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The myoepithelial cell: its role in normal mammary glands and breast cancer

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Mammary gland epithelium is composed of an inner layer of secretory cells (luminal) and an outer layer of myoepithelial cells (MEC) bordering the basal lamina which separates the epithelial layer from the extracellular matrix. Mature MECs morphologically resemble smooth muscle cells; however, they exhibit features typical for epithelial cells, such as the presence of specific cytokeratin filaments. During lactation, secretory cells synthesize milk components, which are collected in alveoli and duct lumen, and transported to the nipple as a result of MEC contraction. Although the induction of MEC contraction results from oxytocin action, also other, still unknown auto/paracrine mechanisms participate in the regulation of this process. As well as milk ejection, MECs are involved in mammary gland morphogenesis in all developmental stages, modulating proliferation and differentiation of luminal cells. They take part in the formation of extracellular matrix, synthesizing its components and secreting proteinases and their inhibitors. In addition, MECs are regarded as natural cancer suppressors, stabilizing the normal structure of the mammary gland, they secrete suppressor proteins (e.g. maspin) limiting cancer growth, invasiveness, and neoangiogenesis. The majority of malignant breast cancers are derived from luminal cells, whereas neoplasms of MEC origin are the most seldom and usually benign form of breast tumours. MECs are markedly resistant to malignant transformation and they are able to suppress the transformation of neigh boring luminal cells. Therefore, a deeper insight into the role of MECs in the physiology and pathology of mammary glands would allow a better understanding of cancerogenesis mechanisms and possible application of specific MEC markers in the diagnosis and therapy of breast cancer. (Folia Morphol 2010; 69, 1: 1–14)

Key words: myoepithelial cell, mammary gland, milk ejection, breast cancer, myoepithelial tumours

LOCALIZATION AND STRUCTURE OF MAMMARY GLAND MYOEPITHELIAL CELLS

Normal mammary glands are composed of a branched system of excretory ducts and secretory alveoli organized in lobules separated from each other by stroma. The epithelium of ducts and secretory alveoli consists of two layers of cells: a layer of luminal cells, which is responsible for the synthesis and secretion of milk components, and a layer of myoepithelial cells (MEC). The key role of MECs is their participation in the process of milk ejection. However, a great body of evidence has been accumulated suggesting their engagement in a wide

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variety of other important physiological processes, i.e. the regulation of mammary gland growth, development, and differentiation, as well as the control of cancerogenesis [23].

In excretory ducts, MECs are arranged in an almost continuous layer, with their longer axis being laid parallel to the ducts. In secretory units, they tend to acquire a basket-like shape (Fig. 1). In both cases, MECs are located between the basement lamina and luminal cells, and attached to the luminal cells by desmosomes, and by hemidesmosomes to the basement lamina [28].

Basement lamina is mostly a product of MECs and consists of collagen IV, fibronectin, laminin, nidogen, glycosaminoglycans, and proteoglycans. It forms a continuous layer separating myoepithelial cells from the stroma. Receptors of basement lamina components, especially integrins, present in MECs, are responsible for interactions with the matrix and neighbouring cells [33].

MEC exhibit expression of proteins typical for the contractile apparatus of smooth muscles cells, α-actin of smooth muscles (SMA), myosin heavy chains, α-actinin, vinculin, and calponin. MEC cytoplasm is filled with bundles of actin microfilaments and myosin filaments responsible for cell contraction. Sub-membrane dense plaques and cytoplasmic dense bodies organize the spatial arrangement of the contractile apparatus. In MEC processes, bundles of actin microfilaments are concentrated on one surface of the cell bordering with basement lamina (Figs. 2, 3).

Unlike in smooth muscle cells, intermediate filaments consist mostly of cytokeratins (CK5, CK14, and CK17) which create a net around the nucleus, extend towards the cell surface, and finally, as parallel bundles enter the processes, reinforcing their structure. In particular, cytokeratins 5 and 14 are involved in the maintenance of MEC cytoarchitecture, as well as the formation of desmosomes and hemidesmosomes [36].

Plasma membrane forms numerous invaginations (caveolae) and subsurface vesicles which are characteristic for both myoepithelial and smooth muscle cells (Fig. 3). The function of caveolae is debatable; they are supposed to act as a kind of storage for extracellular calcium ions, participate in the process of endocytosis, or mediate the transport of molecules between luminal cells and the extracellular matrix. Numerous caveolae arranged in parallel rows and located between bundles of microfilaments at the basal cell surface bordering with basement lamina suggest their

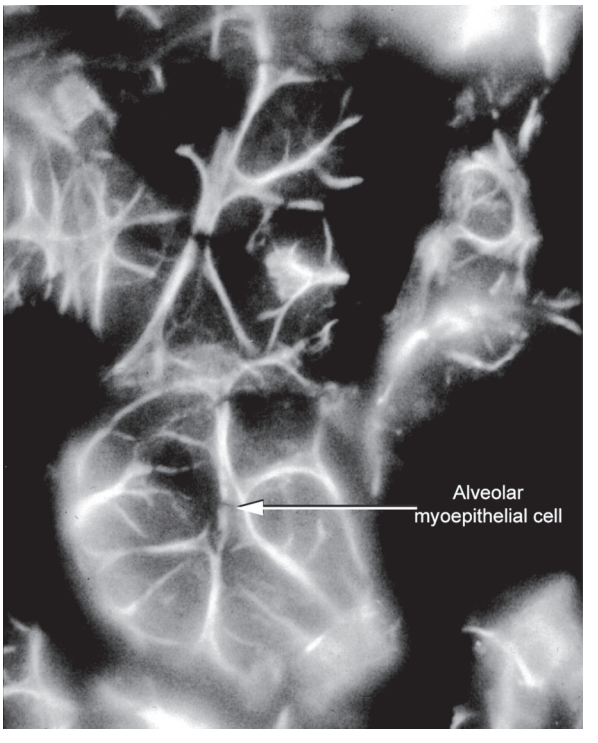

Figure 1. Three-dimensional arrangement of myoepithelial cell morphology in whole mammary gland tissue from a lactating mouse, visualized by the fluorescent stain NBD-phallicidin, which binds specifically to actin filaments. The cells display long, thin processes that radiate from the cell body (×600).

