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Myoepithelial Cells: Their Origin and Function in Breast Morphogenesis and Neoplasia

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Abstract

The human breast epithelium is a branching ductal system composed of an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells that terminate in distally located terminal duct lobular units (TDLUs). While the luminal epithelial cell has received the most attention as the functionally active milk-producing cell and as the most likely target cell for carcinogenesis, attention on myoepithelial cells has begun to evolve with the recognition that these cells play an active part in branching morphogenesis and tumor suppression. A major question that has been the subject of investigation pertains to how the luminal epithelial and myoepithelial lineages are related and precisely how they arise from a common putative stem cell population within the breast. Equally important is the question of how heterotypic signaling occurs between luminal epithelial and surrounding myoepithelial cells in normal breast morphogenesis and neoplasia. In this review we discuss data from our laboratories and from others regarding the cellular origin of human myoepithelial cells, their function in maintaining tissue polarity in the normal breast, and their role during neoplasia.

Keywords

breast myoepithelial cells; progenitors; polarity; collagen; laminin-1

INTRODUCTION

The human breast contains a branching ductal network composed of two epithelial cell types: an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells, separated from the collagenous stroma by a laminin-rich basement membrane. The ductal network terminates in lobular units commonly referred to as the terminal duct lobular units (TDLUs) (1). The TDLUs, composed of lobules that contain acini that function to secrete milk during lactation, are formed from tubular epithelial structures through a process called branching morphogenesis which is a highly conserved developmental process seen across the animal kingdom. This process gives rise to the airways of the lungs, the urine-collecting ducts, the prostate and salivary glands as well as the functional unit of the breast (2). There is ample

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evidence from both mice and humans that the two different epithelial cells that make up the TDLU, luminal epithelial and myoepithelial cells, are derived from common ancestors, namely the breast epithelial precursor cells positioned within the luminal epithelial compartment (3, 4). Although not yet unequivocally identified, breast epithelial stem cells are thought to be responsible for continuous cell renewal, growth, and branching throughout the reproductive period, as well as the massive epithelial expansion seen during pregnancy (reviewed in (5,6)).

Because breast cancer arises mainly in the luminal epithelial compartment of the TDLU (7), until recently little attention has been paid to the surrounding myoepithelial cells. Myoepithelial cells are localized between luminal epithelial cells and the stroma, which ideally positions them to communicate with both compartments. Moreover, recent studies indicate that myoepithelial cells may function as a guardian of tissue integrity in the human breast by maintaining tissue polarity (8,9). Myoepithelial cells, which are present in the normal and premalignant breast, and which generally continue to surround preinvasive *in situ* carcinomas, rarely transform; however, when they do transform, they generally give rise to tumors of low malignancy (10). During breast cancer progression, the fully differentiated myoepithelial cells are outnumbered by cancer cells and gradually disappear (see discussion below) (11). This loss is consistent with the hypothesis that fully differentiated myoepithelial cells are natural tumor suppressors. Furthermore, both *in vitro* and *in vivo* studies have confirmed the ability of myoepithelial cells to suppress tumor growth and invasion (10,12). Thus the overall interest in myoepithelial cell biology is increasing rapidly along with an appreciation of the importance of the myoepithelial cell involvement in normal breast morphogenesis and tumor suppression. In this review, we focus on the origin of myoepithelial cells in the adult breast and their role in maintaining epithelial cell polarization, but first we provide a portrait of the myoepithelial cell in brief.

