

## Review

# The importance of being a myoepithelial cell

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### Abstract

The mammary myoepithelial cell was named the 'Cinderella of mammary cell biology' in light of the earlier focus on the luminal cell. Mammary myoepithelial cells have recently been described as 'natural tumour suppressors'. We now need to understand more about their origin and to reconsider their place in the complex process of mammary morphogenesis. In the present review, we discuss the lineage segregation of mammary myoepithelial cells and their functions in mammary gland development. These functions include their effects on luminal cell growth and differentiation, their key role in the establishment of the polarised mammary epithelial bilayer and the control of stromal invasion in breast cancer.

**Keywords:** breast cancer, cell differentiation, mammary gland, morphogenesis, myoepithelial cell

### Introduction

The mammary gland consists of secretory alveoli connected by a system of branching ducts embedded in connective tissue. The epithelial cells that compose the gland are arranged in two layers, the luminal epithelial layer and the basal myoepithelial layer. The whole structure is surrounded by a basement membrane.

The myoepithelial layer is organised differently in the ducts and in the lobules. In the ducts, elongated myoepithelial cells form a more or less continuous layer and are in direct contact with the basement membrane, and hence with the stroma. The interaction between ductal luminal cells and the extracellular matrix (ECM) is largely mediated by the myoepithelium, although some of the luminal cells in the mammary ducts may reach the basement membrane. Alveolar myoepithelial cells are of stellate shape and form a basket-like structure around the acini, resulting in the exposure of most of the basal surface of the luminal cell to the basement membrane.

Differentiated myoepithelial cells are highly contractile and their ultrastructure is reminiscent of that of smooth muscle cells. Myoepithelial cells contain large amounts of microfilaments, dense plaques (cell-matrix adherens junctions characteristic of smooth muscle cells) and smooth muscle-specific cytoskeletal and contractile proteins. They are true epithelial cells, however, because the major components of their intermediate filament system are the cytokeratins 5 and 14 (K5 and K14), because they form desmosomes, hemidesmosomes and cadherin-mediated cell-cell junctions, and because they are permanently separated from the connective tissue by the underlying basement membrane.

The contractile properties and the central role in milk ejection during lactation are the most studied aspects of mammary myoepithelial cell function. In addition, the tumour suppressor potential of mammary myoepithelial cells has recently received considerable attention. However, the role played by the myoepithelial cells in

mammary development remains poorly investigated. The vast majority of studies devoted to various aspects of mammary gland development to date have focused on the mechanisms controlling luminal cell differentiation and growth, whereas basal myoepithelial cells have been largely neglected.

In the present review, we refer to the studies carried out in the mouse, the rat and the human. Although mammary gland development, function and pathology are obviously not identical in different mammalian species, the data obtained in rodent models, when considered at the cellular and molecular levels, provide essential information relevant to human breast disease.

### **Differentiation of the mammary myoepithelial cell**

In the rat, for most of the embryonic period of development, the basally located cells of the mammary buds originating from the epidermis do not express smooth muscle markers, and the future myoepithelial cells gradually acquire features characteristic of smooth muscle differentiation during the postnatal mammary gland development [1]. Acquisition of the differentiated phenotype is accompanied by changes in adhesion systems (i.e. upregulation of specific integrin and laminin variant expression) [1].

In the human foetal breast, the first smooth muscle marker ( $\alpha$ -actin) was found in basally located cells after 23 weeks of gestation [2]. In the adult mammary gland, the smooth muscle marker expression of myoepithelial cells appears to be heterogeneous. In the quiescent human breast, heavy caldesmon (an important regulator of the contractile function) is present in the myoepithelial cells of large ducts and galactophorous sinuses, but is not found in intralobular small ducts and acini [3]. Similarly, in the lactating rat mammary gland, ductal and alveolar myoepithelial cells display different patterns of expression of contractile, cytoskeletal and ECM smooth muscle markers [1]. This heterogeneity may reflect differences in functional properties and the diverse origins of the myoepithelial cells residing in the various parts of the mammary tree.

