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Myoepithelial Cells: Pathology, Cell Separation and Markers of Myoepithelial Differentiation

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Until recently the myoepithelial cell has been studied relatively little in terms of its role in breast cancer. A number of malignancies showing myoepithelial differentiation have been reported in the literature, although they are still thought to be relatively rare and only limited studies are published. As a result of recent expression profiling experiments, one type of tumor with myoepithelial features, the so-called 'basal' breast cancer, has received a renewed interest, although it has been known to pathologists for more than two decades. These tumors, which express markers of both luminal and myoepithelial cells, are now being studied using antibodies against some new molecules that have emerged from studies of sorted normal luminal and myoepithelial cells. These immunohistochemical data, combined with genomic studies, may lead to better identification and management of patients with 'basal' tumors.

KEY WORDS: cell separation; myoepithelial tumors; basal-like tumors; basal markers.

INTRODUCTION

The normal duct-lobular tree of the breast comprises an inner luminal (secretory) cell layer and an incomplete, outer myoepithelial (contractile) cell layer. It has been a dogma of breast pathology that the vast majority of benign and malignant disease arises as a result of changes in the luminal/secretory cell of the terminal duct-lobular unit (TDLU) *(1)*. As such, the myoepithelial cell has been thought of as the "Ugly Sister" rather than as the "Cinderella" of breast biology *(2)*, providing a function in lactation but otherwise deemed irrelevant. Hence, it has received relatively little attention in terms of its role in breast cancer. Nevertheless, over the last 2–3 decades, malignancies showing myoepithelial differentiation, including pure myoepithelial carcinoma, adenomyoepithelioma, adenoid cystic carcinoma, adenosquamous carcinoma, metaplastic carcinomas and poorly differentiated myoepithelial-rich carcinoma have been increasingly reported in the literature. Tumors exhibiting myoepithelial differentiation were first described in the late 1960s when ultrastructural studies revealed that a proportion of breast cancers had features of myoepithelial cells *(3)*. Since then, myoepithelial tumors, which are still thought to be relatively rare, have been examined in a handful of limited studies *(4–7)*. Although there are relatively few total cases in the literature, and even fewer studies of individual subtypes, it appears that about half of pure myoepithelial carcinomas have an aggressive clinical course with regional and distant metastasis.

Due to the small number of reported cases, it is currently hard to define the true natural history of

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Abbreviations used: TDLU, terminal duct-lobular unit; CGH, comparative genomic hybridization; CK, cytokeratin; SMA, smooth muscle actin; SMM, smooth muscle myosin; ER, estrogen receptor; PR, progesterone receptor; FACS, fluorescence-activated cell sorting; EMA, epithelial membrane antigen.

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any of the subtypes, and hence management of patients with these tumors remains problematic. Currently, patients are managed pragmatically based on the pathologist's assessment of the grade of the tumor and the clinical and radiological stage. The choice of treatment is also currently arbitrary, in that such tumors are generally regarded for decision purposes as high-grade breast cancers.

There are few molecular data on this group of tumors. Interestingly, a study of 10 pure myoepithelial carcinomas (spindle cell tumors) showed relatively few genetic alterations (mean 2.1) by comparative genomic hybridization (CGH) compared to ductal carcinomas of no special type (Grade I, mean 5.4; Grade III, mean 11.7) *(8)*. All of the alterations in the myoepithelial tumors have previously been described in invasive breast carcinoma with a luminal phenotype, but several commonly found alterations were not found in the myoepithelial tumors, such as gains of 1q, 8q, and 20q, and losses of 1p, 8p, and 13q. Interestingly, a CGH study of myoepithelial tumors of the salivary gland also showed a low number of alterations (mean 2.4) *(9)*. It is interesting to speculate why an aggressive tumor has so few alterations and whether the regions altered are the critical to tumorigenesis.

PATHOLOGY OF "BASAL" BREAST CANCER

The type of breast cancers with myoepithelial differentiation that has received the most publicity as a result of recent expression profiling experiments is the so-called "basal" breast cancer. This group has been known to pathologists for two decades prior to the microarray studies. They comprise 2–18% of invasive ductal carcinomas and up to 25% of grade III invasive cancers in various published studies *(10,11)*. The "basal" breast cancers have been identified using a variety of molecules found in normal myoepithelial cells of the breast. These molecules include the intermediate filaments cytokeratins (CK) 5, 14, and 17, muscle components such as smooth muscle actin (SMA) and smooth muscle myosin (SMM) and other markers of myoepithelial cells such as s100 and p63. Tumors exhibiting such a phenotype have also been variously known as "basal-like" or "tumors with basal/myoepithelial phenotype."