THE BREAST MYOEPITHELIAL CELL AT A GLANCE

In vivo, myoepithelial cells are attached to the basement membrane (BM) by hemidesmosomes and to the adjacent luminal epithelial and myoepithelial cells by desmosomes (13). The myoepithelial cells lining the ducts are spindle-shaped cells oriented parallel to the long axis of ducts as a continuous layer. The myoepithelial cells in TDLUs are discontinuous, stellate-shaped, and form a basket-like network around acini, allowing some luminal epithelial cells to directly contact the BM (11,14). The myoepithelial cells express cytokeratins (CK) characteristic for the basal layer of stratified epithelia, such as CK 5, CK 14, and CK 17 (1, 15). The CK 5 and 14 have an important role in the cytoarchitecture of myoepithelial cells, as they are connected to desmosomes and hemidesmosomes which mediate the connection of myoepithelial cells to adjacent cells and the underlying BM, respectively (8,16). The plasma membrane of myoepithelial cells is characterized by the presence of pinocytic vesicles (17), which are now classified as caveolae (18). Interestingly, Hamperl in his 1970 review suggested that myoepithelial cells may control transport and metabolism of molecules between luminal epithelial cells and the basement membrane (19). The myoepithelial cell cytoplasm is filled with actin and myosin, which are responsible for the contractile phenotype mediated by oxytocin during lactation (20,21). In fact, the most striking myodifferentiated phenotype of myoepithelial cells is their expression of the filamentous α -smooth muscle actin (α -sm actin) and heavy chain-myosin (hc-myosin) (22,23). Myoepithelial cells contribute significantly to basement membrane production by expression and deposition of fibronectin, collagen IV, nidogen, and the bioactive laminins (9,11,24). They also possess BM receptors, including integrins, which mediate cell–BM attachment and occasionally cell–cell interactions (25). In particular, β 4 and α 1 integrins are expressed in myoepithelial cells (23,26). Furthermore, a number of tumor suppressor proteins, including p63, p73, 14-3-3 sigma, maspin, and Wilms Tumor have been preferentially detected in myoepithelial cells (27–29), consistent with the apparent endogenous tumor suppressor function of myoepithelial cells. The above description is focused on the fully differentiated myoepithelial cell, but recent data indicate the existence

of a morphologically distinct myoepithelial cell, which lacks expression of α -sm actin and in some cases also lack CK 5, 14, and 17 (28), indicating that a hierarchical differentiation pattern may exist within the myoepithelial lineage. It has been suggested that these minimally or partially differentiated myoepithelial cells may affect the function and biological behavior of adjacent epithelial cells (28), highlighting the need to better understand the origin and lineage development of myoepithelial cells in the human breast.

THE ORIGIN OF MYOEPITHELIAL PROGENITOR CELLS

The question of how myoepithelial cells arise in the mammary gland has gained increased interest in light of accumulating findings that they play a pivotal role in the development and maintenance of breast tissue and that they may exert an important tumor suppressive role (reviewed in (30)). The cornerstone for successful studies concerning lineage relationships and the function of various cell types within the breast was the introduction and improvement of cell separation techniques. In order for researchers to unequivocally identify myoepithelial precursor cells, it has been necessary to separate different cell lineages from the human breast and culture them separately under conditions that maintain their phenotypic marker profile observed *in situ*. On the basis of antibodies against lineage-specific cell surface markers, a number of laboratories have succeeded in purifying primary luminal epithelial and myoepithelial cells from both the rodent mammary gland and the human breast (3,^{15,31–35}).

We have shown that luminal epithelial and myoepithelial cells can be cultured in serum-free media (CDM6 and CDM4, respectively) for up to seven passages without losing their lineage-specific characteristics; the exception is that myoepithelial cells down-regulate α -sm actin and CALLA in culture (3). However, the myoepithelial cells will reexpress the α -sm actin upon addition of serum at confluence (3). Using the purified cell populations, it was possible to address which cell type gave rise to the other by switching the culture medium. Upon addition of CDM6, the myoepithelial cells kept their original phenotype until entering senescence. In contrast, when purified luminal epithelial cells were cultured in CDM4, a subpopulation showed gradual conversion in their differentiation pattern towards the myoepithelial lineage. This switch was judged by monitoring the gradual loss of CK 18 and 19 and the gain of vimentin, CALLA, and serum-inducible α -sm actin ((3); reviewed in (36)).

These data are supported by results from other laboratories (34,^{37,38}). Smalley *et al.* demonstrated that sorted mouse myoepithelial cells give rise only to a single type of clones, whereas sorted luminal epithelial cells give rise to three morphologically distinct clonal types which show progressive acquisition of myoepithelial markers (34,37). In another study, albeit not using cell separation protocols, Kao *et al.* defined two breast epithelial cell types by clonal dilution culture. Type I cells express luminal epithelial markers and stem cell characteristics by being able to both differentiate into other cell types and to form budding structures on reconstituted basement membrane (rBM). Type II cells exhibit a myoepithelial phenotype and form spherical organoids in rBM (38). Interestingly, only type I cells could convert to type II cells not type II to type I (38). Thus it seems reasonable to conclude that the luminal epithelial and myoepithelial cell lineages are related, and that luminal epithelial cells give rise to myoepithelial cells, at least in the adult gland. This in turn suggests that bipotent progenitor cells may reside within the luminal epithelial compartment.