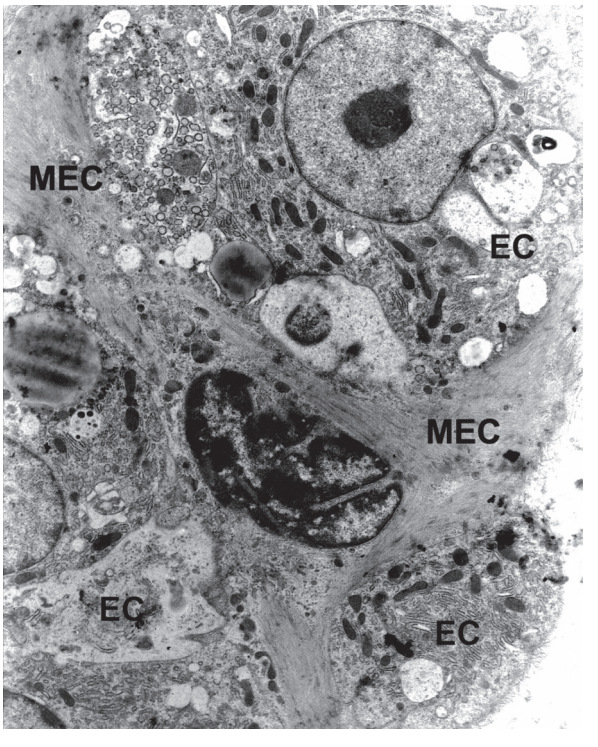

Figure 2. Transmission electron micrograph of lactating mouse mammary epithelium. Myoepithelial cell (MEC) processes radiating from the cell body are interposed between the basal surface of epithelial cells (EC) and the basal lamina (×6000).

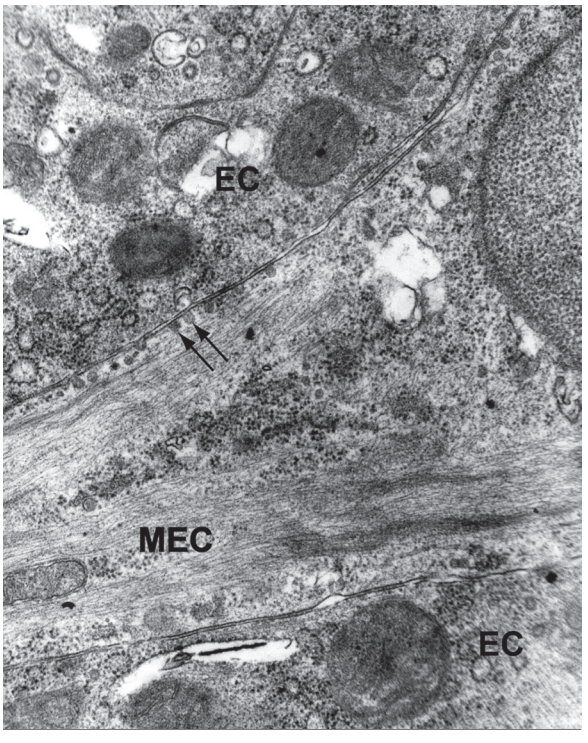

Figure 3. A higher magnification of mammary epithelium. Myoepithelial cell (MEC) is shown to be packed with microfilaments. In perinuclear cytoplasm there are clusters of cellular organelles, many ribosomes and multiple vacuoles. Plasma membrane forms numerous invaginations — caveolae and subsurface vesicles (arrows); EC — epithelial cells (×14000).

possible participation in MEC cytoskeleton arrangement [45].

MEC oval nuclei are filled with dispersed chromatin and clearly separated nucleoli. In perinuclear cytoplasm there are clusters of cellular organelles, cisterns of endoplasmic reticulum, numerous ribosomes, well-developed Golgi apparatus, multiple vacuoles, and vesicles. Mitochondria are present in both the perinuclear part of the cell and in processes (Fig. 3). These MEC features point toward their activity in the synthesis of structural proteins of contractile apparatus, components of basement lamina, and numerous regulatory and suppressor proteins (Table 1).

MYOEPITHELIAL CELLS DURING LACTATION

The basic physiological function of MECs during lactation is milk ejection. Oxytocin triggers the contractions of MECs located around the milk-storing alveoli and excretory ducts, which leads to self-induced milk expulsion.

Although the mechanisms of MECs and smooth muscle cell contraction are very similar, their induction is different. MEC contraction is stimulated by oxytocin binding and activation of signalling pathway mediated by $G\alpha_{q\text{-}11}$ protein and phospholipase C. The breakdown of phosphatidylinositol bisphosphate results in calcium concentration increase, phosphorylation of myosin, and finally the contraction of the cell [50, 52]. Unlike in myocytes, the main source of calcium ions in MECs is the extracellular influx. In addition, intracellular compartments may be engaged in the maintenance of the constant calcium level during the contraction. In contrast to uterine smooth muscles, oxytocin binding by MECs is not accompanied by mitogen-activated protein kinase (MAPK) activation nor by prostaglandin release [55].

