Review

Myoepithelial cells: good fences make good neighbors

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Abstract

The mammary gland consists of an extensively branched ductal network contained within a distinctive basement membrane and encompassed by a stromal compartment. During lactation, production of milk depends on the action of the two epithelial cell types that make up the ductal network: luminal cells, which secrete the milk components into the ductal lumen; and myoepithelial cells, which contract to aid in the ejection of milk. There is increasing evidence that the myoepithelial cells also play a key role in the organizational development of the mammary gland, and that the loss and/or change of myoepithelial cell function is a key step in the development of breast cancer. In this review we briefly address the characteristics of breast myoepithelial cells from human breast and mouse mammary gland, how they function in normal mammary gland development, and their recently appreciated role in tumor suppression.

Introduction

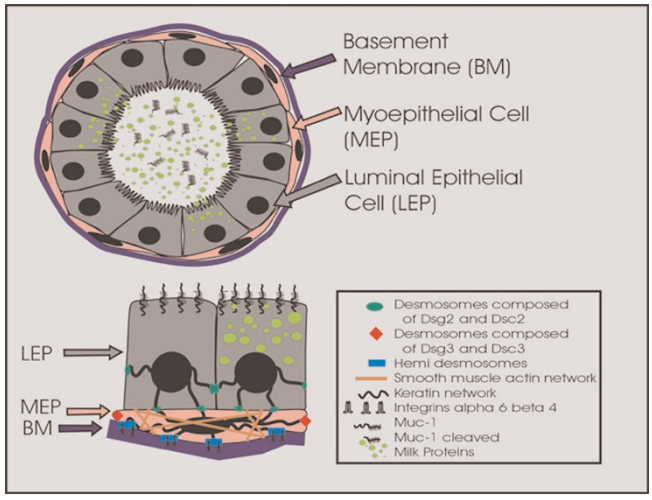
The mammary ductal tree is a bilayered structure that consists of an iterative repetition of basic functional elements. However, when comparing the mouse and human mammary glands, differences emerge. In the mouse the mammary epithelial cells are encased by a periductal stroma that is surrounded by fat tissue, whereas human breast epithelial cells are directly encompassed by highly vascularized intralobular loose connective tissue, and are separated from the adipose tissue by dense interlobular fibrous connective tissue [1]. Moreover, in the mouse, branching ducts terminate in end buds that differentiate during pregnancy and lactation into lobular acini (for review [2]), whereas the human breast exhibits a higher level of differentiation, with terminal ductal lobular units present in the resting state; these lobular acini differentiate further during pregnancy and lactation to secrete milk (for review [1]).

The ductal network in both mouse and human is comprised of two epithelial cell types: luminal epithelial and myoepithelial cells. Ductal myoepithelial cells are spindle shaped and oriented parallel to the long axis such that they form a continuous layer around the luminal cells, especially in the ducts (Fig. 1); upon contraction the myoepithelial cells decrease the length and increase the diameter of the ducts to eject the milk [3]. In contrast, acinar myoepithelial cells are stellate shaped, forming a discontinuous basket-like network around the luminal cells, although during pregnancy and lactation the myoepithelial cell body and processes extend to fully encompass the expanded alveolar epithelial cells [3]. Functionally, myoepithelial cells are a hybrid of both smooth muscle ('myo') and epithelial cells (Table 1). Like muscle cells, myoepithelial cells express filamentous smooth muscle actin and smooth muscle myosin, and exhibit contractile properties; like epithelial cells, myoepithelial cells express intermediate filaments (the epithelial keratins) [4-6] and have cadherin-mediated cell–cell junctions [1,4,7,8]. Structurally, myoepithelial cells form distinct desmosomes with both luminal cells and other myoepithelial cells, generate gap junctions and cadherin–cadherin interactions with other myoepithelial cells, and adhere to the basement membrane (BM) via hemidesmosomes [9-12].