Although a comprehensive characterization, definition and consensus of basal tumors is lacking, work from our own laboratory as well as those of others have shown some distinct features, different from the common infiltrating ductal carcinomas. Morphologically, they appear to be predominantly high-grade (grade III) tumors *(11,12)* and are reported to frequently contain large central acellular zones composed of necrosis, tissue infarction, collagen, and hyaline material on their cut surfaces *(13,14)*. In addition to expressing a number of myoepithelial/basal markers, these tumors are predominantly estrogen receptor (ER), progesterone receptor (PR), and ERBB2 (HER-2) negative *(11)*, an immunophenotype resembling BRCA1-associated tumors *(15)*. The histology and immunophenotype of some basal breast tumours is illustrated in Figure 1. Microarray analysis has also demonstrated a similarity between sporadic (nonfamilial), basal-like tumors and those familial tumors harboring a BRCA1 germline mutation, based on their patterns of gene expression *(16)*.

The pathogenesis of such lesions appears to indicate a poor prognosis. As well as the association with high histological grade and steroid receptor negativity, the basal phenotype has been reported in a single series to be associated with a high risk of brain and lung metastases and of death by cancer independent of nodal status and tumor size *(13)*. The experience from our laboratory is that brain metastases are indeed more common in patients with "basal" tumors compared to those with grade-matched nonbasal tumors. Lung metastases are equally likely to occur in both groups, however the time to lung metastasis is shorter in the basal group (Fulford and Lakhani personal communication).

In addition to differences in metastatic pattern, the basal-like phenotype has been linked to shorter overall survival. A basal-like group identified by expression profiling experiments in the literature conferred a shorter survival time than the other tumor groups described *(17)*, and tissue microarray analysis of basal keratins 5 and 17 has also shown a poorer clinical outcome in node-negative tumors expressing one or both of these markers *(18)*. Work from our own laboratory, however, suggests that a bad prognosis for all basal tumors is an oversimplification. We studied the patterns of genetic alterations in basal and nonbasal tumors by CGH analysis. In this study the CK14-positive areas of the basal tumors showed fewer CGH alterations compared to the nonbasal group, with an increased prevalence for losses at 16p, 17q, and 19q, all alterations associated with pure myoepithelial carcinomas *(8)*. In contrast, the areas of the basal tumors that were negative