As demonstrated in some experiments, oxytocin availability is not the only prerequisite for MEC contraction, and the induction itself requires other, as yet undefined factors, which might work in an auto/paracrine way. Oxytocin binding by MECs is accompanied by an increase in cytoplasmic cAMP concentration; however, there is no evidence suggesting the direct dependence between elevated cAMP and myosin phosphorylation levels. These effects seem to be mediated by different signalling pathways including calcium increase, cAMP formation, and MAPK activation [55].

Morphological observations of MEC contraction suggest that physiological concentrations of oxytocin do not stimulate the contraction of all MECs in lactating mammary glands but only the cells surrounding the alveoli filled with milk. Milk in particular secretory alveoli is produced in an asynchronous way. If availability of oxytocin was the only contraction inducer of MECs, then all of them should contract independently of milk level, which seems to be irrational from a physiological point of view [43].

It is assumed that the modulator of MEC contraction, working in an auto/paracrine way, is parathormone related peptide (PTHrP), like in vascular smooth muscle cells. PTHrP is synthesized in luminal cells and MECs of the lactating mammary gland, and its receptors are present only in MECs. In vascular smooth muscle cells, the PTH/PTHrP 1 receptor, like other vasodilators, activates the phosphatidylinositol-Ca^{2+} signalling pathway. The presence of PTH/PTHrP type I receptor, as demonstrated in our own studies, in the Hs578Bst myoepithelial cell line established from normal human breast tissue, as well as the capability of PTHrP synthesis by these cells, indicate the autocrine mechanisms of PTHrP action [65]. Seitz et al. [60] suggests that the action of

Table 1. Proteins and biomolecules specific for myoepithelial cells (MEC)

Molecule	Function	Diagnostic or clinical significance	References
Structural proteins			
Smooth muscle actin (SMA)	Involved in MEC contraction. Identical as in smooth muscle cells, myofibroblasts and pericytes	SMA expression is observed in 95% of MECs of the normal mammary gland, and non-invasive cancers. No expression is detected in invasive cancers. Diagnosis of invasiveness should be confirmed with a simultaneous expression of SMA and collagen IV	[10]
Smooth muscle myosin (heavy chains) — SMMHC	The main component of MEC contractile apparatus	Similar to SMA, a useful marker in differentiation between invasive and non-invasive breast cancers	[14]
Calponin	Binds tropomyosin and F-actin. Participates in MEC contraction	A useful marker differentiating MECs from spindle cells of the stroma, and invasive from non-invasive cancers	[14]
H caldesmon (HCD)	Cytoskeletal protein binding to actin	Strong expression is specific for myoepithelial and smooth muscle cells of small blood vessels	[42]
P-cadherin	Is a Ca^{2+}-dependent cell adhesive molecule playing a key role in the maintenance of mammary gland epithelium structure	Expressed in MEC of the normal mammary gland, and occasionally in hyperplastic tissues, and some non-invasive cancers	[24]
Cytokeratins 5, 7, 14, 17	Specific components of MEC intermediate filaments	Enable identification of MECs in the normal mammary gland and breast cancers, and differentiation of MEC precursors during mammary gland morphogenesis.	[41, 78]
Desmoglein (Dsg3) and desmocollin (Dsc3)	Dsg3 and Dsc3 desmosomal cadherins are specific for MEC desmosomes and hemidesmosomes	Responsible for the positioning of MECs and maintenance of bilayer structure of the mammary gland epithelium	[57]
Non-structural molecules			
Maspin	Mammary gland specific serpin (serine proteases inhibitor) present exclusively in normal mammary gland MEC, and breast cancer epithelial cells	Tumour suppressor protein, the expression of which decreases with the level of malignancy. Maspin inhibits tumour growth and invasiveness inducing apoptosis and inhibiting cell mobility and angiogenesis	[54]
p63, p73	Nuclear proteins showing close homology to p53, p63, and p73 expression in mammary gland is comprised to MEC nuclei	Proteins responsible for the maintenance of progenitor cell populations in mammary gland epithelium. Participate in mammary gland morphogenesis and the maintenance of the normal structure of the gland	[5, 82]
WT-1 (Wilms tumour 1)	A transcription factor involved in gene expression, similarly as p53	WT-1 expression is constantly observed in MECs, whereas in breast cancers it is negatively correlated with tumour progression	[19, 59]
S 100	Protein belonging to the big family of proteins containing at least one Ca^{2+} binding motif	S 100 protein is constitutively expressed in MECs and is often present in mammary gland luminal cells. In breast cancers, expression of S 100 protein is often elevated and associated with tumour progression and poor prognosis	[18, 27]

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Table 1 (continued). Proteins and biomolecules specific for myoepithelial cells

Molecule	Function	Diagnostic or clinical significance	References
CD10 (CALLA — common acute lymphoblastic leukaemia antigen)	Metalloendopeptidase present on the surface of the cells responsible for the inactivation of many biologically active peptides. Present on the lateral surface of MECs	The enzyme exhibits stable expression in normal mammary gland MECs. During breast cancer growth and progression the number of CD10 positive MECs undergoes reduction, and the intensity of immuno-cytochemical reaction decreases	[44]
CD44	The molecule secreted by MECs, inhibits cancer cells adhesion and migration	Marker applied in breast cancer prognosis	[2, 37]
CD 109	Participates in TGF-β signalling pathway inhibition	Present in mammary gland MECs, but not expressed in secretory and ductal epithelial cells. MEC marker applied in invasive cancer breast diagnosis	[26]
14-3-3 sigma	The product of cancer suppressor gene transactivated by p53 in response to DNA damage	Expressed mainly in MECs of benign and pre-invasive breast cancers. Applied in breast cancer prognosis	[63]
NRP-1 (neuropilin)	A specific receptor for vascular endothelial growth factor in MECs	MECs in hyperplastic and neoplastic tissues of the mammary gland exhibit higher NRP-1 expression than normal tissue	[69]
PTHrP/PTHrPR (parathyroid hormone-related protein/ /parathyroid hormone-related protein receptor)	PTHrP is produced both by myoepithelial and luminal cells, but in mammary glands only MECs exhibit its receptor	PTHrP inhibits the growth and branching of excretory ducts during development. Exhibits proapoptotic and antiproliferative properties in hyperplastic tissues of the mammary gland	[16, 81]

PTHrP on MEC cells leads to their relaxation by stopping the oxytocin triggered influx of calcium ions.