The structural and functional elements of myoepithelial cells are inextricably linked. During lactation, myoepithelial cells contract in response to oxytocin and move milk into the ducts (for review [13]), and gap junctions and cadherin-based interactions connecting myoepithelial cells function to coordinate the ejection of milk smoothly (for review [14]). During development, myoepithelial cells also act to induce luminal cell polarity [5,15] and to regulate ductal morphogenesis [16]; here, connection to the BM and the desmosomal interactions with the luminal epithelial cells facilitate paracrine regulatory mechanisms. Proper coordination of all of these activities is necessary to maintain normal breast function; accordingly, it is unsurprising that the loss of

BM = basement membrane; DCIS = ductal carcinoma *in situ*; Dsc = desmocollin; Dsg = desmoglein; ECM = extracellular matrix; ER = estrogen receptor; HGF = hepatocyte growth factor; MMP = matrix metalloproteinase; MMTV = mouse mammary tumor virus; PTHrP = parathyroid hormone-related peptide; TEB = terminal end bud.

Figure 1



Cross-section of a bilayered duct. Secretory luminal cells (LEPs) are apically located to contractile myoepithelial cells (MEPs) and the basement membrane (BM). Milk proteins and cleaved Muc1 are secreted into the luminal space during lactation. Desmosomes containing desmoglein (Dsg)2 and desmocollin (Dsc)2 form between adjacent luminal cells and between adjacent LEPs and MEPs. Desmosomes between MEPs contain Dsg3 and Dsc3. MEPs as contractile cells contain smooth muscle actin and adhere to the BM via hemidesmosomes.

Table 1

Phenotypic traits of normal human breast myoepithelial cells

Myoepithelial markers	Ref.
CK5	[75]
CK14	[85]
CK17	[77]
BG3C8	[86]
Vimentin	[20]
GFA	[87]
α-Smooth muscle actin	[88]
Smooth muscle–MHC	[89]
Calponin	[89]
CALLA	[90]
Thy-1	[15]
P-cadherin	[91]
α_1 Integrin	[89]
α_6 integrin	[92]
β_4 integrin	[93]
Connexin-43	[94]
bFGF	[55]
Laminin	[95]
Maspin	[68]
Methallothionein	[96]

Adapted from Ronnov-Jessen and coworkers [1].

myoepithelial function is almost universally associated with breast cancer [1,15,17].

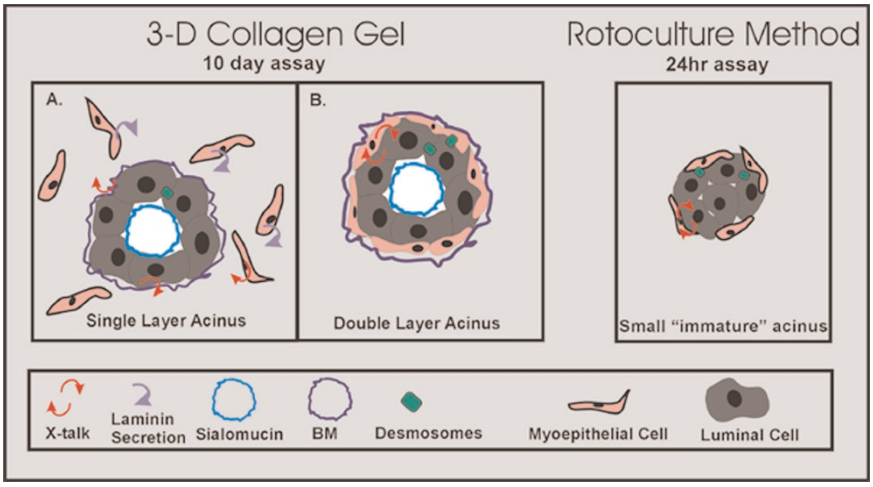
Myoepithelial function in normal breast

The functional interactions that define the bilayered acinus have been explored using three-dimensional culture systems. When phenotypically normal human or rodent luminal cells are grown in laminin-rich extracellular matrix (lrECM) gels, they recreate the structure and function of the acinus found *in vivo* even in the absence of myoepithelial cells [6,18]. We believe that this is possible, in part, because cultured luminal cells express a number of proteins that are characteristic of myoepithelial cells *in vivo* (e.g. β_4 integrin [10], epidermal growth factor receptor [19], vimentin [20], maspin [21], and others; for review [1]). It may be that luminal cells can form acinar structures in culture because of this ability to become luminal/myoepithelial 'hybrids'. The possibility that expression of specific myoepithelial proteins confers distinctive signaling cues that promote cell survival and proper apicobasal polarity is an active area of investigation in our laboratory and those of our collaborators [15,18,22,23].