IDENTIFICATION OF A PUTATIVE STEM CELL COMPARTMENT WITHIN THE HUMAN BREAST

Many laboratories, including ours, have invested a major effort towards identification of putative stem cell compartments within the mammary gland. This stem cell niche is believed to hold the key to the definitive origin of both luminal epithelial and myoepithelial cells, as

well as providing a possible cell population for the origin of breast cancer (reviewed in (5,⁶, 39)).

Stingl *et al.* previously identified a bipotent human breast epithelial cell in culture that is negative or weakly positive for MUC1, a marker of differentiated luminal epithelial cells (40). It is thought that this cell type originates from the luminal epithelial compartment, based on strong expression of epithelial-specific antigen (ESA), a basolateral marker exclusively expressed on luminal epithelial cells in the breast (40). As described above, there is accumulating evidence from work performed on cells in culture that progenitor cells with dual characteristics, i.e., the ability to differentiate into luminal epithelial and myoepithelial cells, originate from a cell in the luminal epithelial compartment that is MUC1 negative. It seems reasonable to believe that these MUC1 negative cells are indeed full members of the luminal epithelial lineage, as they express luminal epithelial markers, such as simple keratins and ESA, and do not express markers of the myoepithelial differentiation (4).

Previous studies in rodents have led to the widely accepted hypothesis that epithelial stem cells have a basal location (reviewed in (41,42)). Initially, ultrastructural studies in the rat mammary gland identified three cell types, luminal epithelial cells, myoepithelial cells, and basal clear cells, the latter of which has been proposed to constitute a stem cell population (43–45). Smith *et al.* described a gradual transition of basal clear cells to fully differentiated myoepithelial cells in human breast, but did not observe any relationship between basal clear cells and luminal epithelial cells (46). In contrast, in the mouse mammary gland, the ultrastructural characterizations, also by Smith's laboratory, led to a proposed model of lineage evolution and identification of the putative stem cells (small light cells, SLCs), first degree progenitor cells (not ultrastructurally distinct from SLCs), second degree progenitors which are still multipotent (undifferentiated large light cells, ULLCs), and two compartments of nondividing, pre-luminal and pre-myoepithelial cells that gradually mature into the fully differentiated lineages (41). At the ultrastructural level, SLCs never reach the acinar lumen, and only a fraction of ULLC do so.

In concordance with the above studies, we have identified a suprabasal MUC1-negative, ESA-positive, and α -sm-actin-negative cell in the human breast (4). The frequency of MUC1-negative, ESA-positive cells in smears of trypsinized, uncultured cells was 8%, which agrees with previous murine data from which the estimated frequency of stem and progenitor cells was also estimated to be about 8–12% (42). Isolation of the MUC1-negative and ESA-positive suprabasal cells resulted in a small population of cells which were then immortalized using retroviral constructs containing the E6 and E7 oncogenes from human papilloma virus 16 (4). The suprabasal-derived epithelial cell line was able to generate both differentiated luminal epithelial cells and myoepithelial cells, as determined by the expression of MUC1 and thy-1, thus indicating their bipotential nature as discussed above. Furthermore, the suprabasal-derived cell line resembled a subpopulation *in situ* of luminal epithelial cells in the normal breast by the restricted expression of CK19. Purification of thy-1 positive cells from the suprabasal-derived epithelial cell line resulted in a population of myoepithelial cells expressing α -sm actin mRNA and protein. When tested for stem cell characteristics in 3D lreCM, the suprabasal-derived cell line gave rise to elaborate structures reminiscent of the functional unit, i.e., the TDLU (Fig. 1), with an inner layer of CK19-positive luminal-like cells and an outer layer of CK14-positive myoepithelial-like cells. Thus when taken together, there is strong evidence that luminal epithelial and myoepithelial cells in the TDLU are derived from a suprabasal cell type, a bona fide member of the luminal epithelial compartment.