Previous observations resulting from studies performed with the mouse mammary gland have provided convincing evidence that the cap cells of the terminal end buds (TEB) can give rise to ductal myoepithelial cells [4]. The TEB are bulbous structures found in pubertal animals. They are located at the endings of mammary ducts advancing into the fat pad, and the cap cells form a basally located monolayer at their tip. Cap cells themselves do not express smooth muscle markers but, as the duct grows into the stroma, they move to the more proximal part of the duct and differentiate into myoepithelial cells. Although the TEB are present only in rapidly growing pubertal mammary glands, cell populations similar to cap cells may exist at

the extremities of all growing buds at various developmental stages. Cap cells are unlikely to be the only source of myoepithelial progenitors, however, as experiments involving the serial transplantation of mammary tissue fragments have shown that precursor cells are distributed throughout the mammary tree rather than concentrated at any particular site [5] (reviewed in [6]).

Several data suggest the existence of bipotent mammary cell precursors that can give rise to both luminal and myoepithelial cells. Stingl *et al.* attempted to characterise the epithelial progenitor populations present in normal adult human mammary tissue by a combination of flow cytometry and *in vivo* colony formation assays [7]. The markers used to identify cells belonging to the two major mammary cell lineages (luminal and basal) included cytokeratins 8/18 and 19, EpCAM and MUC1 for luminal cells, and K14, high levels of  $\alpha$ 6-integrin and low levels of MUC1 for myoepithelial cells. This study by Stingl *et al.* suggested the presence of fate-restricted myoepithelial and luminal precursors, as well as bipotent progenitors. However, the authors did not analyse the expression of myoepithelium-specific contractile and cytoskeletal proteins, such as smooth muscle  $\alpha$ -actin. Furthermore, Lakhani *et al.*'s demonstration of identical genetic changes (loss of heterozygosity) in luminal and myoepithelial cells [8] provides convincing evidence in favour of the existence of a common precursor.

The precise location and molecular characteristics of myoepithelial precursor cells, with the exception of cap cells, are unknown. The results of experiments performed with cultured cells from human and mouse mammary glands suggest that myoepithelial cells may be derived from precursors within the luminal epithelial layer [9–13]. On the contrary, a recent study by Böcker *et al.* performed with human breast specimens describes a K5-positive cell population that might be a precursor of both luminal cells ('glandular epithelial') and myoepithelial cells [14]. Furthermore, Gudjonsson *et al.* isolated a mammary cell population with suprabasal characteristics (i.e. possessing luminal properties but negative for sialomucin, an apical marker of luminal cells) from human breast tissue [15]. These cells were used to establish a cell line displaying progenitor properties: the cells were able, in two-dimensional culture, to form branching structures resembling the terminal duct lobular units of the breast and containing basal and luminal cells. The authors suggested that *in vivo*, consistent with the characteristics exhibited in culture, these bipotent progenitor cells were located suprabasally and resided on myoepithelial cells.

Overall, the location and molecular markers of mammary progenitor cells, as well as the mechanisms controlling the maintenance of stem cells as such and their commitment to one of the two major mammary epithelial lineages, remain to be elucidated.

### The myoepithelial cell layer harbours important regulatory molecules

Numerous molecules implicated in important regulatory processes in other tissues are expressed differently in the two epithelial layers of the mammary gland. Several members of the epidermal growth factor family have been shown to play an important regulatory role in mammary gland development and in the differentiation of mammary epithelium (reviewed in [16]). In the mammary epithelium, the epidermal growth factor receptor expression level found in the cap cells and myoepithelial cells is significantly greater than that in the luminal cells [17,18]. This distribution, together with the results of studies carried out in culture with a mammary epithelial cell line displaying basal characteristics [19], suggests a specific role of epidermal growth factor receptor signalling in the control of the basal myoepithelial cell phenotype.

Gomm *et al.* have analysed the expression of basic fibroblast growth factor (FGF2) and fibroblast growth factor receptor 1 in luminal and myoepithelial cells isolated from the normal human breast [20]. The mRNA for FGF2 was found only in myoepithelial cells, whereas fibroblast growth factor receptor 1, as estimated by immunolabelling, was present in luminal cells and, to a lesser extent, in myoepithelial cells. These results suggest that myoepithelial cell-derived FGF2 may be an important paracrine factor controlling luminal epithelial cell growth.

The Tcf transcription factors are central components of the Wnt/ $\beta$ -catenin signalling pathway, which has been implicated in many aspects of development and tumorigenesis (reviewed in [21]). The members of the Tcf family exhibit differential tissue expression patterns. Tcf4 and Tcf1 have been shown to be present in the mammary epithelium, with nuclear Tcf1 detected specifically in the basal mammary epithelial cells. The most abundant form of Tcf1 lacks the  $\beta$ -catenin binding domain and is therefore likely to act as a negative regulator of Wnt signalling. Consistent with this notion, mice lacking Tcf1 develop mammary adenomas [22]. These findings suggest a potential role for the Wnt signalling pathway in the control of basal mammary cell growth. Different members of the Wnt family have been found in the various mammary gland compartments. In particular, Wnt2 is apparently expressed by myoepithelial cells rather than luminal cells [23].