Another molecule that may be engaged in the regulation of MEC contraction is nitric oxide (NO). In mammary gland MEC, both in the resting and lactation periods, the activity of nitric oxide synthase (NOS-1), as well as the presence of NO receptor-soluble guanylyl cyclase (sGC), were observed. sGC, in an auto/paracrine way, catalyzes the conversion of GTP to cGMP [79]. In smooth muscle cells, the increase in cGMP concentration inhibits the influx of calcium ions and thereby the activity of $Ca2^{+}$--dependent myosin light chain kinase, which leads to the relaxation of cells [46]. Because an identical kinase is present in MEC, a similar mechanism of auto/paracrine regulation of contraction may operate here.

An additional mechanism of the regulation of MEC contraction is variable and diversified localization of oxytocin receptors (OTR). The availability of OTR may be regulated via their localization in specialized membrane microdomains such as lipid rafts and caveolae, which may be connected with the activation of different transduction pathways leading to a diversified response of MECs to oxytocin signal [56].

THE ORIGIN AND DIFFERENTIATION OF MYOEPITHELIAL CELLS

The mammary gland differentiates from ectodermal epithelium, which, during the embryonic period, forms the milk line. The epithelium of milk line invaginates into mesenchyma forming primary and later secondary buds. These buds elongate and grow lateral branches forming a complex system of ducts ending with expanded terminal end buds (TEBs). The TEBs undergo intensive growth and differentiation. A cap cell layer surrounds the body cells constituting the cellular material from which basal MEC and luminal cells differentiate and are therefore thought to be multipotent stem cells (Fig. 4). Some of them remain undifferentiated stem cells which settle in particular niches of excretory ducts and secretory units [80].

Intensive development of the mammary gland takes place during puberty, pregnancy (when the process is stimulated by systemic hormones, oestrogens, progesterone, placental lactogens, and prolactin), and after childbirth, when the combination of systemic hormones, local growth factors, and milk ejection evokes further development of the gland structure [47].

MEC precursors are present on the entire length of branched ducts of the mammary gland, and at

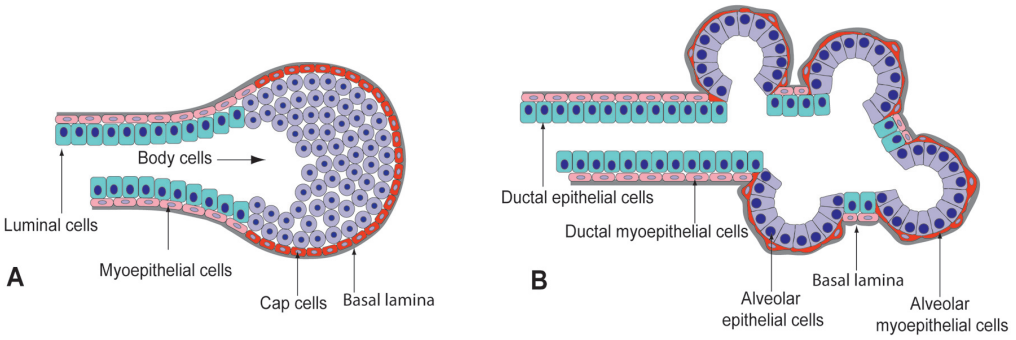

Figure 4. The structure of terminal end buds (TEB) (**A**) and ductal and alveolar cells during pregnancy (**B**). A cap cell layer surrounds the body cells. The cap cells can take on either a myoepithelial lineage or a luminal epithelial lineage and therefore are thought to be multipotent stem cells. Differentiated myoepithelial and luminal epithelial cells line the neck of the TEB and the subtending duct. During midpregnancy the ducts are surrounded by a basal layer of overlapping myoepithelial cells, whereas the alveoli cells are surrounded by a basket-like layer of myoepithelial cells.

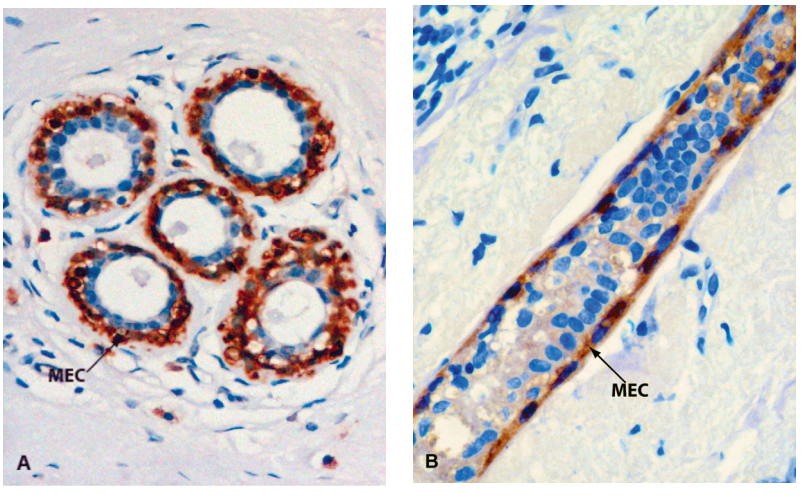

Figure 5. Myoepithelial cells (MEC) surrounding breast excretory ducts highlighted by maspin immunoreactivity. MECs are arranged in a continuous layer with their long axis being laid parallel to the ducts. Cross section (**A**), longitudinal section (**B**) (A and B × 600).