MYOEPIThelial CELLS AS REGULATORS OF TISSUE POLARITY

Myoepithelial cells are juxtaposed between the luminal epithelial cells and the surrounding collagen-rich stroma. Therefore, they are not only situated in an ideal position to communicate between these two compartments, but they are ideally positioned also to provide important regulatory signals for the maintenance of normal breast structure (8,9). Together with the development of techniques to isolate myoepithelial cells from normal breast tissue, it is now possible to conduct ablation experiments to explore the function of myoepithelial cells in breast morphogenesis (9). We have previously shown that mouse and human luminal epithelial cells form polarized acini when cultured in laminin-rich gels (47–49). Because myoepithelial cells are responsible for the expression of many of the basement membrane proteins, including laminins, it was reasonable to determine whether myoepithelial cells were responsible for the polarized acini *in vivo*. Using an ablation-based approach, we confirmed that luminal epithelial cells cultured alone within a 3D collagen I gel form solid acinar-like structures (50), but we showed later that these structures lacked lumina (9). Comparative characterization of luminal epithelial cells cultured in collagen versus IrECM revealed a fundamental difference: luminal epithelial cells cultured in IrECM formed acini an organized BM at the basal pole, whereas luminal epithelial cells cultured within the collagen gels demonstrated reversed polarity. Double staining against MUC1 and ESA as well as MUC1 and the tight junction component, occludin, demonstrated inside-out polarity and an absence of BM deposition when the cells were grown in collagen. Importantly, this aberrant luminal epithelial polarization was corrected by the addition of myoepithelial cells. By admixing myoepithelial cells and luminal epithelial cells in a 1:1 ratio before they were embedded in the Collagen I gels, lumen formation as well as the correct apico-basolateral polarity of the luminal epithelial cells were rescued (Fig. 2). This effect of myoepithelial cells was cell-type-specific, since co-cultures of luminal epithelial cells with other breast cells (resident breast fibroblasts) or non-breast cells (osteosarcoma cells) did not lead to a reversal of inside-out polarity. An important feature of the type I collagen assay was the ability of luminal epithelial and myoepithelial cells to form double-layered acinar structures (9), a feature that has been demonstrated also by rotary co-culture of luminal epithelial and myoepithelial cells (8).

Since Matrigel was initially shown to be sufficient for acini formation (49), we tested whether the laminin isoforms found in the breast epithelial BM, i.e., laminin-1 (the major laminin isoform in Matrigel), laminin-5, and laminin-10/11 could substitute for the myoepithelial cells in the type I collagen assay. These laminins have been reported to be present in different aspects of morphogenesis in a number of tissues (for review, see (51)). Indeed, both laminin-1 and Matrigel (10% of final medium concentration) could reverse polarity of luminal epithelial cells when added to collagen gels, whereas laminin-5 and laminin-10/11 failed to do so. These data would predict that (a) luminal epithelial cells do not make sufficient laminin-1 in collagen gels to allow them to polarize and (b) that normal myoepithelial cells are the source of the polarizing principle through synthesis of laminin-1. To test this idea, we analyzed by RT-PCR the synthesis of BM components by myoepithelial and luminal epithelial cells. These experiments revealed that the only difference between the myoepithelial and luminal epithelial cells in the production of BM components measured was the lack of laminin-1 expression in the latter, as evidenced also by immunocytochemistry (9). These data are consistent with results reported for other organs. It has been shown previously that $\alpha 1$ -chain of laminin-1 is associated with polarization of epithelial cells in the kidney (52) and lung (53). Furthermore, laminin-1 has been shown to polarize and induce acinus formation in human submandibular epithelial cells (54,55) and human prostate epithelial cells (56). Hoffman *et al.* have shown that a peptide fragment (AG-73) derived from the $\alpha 1$ -chain of laminin-1 could induce acinar-like structures in human submandibular gland cells (55). Bello-DeOcampo *et al.* compared the ability of Matrigel, laminin-1, collagen IV, and fibronectin to induce acinus formation in prostate

epithelial cells and found that Matrigel and laminin-1 were equally effective inducers of acinus formation, whereas no effect was seen with collagen IV or fibronectin (56).

