibition of LOX involved in collagen cross-linking and fiber formation dramatically reduces invasion and metastasis in a mouse model of breast cancer (Erler et al., 2006).

Given that the ECM profoundly modulates tissue morphogenesis and that the stroma changes dramatically during breast tumorigenesis, it is logical to ask if modifications in matrix organization and stiffness could drive tumor invasion and contribute to metastasis. Consistently, we have shown that the mammary gland progressively stiffens during tumor progression and that is associated with increased collagen deposition, cross-linking and reorientation (Paszek et al., 2005). In concordance with these results, matrix stiffness, in association with growth factors, enhances ERK activation and increases cell contractility (Fig. 2B) (Paszek et al., 2005). Interestingly, force-generated activation of ERK may also influence cell proliferation, alter tissue behavior and drive anti-estrogen resistance during breast cancer treatment, through phosphorylation of ER α , recruitment of ER α co-activators or enhanced transcriptional activity, in a ligand-independent manner (Likhite, Stossi, Kim, Katzenellenbogen, & Katzenellenbogen, 2006). Indeed, tamoxifen, the anti-estrogen used in

the treatment of breast cancer, has been shown to reduce breast density and therefore the risk of recurrences (Atkinson, Warren, Bingham, & Day, 1999).

We showed that matrix stiffness and/or exogenous force independently induce cell-generated contractility to promote focal adhesion maturation and enhance integrin-dependent signaling thus compromising multi-cellular tissue morphogenesis and promoting a tumor-like behavior in mammary cells (Paszek et al., 2005). This suggests that matrix stiffness likely promotes breast tumorigenesis through altering integrins and their adhesion interactions. Conversely, we found that blocking integrin-dependent cell contractility reverted the malignant phenotype in culture (Paszek et al., 2005; Weaver et al., 1997). Consistent with these findings, ectopic expression of $\beta 1$ integrin mutants with increased transmembrane molecular associations (V737N and G744N), elevated cellular contractility and forced focal adhesion maturation increase integrin/growth factor-dependent signaling, again to compromise multi-cellular tissue morphogenesis and promote tumorigenic behavior in culture and *in vivo* (Paszek et al., 2005).

Investigating ECM composition and stiffness as a risk factor is critical given the profound changes in the mammary stroma associated with breast cancer and the diversity of diseases associated with changes in collagen deposition, orientation and cross-linking. Indeed, inhibition of matrix modifying ECM proteins can dramatically reduce tumor progression through effects on invasion and metastasis. It is plausible that as a consequence of matrix rigidity, altered mechanotransduction constitutes a key mechanism in regulating malignant transformation of epithelial cells. Moreover, there is evidence to suggest that changes in mammary ECM modify treatment responsiveness to anti-estrogens promoting the progression of breast carcinoma and thereby decreasing patient survival. Understanding the molecular mechanisms behind this process could lead to the design of a unique, targeted strategy to diagnose and treat this disease.

Acknowledgements

We apologize to the many authors whose work is not cited due to space limitations. This work was supported by NIH grants CA078731 to VMW, R01 GM072002-04 to MD, and DOD grants DAMD1701-1-0368, 1703-1-0496, and W81XWH-05-1-330 to VMW.

Abbreviations

ECM	extracellular matrix
MMP(s)	metalloproteinase(s)
EGFR	epidermal growth factor receptor
ERα	estrogen receptor alpha
PRL	prolactin
BM	basement membrane
MEC	mammary epithelial cell

2D or 3D

two or three dimensions

TIMPs

tissue inhibitors of metalloproteinase(s)

Stat5

signal transducers and activators of transcription protein 5

LOX

lysyl oxidase

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Fig. 1.

Mammary epithelial growth and morphogenesis is regulated by matrix stiffness. (A) 3D cultures of normal mammary epithelial cells within collagen gels of different concentration. Stiffening the ECM through an incremental increase in collagen concentration (soft gels: 1 mg/ml Collagen I, 140 Pa; stiff gels 3.6 mg/ml Collagen I, 1200 Pa) results in the progressive perturbation of morphogenesis, and the increased growth and modulated survival of MECs. Altered mammary acini morphology is illustrated by the destabilization of cell-cell adherens junctions and disruption of basal tissue polarity indicated by the gradual loss of cell-cell localized β -catenin (green) and disorganized β 4 integrin (red) visualized through immunofluorescence and confocal imaging. On the right side of the panel is an illustration of

a mammary ductal structure (luminal and myoepithelial cells surrounded by BM), and depicting MEC morphogenesis in soft and stiff gels. Scale bars represent 25 μm . (B) Confocal immunofluorescence images of MEC colonies on soft and stiff gels (140 vs. >5000 Pa) stained for β -catenin (red) and E-cadherin (green), and counterstained with DAPI (blue) after triton X-100 extraction. β -catenin could be extracted from the sites of cell–cell interaction in MEC colonies formed on a stiff but not on a soft gel, indicating that adherens junctions are less stable in MEC structures formed on stiff gels. White arrowheads indicate diffuse staining patterns of β -catenin and E-cadherin. Modified from Paszek et al. (2005).

Fig. 2.

Malignant transformation of mammary epithelial cells is regulated by matrix stiffness. Breast transformation ensues through progressive acquisition of genetic alterations in the luminal epithelial cells residing within the mammary ducts. The tissue stroma responds to these epithelial alterations by initiating a desmoplastic response that is characterized by activation and transdifferentiation of fibroblasts, infiltration of immune cells, increased secretion of growth factors and cytokines, and elevated matrix synthesis and remodeling that manifests as matrix stiffening. (A) Cartoon depicting the stages of breast tumorigenesis (from left to right; normal ducts, ductal carcinoma *in situ* and invasive phenotype), highlighting key desmoplastic changes within the tissue stroma. (B) Force-dependent focal adhesion maturation mediated by elevated tumor matrix stiffness. Integrins are bidirectional mechanosensors that integrate biochemical and biophysical cues from the matrix and the actin cytoskeleton and transduce cell-generated force to the surrounding microenvironment. Activated integrins bind to ECM proteins via cooperative interactions between their alpha and beta extracellular domains and form nascent highly dynamic adhesion signaling complexes. In response to external mechanical force or elevated cell-generated contractility integrin clustering is enhanced and the recruitment of multiple integrin adhesion plaque proteins including talin and vinculin is