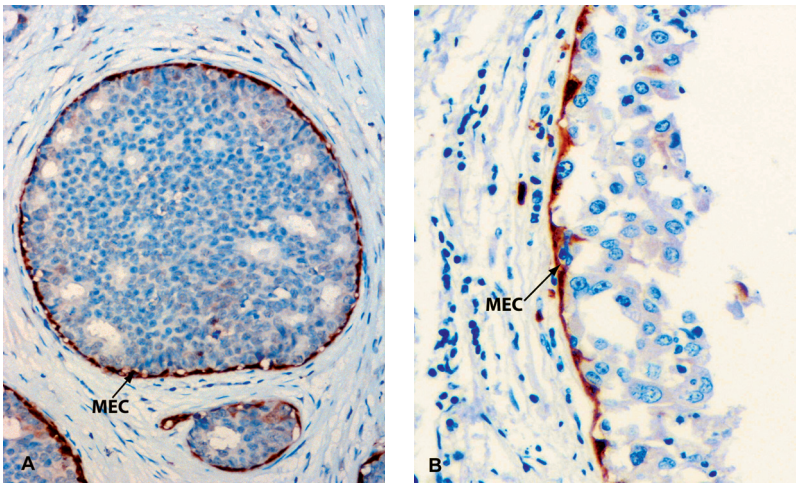

Figure 6. Ductal carcinoma in situ (DCIS). The proliferating DCIS cells are localized within the large distended duct, which is still lined by an intact layer of myoepithelial cells (MEC) exhibiting intense maspin immunoreactivity (arrows) (**A** ×400; **B** ×600).

every stage of development. The analysis of mammary gland ultrastructure demonstrated the presence of three types of epithelial cells: luminal cells, myoepithelial cells, and basal pale cells, which, as the authors suggest, may constitute the population of stem cells. In human mammary glands, a gradual transition of pale cells into fully differentiated MECs was demonstrated, but there are no observations of the transition of these cells into differentiated luminal cells [64, 75].

Isolated suprabasal cells in three-dimensional (3D) cultures in laminin-rich media form structures similar to functional units of the mammary gland (commonly referred to as terminal duct lobular units [TDLUs]) with an internal layer of CK19 positive luminal cells and an external layer of CK14 and α-SMA positive cells similar to MECs. There is strong evidence that luminal epithelial and myoepithelial cells are derived from a suprabasal cell type [22].

The presence of cells with simultaneous CK19 and CK14 expression was observed in mammary gland epithelium. These cells located within duct bifurcations are relatively weakly differentiated and dye-resistant. Planted into pure fat tissue they form follicular and tubular structures, which proves that they have full potential of differentiation [3]. The analysis of human mammary gland specimens exhibited a population of bipotential CK5 positive cells differentiating either into luminal or myoepithelial cells [9].

Recently, several populations of stem cells differing in determination level have been identified in human mammary gland. A helpful combination of markers, applied for the sorting of mammary gland stem cells, is made up of EpCam (epithelial adhesion molecules, also known as epithelial specific antigen — ESA), CD49f, and MUC1 (luminal cells-specific glycoprotein). EpCam displays a strong expression in luminal cells and poor expression in basal cells, and the expression pattern for CD49f is reversed. Bipotential basal stem cells, capable of differentiation into luminal or myoepithelial cells, exhibit poor expression of EpCam, strong expression of CD49f, and no expression of MUC1, whereas luminal progenitor cells display strong expression of EpCam and the presence of $CD49f^-$ and $MUC1^+$ [17, 72].

In a suspension of cells freshly isolated from mammary gland, 1% display the ability to proliferate and form three types of colonies in the medium. Most of them (70%) create tight clusters of cells displaying expression of CK18, CK19, and MUC1 antigens specific for luminal cells, but no expression of CK14 typical for basal cells. A further analysis showed the cells to present luminal cell phenotype, high expression of EpCam, and the presence of CD49f and MUC1 antigens. The second population, in terms of quantity (25%), is formed by bipotential progenitor cells with poor expression of EpCam, strong expression of CD49f, and no expression of MUC. These cells created colonies characterized by the presence of cells with CK14–, K18+, K19+, and MUC1 in their central area, and CK14+ basal cells in the peripheral area. Both cell types, as demonstrated using clonal analysis, descended from common progenitor cells. The third type of cells included stem cells of MEC forming colonies consisting only of basal cells of CK14+, CK18–, CK19–, and MUC1-phenotypes. Precursors of MEC, as further analysis showed, originate directly from bipotential stem cells [71].

MYOEPITHELIAL CELLS IN MORPHOGENESIS AND ORGANIZATION OF MAMMARY GLAND STRUCTURE

The location of MEC between the luminal cells and the extracellular matrix suggests their active role in the exchange of information between the extracellular matrix and the luminal epithelium of the gland, and therefore their participation in the regulation of growth, morphogenesis, and the maintenance of the proper two-layered structure of the mammary gland (Fig. 5).