Whereas laminin-1 is undoubtedly a key regulator for tissue morphogenesis and maintenance of proper polarity in the breast and other organs, other myoepithelial-derived molecules have also proven to be pivotal for breast tissue polarity. Runswick and colleagues have demonstrated a role for desmosomal adhesion molecules in acinar positioning in the breast (8). Desmosomal cadherins are composed of desmocollins (Dsc) and desmogleins (Dsg). Luminal breast epithelial cells express only Dsc2 and Dsg2, whereas myoepithelial cells also express Dsc3 and Dsg3. When isolated and recombined in rotary culture, luminal epithelial and myoepithelial cells undergo positional sorting, resulting in formation of an elegant bi-layer structure reminiscent of their *in vivo* counterparts. Treatment with function-blocking peptides to the myoepithelial-specific Dsc3 and Dsg3 disrupted the bi-layer structure and its polarity (8,57). These data indicate that desmosomal cell–cell interactions are also important in myoepithelial-induced polarity in the mammary gland. The relationship between these two morphogenetic events, as well as their relative hierarchy in determining acinar polarity still needs to be established (58).

MYOEPIHELIAL CELLS IN NEOPLASIA

Form and function are inexorably related in the mammary gland (59), and one of the hallmarks of breast cancer progression is the loss of normal tissue architecture, including polarity. It is also postulated and generally accepted that primary breast carcinomas show a dramatic increase in the ratio of luminal-to-myoepithelial cells, and that many invasive breast carcinomas essentially lack myoepithelial cells entirely (60). If the criteria for identification of myoepithelial cells were the expression of a complete differentiation program, then this strongly held belief is reasonable and correct. However, many earlier studies have focused exclusively on the presence of an intact BM in general and expression of laminin-1 in particular, which within the limitations of the antibodies used, are usually absent in invasive breast cancer (9,¹¹,61). However, in the current literature, myoepithelial markers are more frequently reported in breast cancer. Thus, CK14, CK17, and vimentin have been detected in 20–33% of invasive breast carcinomas (62,63). Similar frequencies of ultrastructurally identified myoepithelial cells have been found in breast cancer (reviewed in (64)). Recent microarray studies have shown that the phenotypic diversity observed in breast cancers correlates with unique gene expression patterns that can be used to divide breast cancers into at least four main subgroups, normal-like, luminal-like, ERBB2-overexpressing, and basal-like, all with different clinical outcomes (65). The worst prognosis is of basal-like breast cancers, an estrogen receptor negative cancer, showing expression of both luminal and myoepithelial markers (66,67). However, despite the presence of cells with a partial myoepithelial differentiation, the luminal epithelial cells remain largely disorganized (9,⁶²,68).

Having illustrated the functional role of normal myoepithelial cells within breast morphogenesis, we asked whether cancer-associated myoepithelial cells were deficient in their ability to signal to luminal epithelial cells for polarity and whether this deficiency could be related to their inability to synthesize laminin-1. Initially we used a breast cancer cell line (HMT-3909S13) (69,70) that appears to be a progenitor cell line and produces myoepithelial cells at the stromal–epithelial junction in tumors generated in nude mice. These tumors resemble bi-phasic/basal human breast carcinomas by double staining with human-specific antibodies to vimentin and keratin.

By immunomagnetic cell sorting using an $\alpha 1$ -integrin antibody, it was possible to purify the myoepithelial cells from HMT-3909S13 cells. These cancer-derived myoepithelial cells, referred to as HMT-3909S16, resemble normal myoepithelial cells by their marker expression

(71). Nevertheless, they completely fail to correct the polarity of luminal epithelial cells in collagen gels even at cell numbers 10 times higher than those sufficient for normal myoepithelial cells to elicit this function (9,71). Since these cells were immortal and the normal myoepithelial cells used in the collagen assay were not, we asked whether their inability to reverse polarity was related to immortalization. We therefore used a normal breast-derived E6/E7 immortalized myoepithelial cell line; co-culture in the type I collagen gel assay still resulted in correct polarization when these cells were used (9,71).