Activins belong to the transforming growth factor beta superfamily, many members of which are important regulators of differentiation and development, particularly for various smooth muscle and epithelial cell types. Human mammary myoepithelial cells in primary culture have been found to express both activin  $\beta$ A and activin type II receptor, whereas other breast cell types do not [24]. The targeted expression of transforming growth factor beta in luminal cells results in the inhibition of mammary develop-

ment [25]. To our knowledge, however, the involvement of the transforming growth factor beta signalling pathway in the differentiation of mammary myoepithelial cells has not yet been studied.

The most recently discovered members of the p53 family are p63 and p73. These transcription factors play a central role in the control of cell growth and survival, and they act as tumour suppressors. Immunohistochemical studies have shown that, in the mammary epithelium, both p63 and p73 are restricted to the myoepithelial cell layer [26–29]. A recently described human mammary epithelial cell line with basal properties has been shown to express a p63 variant,  $\Delta$ N-p63- $\alpha$  [27]. The specific location of p63 in a subset of basal mammary cells [27] is particularly intriguing because, in the epidermis, this p53 homologue identifies keratinocyte stem cells [30]. Moreover, p63 expression is critical for the maintenance of the progenitor cell population necessary for development and morphogenesis. All squamous epithelia and their derivatives, including the mammary gland, have been shown to be absent in p63-deficient mice [31,32]. Additional studies are required to elucidate the role of p63 in mammary development.

### Perturbation of the myoepithelial cell-specific protein expression pattern

There is a growing body of evidence to suggest that interference with the normal pattern of expression of genes encoding molecules specific to the myoepithelium, by ablation or overexpression, perturbs the growth and differentiation of the entire mammary epithelium. Several examples are now described.

P-cadherin is restricted to the basal layer of stratified and pseudostratified epithelia, and it is present only in the myoepithelial cells in the mammary gland. P-cadherin-deficient virgin mice display precocious mammary gland development [33]. They contain alveolus-like buds similar morphologically to those observed in early pregnant animals, with luminal cells producing milk proteins (caseins). This suggests that perturbation of the pattern of gene expression in myoepithelial cells may affect the growth and differentiation of luminal cells.

Parathyroid hormone-related protein (PTHrP) is implicated in a wide variety of biological processes during embryonic development and in adults. In the mammary gland, it is produced by both luminal and myoepithelial cells. However, only myoepithelial cells are responsive to PTHrP. The targeted overexpression of PTHrP in myoepithelial cells under the control of the *K14* gene promoter results in mammary hypoplasia characterised by deficient ductal branching in virgin mice, due to the high rates of apoptosis and the low rates of proliferation observed in TEB [34,35]. These findings provide further evidence that

myoepithelial cells can participate in the control of growth, differentiation and morphogenetic events involving luminal cells, thereby demonstrating the contribution of these cells to normal mammary gland development.

Epithelial basal cell-specific promoters, such as those of the *K5* and *K14* genes, are often used to create transgenic mice for studies of developmental processes in the epidermis and hair follicles. Unfortunately, with few exceptions, the mammary phenotypes of mice bearing transgenes under the control of *K5* or *K14* promoters have not been analysed. In addition to the results obtained with the *K14-PTHrP* transgenic mouse line described earlier, a recently published study illustrates that basal keratin promoters can be used to address important questions concerning the mammary gland. Jonkers *et al.* showed that mice carrying conditional *Brca2* and *Trp53* alleles and a *cre* transgene under the control of the *K14* gene promoter developed mammary tumours with luminal and myoepithelial characteristics [36]. It is currently unknown whether these tumours develop due to inactivation of the *Brca2* and *Trp53* genes in differentiated myoepithelial cells, or whether they result from the ablation of these genes in some *K14*-expressing early mammary precursor cells, as the *K14* promoter is active early in embryonic development.