MECs show a strong expression of receptors of integrins and growth factors such as EGF and FGF-2, which may suggest their regulatory function. Activin, belonging to the TGF-β superfamily, is expressed only in MECs of mammary gland and plays a role in the regulation of ducts growth [38]. Maspin, being exclusively expressed in MECs, has been shown to play a significant role in the morphogenesis, development, and functioning of mammary gland. Maspin gene overexpression coupled with WAP promoter (responsible for the regulation of milk protein expression, and active since half way through gestation until the end of lactation) led to the inhibition of gland growth and disturbances in the formation of mature gland structures. Transgenic mice with maspin overexpression had a decreased number of alveolar structures unable to synthesize milk components [83].

In normal mammary gland, only MECs display the expression of OTR and are the hormone target [11]. Benson and Folley [8] were the first to demonstrate the vital role of oxytocin in the regulation of differentiation, growth, and involution of mammary gland. Subsequent studies, performed on knocked-out mice

unable to synthesize oxytocin, demonstrated their inability to form the proper lobulo-alveolar structure of mammary gland in the period of puberty, and the impairment of the further development of the gland during the postpartum period [77]. Oxytocin injection into non-lactating mice induces proliferation and differentiation of MECs. This phenomenon was observed only in the mice treated previously with progesterone and oestrogens. These findings suggest that the proliferational effect of oxytocin depends on the level of mammary gland development and works at the hormonal stage corresponding to the pregnancy period [58].

The expression of both oxytocin mRNA and peptide observed in MEC primary cultures [12] suggests that these cells may act as a local source of oxytocin synthesis, and therefore may be involved in auto/ /paracrine regulation of proliferation and differentiation of mammary gland cells at all stages of its development.

The integrity of mammary gland epithelium is maintained by a complex system of cell/cell and cell/ /extracellular matrix interactions mediated by cadherins and integrins.

In mammary gland P-cadherins are located only in MECs and the cells of terminal end buds, while E-cadherins are exclusively in luminal cells [15]. Virgin mice with P-cadherin deficiency display accelerated mammary gland growth, and luminal cells initiate the synthesis of casein, likewise during the period of early gestation. It finally leads to hyperplasia and dysplasia of the gland epithelium. These observations indicate that P-cadherins mediate interactions between myoepithelial and luminal cells and participate in the control over growth and differentiation of mammary gland epithelium [53].

In epithelial cells, β-catenin is a component of cadherin-containing intercellular junctions. The interactions with MECs trigger the Wnt/β-catenin signalling pathway in luminal epithelium, the main mediators of which are Tcf transcription factors. The presence of both Tcf4 and Tcf1 was observed in mammary gland epithelium; however, only in MECs was the nuclear Tcf1 localization demonstrated [57].

A significant role in the formation of normal mammary gland structure is played by desmosomal cadherins: desmoglein (Dsg) and desmocollin (Dsc). They display different expression in luminal and myoepithelial cells. Dsg2 and Dsc2 are present in both layers of the cells, while Dsg3 and Dsc3 occur only in MECs. Co-culture of myoepithelial and luminal cells form bilayered structures resembling their *in vivo* arrangement. The introduction of specific peptides inhibiting myoepithelial Dsc3 and Dsg3 to the co-culture causes distortions in the formation of the proper bilayer epithelium structure and disturbs the polarization of luminal cells [21]. These observations prove that MECs play a significant role in the arrangement of mammary gland structure and polarization of luminal cells via direct interactions between cells mediated by desmosomes [25].

Isolated mammary luminal cells in 3D cultures in collagen I gel form lumenless alveolar structures. Double labelling for MUC1/ESA and then for MUC1/ /occludin demonstrated that cells form clusters with reversed polarity. Introducing MECs to the culture caused the correct polarization of epithelial cells and the forming alveolar structures to have clearly distinguished lumen.

In addition, the presence of laminin-1 (but not other laminin isoforms; 5, 10, or 11) in the culture gel allowed the formation of correct epithelium polarization. MECs are the only cells synthesizing laminin-1 (among other main components of the basement lamina), and their introduction to the culture makes up for laminin-1 deficiency, which leads to the appropriate formation of alveolar epithelial structures [22].

Although laminin-1 is the key regulator of correct polarization of epithelium and the morphogenesis of mammary gland, other molecules produced by MECs may also take part in this process.

Except for the occurrence of all integrins present in luminal cells, mammary gland MECs show an exclusive strong expression of $\alpha 1\beta 1$ and $\alpha 5\beta 5$ integrins. Inactivation of $\beta 1$ integrin in virgin mice leads to disturbances in the formation of ducts and their branching, resulting in an improper arrangement of the lobulo-alveolar structure of the mammary gland during the gestation [20].

In MECs and cap cells in terminal end buds (TEB), the presence of neogenin — the receptor of netrin-1 (which plays a significant role in the development of the nervous system by directing neuronal processes to their target locations) — has been detected. The expression of netrin1 has also been observed in internal cells of TEB and luminal cells. The analysis of knockout mice devoid of neogenin or netrin-1 expression show structural abnormalities in TEB; cap cells separate from internal cells and the basement lamina loses its integrity. Netrin-1/neogenin interaction plays a significant role in the formation of mammary gland cytoarchitecture both via the regulation of cell adhesion and via some unknown signalling pathways [68].

MYOEPITHELIAL CELLS AS SUPPRESSORS OF BREAST CANCER

MECs present in normal mammary glands and benign forms of tumours are considered natural suppressors of breast cancer. They inhibit the growth of tumours and neoangiogenesis, induce apoptosis, and limit the mobility of cancer cells. MEC localization between the basement lamina and the layer of luminal cells suggests their paracrine action on adjacent luminal cells as well as on the connective tissue and endothelial cells [48].

A commonly accepted hypothesis states that with the increase in cancer malignancy the ratio of luminal to myoepithelial cells rises, while the invasive cancers are almost devoid of the latter. In invasive cancers, MEC markers — CK14, CK17, and vimentin — are present in less than 20% of all tumours [76].