Of the molecules produced by myoepithelial cells to regulate luminal cell function, laminin-1 and desmosomal proteins have emerged as key mediators. Laminin-1 is a heterotrimer of α1, β1 and γ1 chains, and is a major component of BM (for review [24]). Embryos derived from murine embryonic stem cells null for the laminin-1 β1 and γ1 chains are embryonically lethal at day 5.5 and lack BM [25,26]. Interestingly, embryos derived from murine embryonic stem cells null for the laminin-1 α1 chain or the α1 LG4-5 domains are also embryonically lethal; however, these null embryos do form an embryonic BM, possibly because of compensation by the α5 chain from laminin-10 (α5β1γ1) [26-28].

Cell/laminin-1 interactions were previously implicated in tissue morphogenesis and maintenance of polarity in kidney, salivary gland, and intestine and mammary epithelial cells [29-32], and we showed that interactions with laminin-1 are important for the functional mammary cell differentiation to produce the milk protein β-casein [33]. Disruption of signaling by β_1 integrin inhibitory antibodies or by the E-3 fragment of laminin-1 inhibits the expression of β-casein [33,34], and subsequent experiments have suggested that organized polymerization of laminin-1 is required for functional mammary differentiation [35-37]. We previously showed that human breast luminal cells, when grown in three-dimensional type I collagen as opposed to laminin-rich gels, form structures with altered integrins [38] that have reversed polarity and lack central lumina [15]; however, if these same cells are cocultured with myoepithelial cells in collagen I gels

Figure 2



Three-dimensional culture method versus rotary culture. The methods shown utilize isolated purified human breast luminal and myoepithelial cells from reduction mammoplasty. In the three-dimensional culture method, coculture of purified luminal and myoepithelial cells in collagen I gel results in the formation of two different types of structures. The majority are **(a)** a single layer of cells that form acinar structures in which the secretion of laminin-1 by surrounding myoepithelial cells signals to luminal cells to polarize correctly, and the minority are **(b)** double layer acinar structures that are more reflective of the acinus *in vivo*. Gudjonsson and coworkers [15] showed that myoepithelial cells were able to induce correct luminal polarity via the synthesis of the basement membrane (BM) component laminin-1. In contrast, in the **(c)** rotary culture method, purified luminal and myoepithelial cells are grown in suspension. Acinar structures form, albeit at a smaller size compared with the three-dimensional method. Runswick and coworkers [5] showed that blocking desmosome adhesion via blocking peptides inhibited acinar formation.

they exhibit correct apicobasal polarity, as they do when cultured in lrECM gel [15] (for review [39]). It was revealed that the myoepithelial cells are the only epithelial cells in the breast that produce the α1 chain of laminin-1, and thus they are a key determinant for correct luminal cell polarization in three-dimensional collagen [15]. Although the experiments described above demonstrated that laminin-1 could direct the formation of acinar structures in three-dimensional cultures, it was not clear whether laminin-1 was the molecule that directed this morphogenic process *in vivo* or whether other molecules are also involved.

Parallel studies by others using a rotary culture system have suggested an alternative solution in which cell–cell adhesion may be the ultimate regulator for establishment of the acinar structure (Fig. 2). Runswick and coworkers found that inhibition of myoepithelial-specific desmosomal cadherins, desmocollin 3 (Dsc 3) desmoglein 3 (Dsg 3), prevented morphogenesis of the bilayered acinus structure and disrupted the basal positioning of myoepithelial cells [5,39]. These experiments suggested that functional desmosomes between adjacent myoepithelial cells and epithelial cells are involved in the formation of acinar-like structures. It remains to be shown whether laminin or desmosomal proteins are sufficient for polarity or whether both are required; this question is under investigation in our laboratory.