Several members of the family of ephrin receptor tyrosine kinases and their ligands have been implicated in the regulation of pattern formation during embryogenesis. In the normal mouse mammary gland, the ephrin receptor EphB4 is found predominantly in myoepithelial cells, whereas expression of its ligand (ephrin-B2) is restricted to luminal cells [37]. In the rapidly growing tumours found in *Wap-ras* transgenic mice, the receptor ceased to be expressed on myoepithelial cells and was instead detected in the tumour cells [38]. The targeted overexpression of EphB4 in luminal mammary cells under the control of the mouse mammary tumour virus promoter affected the mammary gland development. The transgenic glands exhibited, on the one hand, high rates of apoptosis in pregnancy and, on the other, abnormal proliferation during the early stages of involution. Double-transgenic animals expressing the *EphB4* and *neuT* genes developed mammary tumours earlier than did those expressing *neuT* only. Moreover, lung metastases were observed exclusively in the double-transgenic mice.

These data are an example of how the introduction of a myoepithelial cell-specific molecule into luminal cells results in the perturbation of the mammary epithelial cell response to physiological signals that normally induce proliferation, apoptosis or survival.

### **Myoepithelial cells play a key role in the establishment of the mammary bilayer**

The integrity of the mammary epithelium is maintained by several distinct adhesion systems. The adhesive struc-

tures involved in cell–cell contacts at the lateral surfaces of the luminal cells and between the luminal and the basal myoepithelial cells include desmosomes and cadherin-mediated junctions. In contrast, hemidesmosomes and dense plaques specific to myoepithelial cells are localised to the sites of cell–ECM interactions. Within the cell, desmosomes and hemidesmosomes are associated with the intermediate filaments, whereas integrin-containing and cadherin-containing junctions are connected to the actin cytoskeleton, and are often referred to as adherens junctions. In accordance with the specific functions of basal myoepithelial cells in adhesion of the mammary epithelial bilayer to the basement membrane, integrins and cytoplasmic components of the cell–ECM adherens junctions (such as vinculin,  $\alpha$ -actinin, focal adhesion kinase and talin) are much more abundant in myoepithelial cells than in luminal epithelial cells [39].

Mammary myoepithelial cells may be expected to participate in ECM turnover, either permanently or during particular stages of mammary development, such as intensive growth in puberty or gland remodelling during involution. Thus, although most of the ECM-degrading enzymes found in the mammary gland are considered of stromal origin, myoepithelial cells produce several specific or ubiquitous matrix-degrading enzymes, in addition to numerous protease inhibitors (reviewed in [40]). A recently described angiogenesis-related matrix metalloproteinase, MMP19, was expressed by normal breast myoepithelial cells [41].

Gudjonsson *et al.* suggested that myoepithelial cells might play an essential role in the control of polarity in the bilayered mammary epithelium [42]. Indeed, luminal epithelial cells cultured in collagen-I gel formed acinus-like structures with reversed polarity, with apical markers expressed on their external surface and with basal markers expressed on the luminal side. The addition of myoepithelial cells led to the formation of acinus-like aggregates with the correct polarity. The mammary basement membrane component laminin 1 may replace myoepithelial cells in their instructive function, as in the reconstituted basement membrane material (Matrigel), even in the absence of myoepithelial cells, the polarity of the aggregates formed by luminal cells was correct. Myoepithelial cells isolated from breast tumours were not able to produce laminin 1 and, consistently, could not correct the polarity of the aggregates formed by luminal cells in collagen gel. These findings stress the importance of cell–ECM interactions in the establishment of basoapical polarity in the mammary epithelium, and illustrate the critical involvement of the myoepithelial cell as a source of the basement membrane material.

Desmosomes and cadherin-mediated adherens junctions are involved in the adhesion of the two mammary epithelium layers. The luminal cells express larger amounts of

E-cadherin than do the myoepithelial cells, whereas P-cadherin is expressed exclusively by the myoepithelial cells. This differential cadherin distribution may contribute to the segregation of the two major mammary cell types.