To test if the difference between the immortalized normal-derived and cancer-derived myoepithelial cells was their ability to produce laminin-1, we stained the cells for a number of myoepithelial differentiation markers. The major difference in the markers tested was clearly the ability to make laminin-1. This difference was further confirmed by RT-PCR. But since other markers such as desmosomal proteins were also aberrant (unpublished), we purified fresh myoepithelial cells from three primary breast carcinomas, being well aware that these could contain residual normal myoepithelial cells. Nevertheless, in two out of three samples, the cancer-derived myoepithelial cells behaved in a manner similar to the established cancer-derived myoepithelial cell line. The one cancer-derived myoepithelial cell sample that was able to polarize the luminal epithelial cells at a reasonable level stained positive for laminin-1. Furthermore, a BM, albeit fragmented, was deposited, and $\beta 4$ integrin was targeted to the basal cell surface (9).

To test whether the data obtained with tumor-derived myoepithelial cells in culture corroborated observations *in vivo*, we further stained 12 infiltrating ductal breast carcinomas with laminin-1. Seven carcinomas were completely negative for laminin-1. The other five carcinomas showed foci of fragmented laminin-1 staining that was clearly much less pronounced than in normal breast tissue (Fig. 3). Double staining for either of the myoepithelial markers maspin, CK17, or cytokeratin-associated BG3C8 and laminin-1 revealed that tumor-associated myoepithelial cells were either negative or weakly positive for laminin-1. On the other hand, strong positive staining for laminin-1 was consistently observed around normal residual breast tissue whenever normal structures were present within or adjacent to the carcinomas (9).

Our finding that some cancer-derived myoepithelial cells are functionally defective, as characterized by a lack of laminin-1 expression and their inability to polarize luminal epithelial cells, provides a plausible explanation for why breast carcinomas showing partial myoepithelial differentiation do not confer a better prognosis for the patients (68). Accordingly, in some breast carcinomas, neoplastic myoepithelial cells are present and appear to be functional. This is the case with the adenoid cystic carcinoma, which has a better prognosis and does not metastasize to distant sites (72). In these carcinomas, laminin is deposited, and the neoplastic luminal epithelial cells polarize correctly. This result could be interpreted as the ability of normal or “near-to-normal” myoepithelial cells to induce and/or retain polarization of cancer cells via production and deposition of BM. Thus, in grade I carcinoma, which has a favorable prognosis and is often polarized correctly (73), a fragmented BM has been described (11,61) and, at the ultrastructural level, the presence of myoepithelial cells has been reported (74). In this context, it is tempting to speculate that the presence of myoepithelial cells that are capable of producing functional laminin-1 could have kept genetic alterations occurring within the malignant cells in check (on the relation between genotype and phenotype, also please see (75,76)). The myoepithelial cells could thus in theory function as a barrier, preventing the conversion of noninvasive tumors to an invasive cancer, although the evidence for such a hypothesis is scant at best.

It is also interesting to note that, in general, myoepithelial cells themselves are highly resistant to cancer development, and in those cases when cancer arises in the myoepithelial lineage, it

is generally of low malignancy, with the exception of malignant myoepithelioma which is a rare form of breast cancer (77). This finding concurs with our data which show that the cancer-derived myoepithelial cell line was non-tumorigenic in nude mice even after injection of 10 times as many cells as were sufficient for tumors with the parental line (0/6 mice compared to 11/12 mice) (9).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

In this review, we have cited evidence that myoepithelial cells in both mice and human mammary glands originate from a suprabasal cell type within the luminal epithelial compartment within the adult breast. Furthermore, we discuss observations that normal myoepithelial cells are critical for correct polarity of luminal epithelial cells, most likely via production of laminin-1. On the other hand, the myoepithelial cells present in tumors have many traits in common with normal myoepithelial cells, but show either complete absence or reduced expression of laminin-1 and are thus unable to induce the polarization of luminal epithelial cells.