Several transgenic mouse models have provided further insight into the role played by myoepithelial cells during mammary gland morphogenesis. The cell adhesion receptor P-cadherin is localized to myoepithelial cells; among mice that are homozygous null for P-cadherin, virgin mice exhibit precocious mammary gland development similar to the differentiation that is normally present in early pregnant animals [40]. These findings suggest that myoepithelial expression of P-cadherin may provide an inhibitory signal for luminal cell growth [41]. The parathyroid hormone-related peptide (PTHrP) has been implicated in epithelial–stromal interactions during mammary gland development [42]. In the K14-PTHrP transgenic model, overexpression of the peptide hormone PTHrP in myoepithelial cells inhibits side branching, and ductal elongation is stunted compared with wild-type mice, suggesting that perturbing myoepithelial–stromal interactions affects growth and differentiation of luminal cells [43].

These studies provide insight into specific processes by which myoepithelial cells transmit information for apicobasal polarity and branching morphogenesis; future studies will need to focus on the molecular mechanisms by which these factors interact to establish the acinar structure and the hierarchical nature of their activities.

Paracrine regulator during morphogenesis

Ductal elongation requires the production and organization of new BM, and myoepithelial cells play a key role in these processes as well. Myoepithelial cells synthesize BM components such as collagen IV, laminin-1, laminin-5, and fibronectin that regulate ductal growth [44], and facilitate the

sculpting of new BM through the production of matrix metalloproteinases (MMPs), including MMP2 and MMP3 [45]. Myoepithelial cells also express morphogens and growth factors that are activated in a coordinated manner during morphogenesis. Neogenin, a receptor initially identified to act in short and long range neuronal guidance (for review [46]), is expressed by myoepithelial cells and cap cells in terminal end buds (TEBs), a specialized structure at the end of growing ducts [47]. In the mouse, the neogenin knockout is perinatally lethal; however, transplantation studies have shown that mammary glands null for neogenin exhibit altered TEBs that appear disorganized, display breaks in the BM, and contain aberrant subcapsular spaces [47]. It is thought that neogenin may mediate netrin-dependent cell clustering, which is required for the proper formation of the TEB structure [47]. Similarly, the ephrin receptor ephB4 is selectively expressed in myoepithelial cells [48], and mouse mammary tumor virus (MMTV)-ephB4 transgenic mice exhibit defects in mammary gland development with delayed maturation, decreased branching, and decreased alveolar development [49]. Myoepithelial cells also express the heparin-binding growth factor pleiotrophin (also known as HARP), which is active during growth and development [50], and epimorphin, a morphogen that is required for mouse mammary gland branching in three-dimensional culture assays [51]. Over-expression of epimorphin disrupts the organization of the ductal tree in transgenic mice [52]. Furthermore, myoepithelial cells synthesize and secrete basic fibroblast growth factor (bFGF) [53-55] and hepatocyte growth factor (HGF/SF), which function during tubular morphogenesis [56]. (In culture assays HGF is believed to be sufficient to mediate branching [57]; however, we previously showed that it does so only if epimorphin is also expressed [51].) Also, myoepithelial cells may modulate HGF-stimulated branching by expression of activin Ba, a member of the transforming growth factor-β superfamily [58]. Sophisticated branching morphogenesis assays utilizing isolated luminal and myoepithelial cells will be necessary to dissect how these interactions control mammary gland branching.

Myoepithelial cells act in tumor suppression

The majority of breast cancer studies have focused on luminal cells, because these are known to be the source of most carcinomas of the breast (for review [1]). However, progression to carcinoma involves alteration of the entire organized structure of the breast; depending on tumor grade, the changes can include the loss of apicobasal polarity, collapse of the glandular structure, disappearance of normal myoepithelial cells, and disruption of the BM at the epithelial–stromal junction [1]. The mechanisms responsible for the loss of the myoepithelial layer and BM in invasive cancer are unknown. Man and Sang [59] proposed that loss of myoepithelial cells in cancer is due to localized death of these cells; however, this is not proven, and the potential factors responsible for selective cell death are not known. How myoepithelial cells may act to suppress tumor progression *in vivo* and how these functions are compromised during cancer development remain major unanswered questions.