Although the presence of MECs is associated with the maintenance of ductal cancer in situ (DCIS) in the benign form even for a long period, some of these cancers undergo malignant transformation. Comparative analysis of gene expression has shown significant differences between MECs accompanying DCIS and MECs of normal mammary gland ducts. DCIS-associated MECs exhibit lowered expression of oxytocin receptors, laminin-1, and thrombospondin, but higher expression of chemokines responsible for cellular proliferation, migration, and invasiveness of cells such as SDF1/CXCL12 and CXCL14. Moreover, an increase in enzymes engaged in extracellular matrix degradation, such as numerous metalloproteinases, as well as various epigenetic changes connected with higher levels of DNA methylation, were observed in these cells [1]. These properties of DCIS-associated MECs may suggest their paracrine effects on normal glandular epithelium resulting in malignant transformation.

Myoepithelial cells associated with DCIS lose contact with luminal cells, which leads to disturbances in luminal cell polarization and inhibition of the basement lamina component synthesis by MECs. In breast cancers with laminin-1 synthesis deficiency in MECs, even the presence of these cells was not correlated with better prognosis [39]. On the other hand, breast tumours with functioning MECs that synthesize basement lamina components do not give distant metastases and are correlated with better prognosis [32]. Therefore, it can be stated that the presence of MECs in breast tumours inhibits the alterations associated with malignant transformation [7].

A break of integrity of the MEC layer affects the genetic and functional changes in the luminal cells located above it. A decrease in expression of oestrogenic receptors, higher frequency of heterozygosity occurrence, higher cellular proliferation index, and increase in expression of genes related to the active mobility of cells and angiogenesis have been observed in these cells.

Normal MEC and myoepithelial cell lines derived from benign breast tumours produce relatively high numbers of protease inhibitors and anti-angiogenic factors [48]. HMS1 mammary myoepithelial cells (the cell line derived from benign tumours) are characterized by a high ratio of proteinase inhibitors to proteinases, unlike mammary cancer cell lines where the number of proteinases significantly prevail their inhibitors. The analysis of the profile of inhibitors secreted by MECs revealed the presence of tissue proteases inhibitor (TIMP-1), plasminogen activator inhibitor, and trypsin inhibitors such as α1-antitrypsin [70].

Numerous studies point towards a significant role of fibroblasts in the progression and invasiveness of cancers. The majority of metalloproteinases (MMP) participating in cancer progression are fibroblasts produced in response to signals coming from cancerous cells. MECs suppress the pro-invasive dialogue between cancerous and fibroblast cells by the inhibition of MMP expression, the mechanism of which has not been elucidated. There are some suggestions pointing towards growth factors produced by MECs (TGF-β, FGF-2, activin, or components of Wnt signalling pathway) as the mediators reducing the expression of pro-invasive MMP in fibroblasts [30].

A break of integrity of the MEC layer and its atrophy in cancer tissue may be caused by an autoimmunological reaction and the impact of leukocytes and macrophages on the basement lamina, as well as their direct action on MECs. An increase in leukocytes and macrophage numbers is an evident feature of in-situ cancer transition into infiltrated invasive forms, and it coincides with bad prognosis and higher mortality rates. Leukocytes are able to force the barrier of the basement lamina and the MEC layer thanks to the secretion of numerous proteases, effectively degrading their components [40].

In normal mammary gland as well as *in vitro* cultures, MECs are able to synthesize and secret maspin. Breast cancer cells co-cultured with MECs (HMS-1) lose their capability of effective migration. A similar effect was observed by culturing breast cancer cells in medium previously used to culture MECs. The introduction of dexamethasone into the co-culture of MECs and breast cancer cells leads to a maspin synthesis increase and completely inhibits the capabili-

ty of cancer cell migration. Immunoprecipitation of maspin in culture medium with specific anti-maspin antibodies abolishes the suppression effect of the medium on the invasiveness of cancerous cells [6].

In maspin-treated breast cancer cells, the profile of integrin expression on the surface changes in direction promoting binding with collagen and fibronectin (increased expression of α3 and α5 integrins accompanied by the decrease of others) [4]. Alterations caused by maspin also include proteins which are engaged in signalling pathways related to cell mobility (reduced activity of Rac1 kinase and increased activity of ERK1/2 and PI3K kinases) [49]. Another form of maspin impact on cancer cell decreased mobility is the inhibition of proteases engaged in plasminogen activation (responsible for the digestion of the extracellular matrix, which promotes the migration). The mechanism of maspin action consists of binding urokinase plasminogen activator (uPA), its precursor (pro-uPA), and its receptor (uPAR) [62].

Moreover, MECs are able to cleave the surface form of the CD44 molecule generating its soluble form, which inhibits the migration ability of adjacent cancer cells [2].

MECs in normal mammary glands and in DCIS tumours separate the luminal epithelium from the blood vessels of the gland stroma, forming a barrier that is impenetrable to the vessels (Fig. 6). Myoepithelial cells (HMS) derived from benign mammary gland tumours (myoepithelioma) are characterised by high expression of active angiogenesis inhibitors such as TIMP-1, thrombospondin, or maspin, and low level of angiogenic factors. The comparison of xenografts of breast cancer tumours derived from myoepithelial or luminal cells has shown an intensive synthesis of extracellular matrix components and very little neoangiogenesis in the first case, but almost ten times higher neoangiogenesis in the second case. Both HMS-1 myoepithelial cells and HMS-1 concentrated culture medium significantly inhibit migration and proliferation of endothelial cells. This effect is enhanced by phorbol esters but stopped by cycloheximide and dexamethasone [48]. Immunoprecipitative analysis of the culture medium points to maspin and thrombospondin as the key molecules responsible for angiogenesis inhibition by MECs [84].