The task ahead is to explore the potential function of myoepithelial cells in normal function and consequences of aberrant function in breast cancer differentiation. Recent important work from the Polyak laboratory has shown that myoepithelial cells isolated from ductal carcinoma *in situ* (DCIS) are drastically altered and secrete many cytokines and other potential tumor-promoting molecules (78). In addition to those molecules being investigated (see also this issue of the journal), it will be necessary to identify those factors that are responsible for abrogation of BM production and signaling in general and laminin-1 in particular in DCIS and cancer-derived myoepithelial cells. It will be important also to elucidate the molecular mechanisms that regulate the cell fate decisions made by normal mammary stem cells and breast cancer “stem” cells that enable them to undergo full versus incomplete myoepithelial differentiation. In our attempts to understand myoepithelial and luminal epithelial relationships and their respective contributions to breast cancer plasticity, we need to unravel which factors determine the failure of breast cancer cells to differentiate along the myoepithelial pathway in most breast carcinomas; and thus, fail to exhibit the tumor suppressive properties of differentiated myoepithelial cells. From recent data cited above, it is also possible that the tumor suppressive ability of myoepithelial cells depends on their complete differentiation and that changes in their expression pattern can lead to reversal of their function, i.e., that undifferentiated myoepithelial cells may actually promote tumor progression instead of suppressing it. It is intriguing to note that this is the classical pattern of tumor suppressor molecules, such as p53, and it appears that once organ integrity is breached, the cellular partners of epithelial cells may alter their signaling molecules to act like oncogenes. In this context improved and simplified purification protocols will be an indispensable tool for isolation of subpopulations showing different degrees of differentiation to accompany the ultrastructural characterization of different populations reported for mouse mammary epithelial cells (79). In addition to primary epithelial cells that have limited lifespan in culture, there is an urgent need for well-characterized immortal myoepithelial cell lines representing the various differentiation states of the myoepithelial lineage. In this regard, we believe that the suprabasal-derived epithelial stem cell line (4) has the potential to provide valuable information concerning the role of myoepithelial cells in normal breast development and cancer progression.

The data indicating that myoepithelial cells arise from progenitor cells within the luminal epithelial compartment (3,40) and that (an) extrinsic factor(s) may trigger a subset of luminal cells to convert to myoepithelial cells could partially explain why the majority of breast carcinomas express a luminal phenotype. If the extrinsic signal(s) is not present or the cells lose their ability to respond to the microenvironment appropriately (80), the stem/progenitor cells continue to expand along the luminal epithelial lineage, thus avoiding, or partially

bypassing, the myoepithelial route, as postulated previously by Rudland on the basis of the behavior of rat cell lines (81,82). However, although breast cancer cells express mainly the luminal epithelial phenotype, some cancer cells retain the ability to switch to a myoepithelial program, albeit only partially. These observations could open up the possibility of a future differentiation therapy where cancer cells are forced to differentiate along the myoepithelial pathway, thus manufacturing cells of lower malignancy or those that could suppress the aggressive behavior of their more malignant counterparts.

In summary, although much remains to be learned, the role of myoepithelial cells as possible tumor suppressors may include their function as a guardian of 'normalcy,' being a paracrine inhibitor of invasion in early breast cancer as well as a target for differentiation therapy by inducing malignant cancer cells to differentiate along the myoepithelial pathway to a less devastating cell type.

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Fig. 1. Suprabasal epithelial cell lines make an elaborate TDLU-like structure in laminin-rich extracellular matrix (lrECM). Suprabasal epithelial cells embedded as single cells into lrECM form elaborate structures reminiscent of TDLU *in vivo*. Bar, 50 μ m. *Upper left*, schematic drawing of TDLU showing luminal epithelial cells (*red*) and surrounded by myoepithelial cells (*yellow*) and basement membrane (*black line*). (Courtesy Gudjonsson *et al.* (4)).

Fig. 2. Reversal of inside-out acini by addition of myoepithelial cells. (A) Luminal cells make inside-out acini in collagen. Luminal epithelial cells were double-stained for MUC1 (*red*) and ESA (*green*). (B) In the presence of myoepithelial the acinar polarity is rescued as evidenced by apical expression of MUC1 (*red*) and ESA (*green*). Bar, 25 μ m. (Courtesy Gudjonsson *et al.* (9)).

Fig. 3.

Reduced staining of laminin-1 in human breast cancer. Cryostat sections of TDLU (a), a ductal carcinoma *in situ* (b), and four different infiltrating ductal breast carcinomas (IDC) (c–f) stained with immunoperoxidase for laminin-1 and counterstained with hematoxylin. Note the strong staining of laminin-1 in basement membrane of TDLU separating the stromal (S) and epithelial (E) compartments in (a). In carcinomas, laminin-1 staining is localized to the stromal (S)-cancer epithelial (C) interface, but is discontinuous and less pronounced. Bar, 25 μ m. (Courtesy Gudjonsson *et al.* (9)).