It is generally believed that myoepithelial cells rarely become malignant (for review [60]). Recently, Angele and coworkers [61] found that human luminal and myoepithelial cells differ in their DNA repair capacity, and this may contribute to the lower rate of transformation in myoepithelial cells. Additionally, when they do undergo transformation, they usually form benign or low-grade neoplasms. Myoepithelial cells express many ECM structural proteins, proteinase inhibitors and angiogenic inhibitors, and accumulate ECM rather than degrade it, which may explain in part why these lesions are not invasive [62,63].

In addition, myoepithelial cells express a number of type II tumor suppressor genes, defined as factors that affect phenotype through changes in expression rather than through genetic mutation (Table 2) [64,65]. Barsky and coworkers [62,66] were the first to use functional assays to show that myoepithelial cells exhibit many antitumorigenic properties, such as the ability to inhibit tumor cell invasion and angiogenesis. Subsequent studies revealed that myoepithelial-conditioned media inhibited the growth of breast cancer cell lines and induced a G_2/M cell cycle arrest [67]. The ability of myoepithelial cells to inhibit breast cancer cell growth and invasion may in part be attributed to their expression of maspin, a member of the serpin family of serine protease inhibitors. Over-expression of maspin in the breast cancer cell line MDA-MB-435 resulted in inhibition of tumor functions such as growth, angiogenesis, and invasion [68]. In addition, Jones and coworkers [69] showed that myoepithelial cells inhibit invasion through downregulation of MMP expression by tumor cells and fibroblasts. These data suggest that normal myoepithelial cells inhibit tumor cell function through a combined suppression of tumor cell growth, invasion, and angiogenesis.

Do cancer myoepithelial cells have altered function?

The myoepithelial layer appears to remains intact in ductal carcinoma *in situ* (DCIS); despite this, the myoepithelial cells appear to be aberrant because they differ from normal myoepithelial cells in gene expression, and secrete many chemokines and other factors [70]. This indicates that although myoepithelial cells are present, they no longer send the correct signals to luminal cells. This observation raises the question of whether there are differences between normal myoepithelial cells and those myoepithelial cells that are present in DCIS. Gudjonsson and coworkers [15] found that 20% of carcinomas in which myoepithelial cells were present expressed little or no laminin-1, and that purified cancer myoepithelial cells were unable, for the most part, to 'polarize' luminal cells in three-dimensional collagen assays. These data suggested that cancer myoepithelial cells might be unable to transmit the necessary cues to induce correct luminal cell

Table 2

Type II tumor suppressor genes expressed by myoepithelial cells

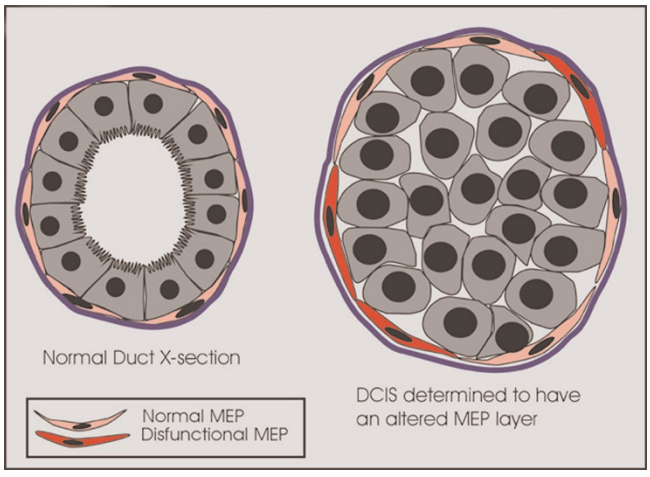
Myoepithelial tumor suppressor genes	Function	Reference
α-Smooth muscle actin	Cytoskeletal structure; suppress cell growth and motility	[97,98]
Cytokeratin-5	Cytoskeletal structure; regulates cell growth	[99]
α_6 integrin	ECM receptor	[100]
Caveolin-1	Regulation of cell growth	[101]
Connexin-43	Gap junction protein	[102]
Maspin	Protease inhibitor	[68]
TIMP-1	Protease inhibitor	[17]
Relaxin	Hormone-regulation, cell growth	[103]
Activin	Hormone regulation	[58]

Adapted from Bissell and Radisky [65].

polarity, at least in part due to their inability to produce laminin-1.