Myoepithelial neoplasm-derived cells produced in culture significantly lower quantities of vascular endothelial growth factor and nitric oxide synthase activity in response to hypoxia, when compared with the cell line derived from luminal cancer [48]. This observation supports the hypothesis assuming that MECs, even those subjected to malignant transformation, exhibit natural anti-angiogenic capabilities.

An important function of MECs in cancer suppression is their involvement in steroid hormone metabolism. Comparative studies on the influence of 17-β estradiol on steroid sulphatase (STS) in normal human MECs and breast cancer cells (MCF-7) have shown STS activity to be more than a hundred times higher in MECs [73]. Exposure to 17-β estradiol led to a 70% reduction in STS activity in MCF-7 cells, and a 9% activity increase in MECs, which suggests that MECs may participate in the conversion of precursors into active steroid hormones. The exposition of MECs to tamoxifen enhances the synthesis of maspin and inhibits maspin-dependent invasiveness of cancer cells. The introduction of 17-β estradiol inhibits the effect of tamoxifen on MECs, which suggests that the mechanism of tamoxifen action is dependent on oestrogen receptors. Since MECs have ERβ receptors (but not ERα), it might be concluded that tamoxifen-induced maspin secretion results from the triggering of the signalling pathway initiated by ERβ receptors, and activation of transcription factors AP1 [61].

The presented data allow the notion suggesting that MECs have a genetic program preventing not only their own malignant transformation but also the transition of noninvasive tumours derived from luminal cells into malignant forms of breast cancer in an autocrine or paracrine way.

However, the changes of the genetic expression profile of MECs, which co-occur with the transformation of benign cancers into their malignant forms, may modify MECs in such a way that allows them to enforce proliferation, migration, and invasiveness of cancerous cells [1].

BREAST CANCERS DERIVED FROM MYOEPITHELIAL CELLS

The analysis of the genetic profile of breast cancers allows their subdivision into four basic histogenetic types: one normal breast tissue-like type, two luminal-like types, one *ERBB2*-overexpressing type, and one basal-like type. Each of the types is characterized by a diverse clinical response [67]. The basal-like type of breast cancer derived from MECs comprises 2–18% of all invasive ductal breast cancers and can be identified with the use of markers specific for MECs, such as cytokeratins (CK5, CK14, CK17), smooth muscle cells actin and myosin, and others, such as p63 or s100 protein [31].

Breast cancers of basal/myoepithelial type differ from other ductal breast cancers in significant mor-

phological features. They usually are high-grade (III) tumours and often contain in their central area a non-cellular substance consisting of necrotic cells debris, collagen, and hyaline substance [74]. Besides the expression of MEC markers, these cancers are also characterized by the lack of expression of progesterone (PR), oestrogenes (ER), and HER-2 receptors [29]. The microarray analysis of the immunophenotype and genetic expression profile shows many similarities between sporadic basal cancers and inherited cancers with BRCA1 mutation [35, 67]. Basal breast cancers, unlike luminal cancers, are characterized by a high expression of gene coding for α2 and γ2 laminin chains and â4 integrin subunits [51].

Further analysis of mammary gland basal cancers has shown 82% frequency of TP53 mutation, while in luminal-type cancers it was only 13%. TP53 mutations are associated with poor prognosis and poor response to therapy [66]. Poor clinical prognosis is also related to the high cancer grade and the lack of expression of steroid hormone receptors. In rare cases, basal cancers are connected with increased risk of brain metastases and higher mortality rates, independently of the lymph node status and tumour size. Numerous clinical trials show that breast cancers with 5 and 17 cytokeratins expression (specific for basal cells) exhibit poor prognosis and shorter survival rates.

However, some authors argue that such an interpretation is too simple because these might not be pure myoepithelial cancers, and the presence of MEC markers within the tumour may be caused by luminal cells exhibiting high phenotypic plasticity, or by stem cells with broad expression of both myoepithelial and luminal cell markers [13].

The basal type of breast cancer with poor clinical prognosis, negative for oestrogen receptors, has markers of both luminal and myoepithelial cells, and MECs are partially differentiated, unlike luminal cells which are highly unorganised. It should be mentioned that MECs are highly resistant to malignant transformation, and even if they undergo the transformation the cancers derived from MECs are benign, except for malignant myoepithelioma which is the least frequent form of breast cancer [34].

SUMMARY AND CONCLUSIONS

Although myoepithelial cells of mammary gland comprise the second population in the gland (with respect to cell number), they have not been the subject of many scientific studies until recently when their important role in the regulation of proliferation, differentiation, and activity of luminal cells, and morphogenesis of mammary gland have been observed.

The layer of MECs and basal lamina (most of the components of which are produced by these cells) form a selective barrier regulating bidirectional exchange of information between mammary epithelium and stroma cells (fibroblasts, endothelial cells). The regulation of luminal cell function and mammary gland development may result from the direct effect of the cells via integrins and cadherins of cell--cell junctions but may also be caused by the paracrine influence on neighbouring cells through numerous regulatory proteins.

MECs of normal mammary glands or benign breast tumours are natural cancer suppressors, responsible for the maintenance of the proper structure of the gland.

In numerous studies, the inhibitory effect of MECs on cancer growth, invasiveness, and angiogenesis has been demonstrated. The elucidation of the mechanisms of MEC differentiation and their involvement in mammary gland morphogenesis and malignant transformation would allow better insight into breast cancer biology resulting in the improvement in cancer diagnosis and therapy.

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