In addition, a recent elegant study revealed the important role played by desmosome components in the positioning of the luminal and myoepithelial cells in the mammary bilayer [43]. In this work, Runswick *et al.* investigated the role of desmosomes in the mammary epithelium organisation, using blocking peptides corresponding to the cell adhesion recognition sites of desmosomal cadherins. Incubation of mammary epithelial cells with a mixture of cell adhesion recognition peptides inhibiting the cadherins in the mammary epithelium prevented alveolar morphogenesis and demonstrated that desmosomes are absolutely required for the formation of multicellular branching structures. The desmosomal cadherins, desmocollin and desmoglein, are differentially expressed in the two mammary epithelial cell layers: Dsc2 and Dsg2 are present in both cell types, whereas Dsc3 and Dsg3 are restricted to the myoepithelium. In rotary culture, human breast epithelial cells can associate to form clusters resembling mammary alveoli, with centrally located luminal cells surrounded by an external ring of myoepithelial cells. The addition of cell adhesion recognition peptides corresponding to the myoepithelial cell-specific desmosomal cadherins, desmocollin Dsc3 and desmoglein Dsg3, interfered with this cell type-specific positioning. These results led Runswick *et al.* to conclude that the cell–cell adhesion might have a more dominant effect than cell–matrix interactions in cell positioning in the mammary bilayer [43].

### Mammary tumours with basal cell characteristics

Most breast carcinomas express phenotypic markers suggestive of a luminal origin. In contrast, breast myoepitheliomas are rather rare. The basal keratins K14 and K17 have, however, been reported to be present in an important subset (20–33%) of invasion breast carcinomas (reviewed recently in [44]). Moreover, analysis of the pattern of gene expression using complementary DNA microarrays has revealed a distinct tumour subclass accounting for 15% of all analysed tumours. In this subclass, expression levels were high for basal keratin genes and for other genes characteristic of basal mammary cells, such as those encoding the  $\alpha$ 3 and  $\gamma$ 2 laminin chains and the  $\beta$ 4 integrin subunit [45].

Further analysis of this basal cell-like tumour subclass has shown that *TP53* was mutated in 82% of the samples analysed, whereas only 13% of the tumours in the luminal subclass contained mutated *TP53* [46]. Previous studies have indicated that mutations in *TP53* are associated with a poor prognosis and a poor response to systemic therapy (see [46] for references). Basal cell-like tumours were

consistently found to be associated with short survival time. Expression of the entire set of basal cell markers by this subclass of breast tumours indicates a possible origin from a mammary cell progenitor with molecular characteristics of basal cells.

### Tumour suppressor potential of myoepithelial cells

Many lines of evidence suggest that differentiated myoepithelial cells are 'natural tumour suppressors' [47] (for reviews, see [48,49]) because they inhibit proliferation in breast carcinoma cells by inducing growth arrest and apoptosis, because they interfere with the invasive behaviour of tumour cells and because they inhibit angiogenesis. Indeed, mammary myoepithelial cells and cell lines obtained from benign myoepithelial tumours produce relatively high levels of protease inhibitors and active anti-angiogenic factors, such as protease nexin II,  $\alpha$ 1-antitrypsin, a 31 kDa serine protease inhibitor, tissue inhibitor of metalloproteinase 1, thrombospondin-1 and the soluble basic fibroblast growth factor receptor [47,50–52].

Maspin, a member of the serpin family of serine proteases, was identified by subtractive hybridisation of cDNAs from normal versus tumourigenic human mammary epithelial cells [53]. This serpin, produced by myoepithelial cells, functions as a tumour suppressor and can inhibit metastasis *in vivo* (reviewed in [54]). A new myoepithelium-specific serine proteinase inhibitor was recently identified and described by Xiao *et al.* [55]. This molecule, when expressed in human breast cancer cells, abolished their growth, decreased their invasive potential and prevented tumour dissemination *in vivo*. In addition to producing these anti-invasive and anti-angiogenic molecules, myoepithelial cells have also been shown to possess CD44 shedding activity and to produce soluble CD44, which blocks the adhesion and migration of human carcinoma cells on hyaluronic acid-coated surfaces [56,57].

Finally, the analysis of myoepithelial marker expression remains a commonly used approach to distinguish between benign and malignant tumours, or to detect stromal invasion (reviewed recently in [58]).

### Conclusions

The mammary myoepithelial cells, due to their specific differentiation programme, are able to integrate multiple signals from the neighbouring cells, from the underlying basement membrane and from the connective tissue. In turn, these cells have a major impact on luminal cell growth and differentiation, and they play a key role in the establishment and the maintenance of the mammary epithelium architecture. However, we currently know very little about the cellular and molecular characteristics of myoepithelial cell precursors, as well as the unique mechanisms that control the myoepithelial cell phenotype and

allow the expression of its double (smooth muscle and epithelial) identity. Moreover, the nature of the signals involved in communication between luminal and basal myoepithelial cells is largely unknown. These unresolved questions provide a vast area for future investigation.

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