In another study, Allinen and coworkers [70] used SAGE (serial analysis of gene expression) to identify gene expression differences between myoepithelial cells isolated from normal and DCIS samples (Fig. 3). Moreover, those investigators found that cancer myoepithelial cells exhibited the greatest changes in gene expression, and that the chemokine CXCL14 was expressed at higher levels in the DCIS myoepithelial cells than in normal myoepithelial cells. Recently, the chemokine CXCL12/SDF-1 and its receptor CXCR4 were implicated in the induction of tumor cell growth and metastasis [71-73]. Thus, cancer myoepithelial cells, rather than being tumor suppressors, may act to induce growth, migration, and invasion of breast cancer cells, and to undermine the integrity of BM.

Figure 3



DCIS myoepithelial cells exhibit an altered gene expression. In the normal breast myoepithelial cells (MEPs) are located between the luminal cells and the basement membrane. By their location they might act as a barrier to tumor invasion. In ductal carcinoma *in situ* (DCIS) the myoepithelial layer is still present; however, Allinen and coworkers [70] recently showed that there appears to be molecular differences between MEPs present in normal breast versus DCIS lesions.

Partial myoepithelial differentiation in invasive cancer

Myoepithelial cells and myoepithelial differentiation are largely absent in breast cancer (for review [74]), although there are exceptions to this rule [75-77]. In the microarray analysis performed by Perou and coworkers [78], the 15% (6/40 cases) of tested breast cancer cases that exhibited partial myoepithelial differentiation were also estrogen receptor (ER) negative, and Keese-Adu and colleagues [79] found that 29% (22/77 cases) of tested ER-negative breast cancer samples also exhibited a partial myoepithelial phenotype. These observations suggest a relationship between the loss of ER expression and acquisition of myoepithelial characteristics in breast cancer cells. The expression of the myoepithelial proteins keratin 14, $\alpha_6\beta_4$ integrin, and Dsg 3 in breast cancer cell lines has been shown to correlate with a more aggressive phenotype in cell culture assays [80]. The role played by myoepithelial cells in normal breast as mediators of cell–ECM survival signaling and controllers of morphogenesis may provide insight into why the loss of myoepithelial cells in tumors appears to be linked to expression of myoepithelial characteristics in some breast cancer cells; expression of myoepithelial proteins such as $\alpha_6\beta_4$ integrin may promote tumor cell survival and metastasis in the absence of tumor suppressive functions of normal myoepithelial cells [22].

Conclusion

A key unknown player is the nature of the myoepithelial precursor cell, identification of which may help to define the

pathways that stimulate myoepithelial differentiation and how these pathways are disrupted during tumorigenesis. We and others have shown that a bipotential progenitor cell may reside in the luminal cell compartment; in cell culture suprabasal luminal cells ($MUC1^-/ESA^+$) are able to generate both luminal and myoepithelial cells [81,82]. If myoepithelial cells are derived from a bipotential cell, then what pathways stimulate myoepithelial fate? Using the mammosphere culture system, Dontu and coworkers [83] showed that Notch signaling stimulates multipotential progenitor cells to adopt a myoepithelial lineage specific commitment. The Wnt signaling pathway has also been implicated in myoepithelial differentiation. Lie and coworkers [84] found that mammary gland hyperplasias and tumors from Wnt-1 transgenic mice contained a population of cells that expressed progenitor cell markers Keratin-6 and Sca-1 [84]. Interestingly, the Wnt-1 tumors stained positive for both luminal and myoepithelial cell markers, and similar results were found with the MMTV–β-catenin and MMTV–c-*myc* transgenic mouse models. Loss of heterozygosity for PTEN was detected in both the luminal and myoepithelial cells, suggesting a common origin [84].

Clearly, the function of myoepithelial cells in the breast is more than just contractility, and myoepithelial cells are more than a fence between the milk-producing luminal cells and the surrounding stroma. It is clear that much remains to be learned about the physiological role of these cells in the normal breast and the functional differences between normal and cancer myoepithelial cells.

Competing interests

The author(s) declare that they have no competing interests.

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