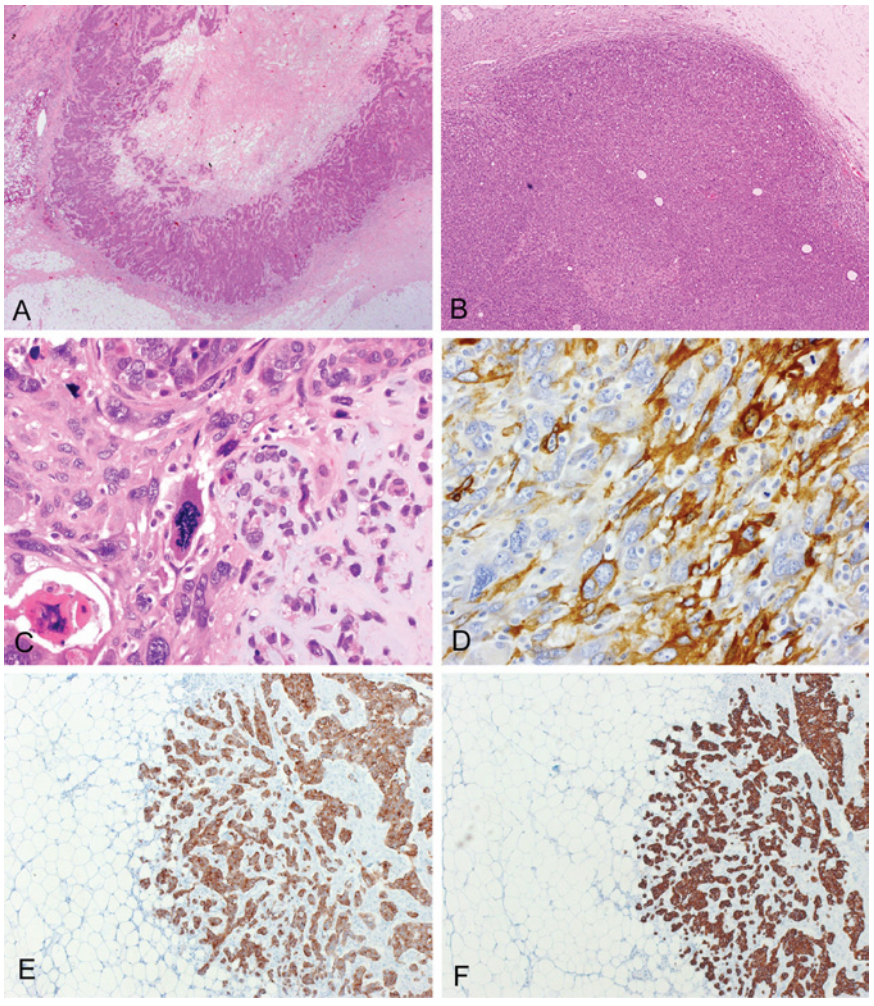

Fig. 1. Histology of myoepithelial tumors. (A) Basal breast cancer with central scarring (H&E). (B) BRCA1-associated tumor with medullary-like features and a pushing invasive edge (H&E). (C) Metaplastic carcinoma with giant cells, squamous metaplasia and chondroid differentiation (H&E). (D) CD10 immunoreactivity in a metaplastic breast carcinoma (DAB). (E,F) Dual myoepithelial CK14 (E) and luminal CK19 (F) immunopositivity in a basal breast cancer (DAB). Such tumors often display bi-differentiation rather than pure basal/myoepithelial or luminal differentiation.

for CK14 showed not only the changes associated with myoepithelial carcinomas, but also further alterations found in invasive ductal carcinomas. The CK14 positive and negative areas had overlap between the CGH changes, suggesting that the different areas arose from a common precursor and subsequently diverged. The basal group could be further divided by its CGH profile into two groups with differing overall survival. One group exhibited a poor prognosis and hence may require a different management approach; however, the other group had a similar prognosis to grade-matched nonbasal tumors *(19)*. Although no individual gains and losses showed prognostic information on their own, gain of 1q and 6p, as well as losses of 3p, 4p, and 8p did indicate shorter disease-free and overall survival times in the whole cohort. The existence of two groups of basal cancers with differing outcomes has also been observed previously in expression array experiments *(20)*. The data from these two pieces of work suggest, therefore, that subgroups of basal breast tumors exist with distinct genetic patterns, which may lead to differences in gene expression and hence biological behavior of the tumor.

With a recent surge of interest in normal cells and stem cells, there has been a tendency in the literature to assign cell types to particular subtypes of breast cancer (a histogenetic model for cancer

classification). This approach represents a significant divergence from previous practice and has implications for pathologists as they use differentiation to classify rather than assign cell types of tumor origin. We believe that while it is interesting to speculate, we should be circumspect about jumping to a histogenetic classification. In this regard, although tumors which express basal/myoepithelial markers may be derived from myoepithelial cells, they could equally well arise from luminal cells that show phenotypic plasticity or even from stem cells capable of expressing a range of markers. There is a paucity of data on normal cell types in the breast and perhaps by combining morphology, immunophenotype and genetic data we can start to address the issue of which tumor types are derived from which cells. This type of approach has been considerably enhanced by the availability of a large range of markers of both the luminal and the myoepithelial phenotype derived from studies using luminal and myoepithelial cells sorted from the normal human breast *(21)* and analyzed by proteomics *(22)* and expression arrays *(19)*.

Options for separating luminal and myoepithelial cells from normal human breast are described in detail below. Techniques have also been published to separate human breast endothelial cells *(23)* and inter- and intralobular fibroblasts *(24)*, but these cell types are not within the scope of this review.

CELL SEPARATION

The epithelial fraction of the human breast may be extracted in the form of lobular and ductal fragments (organoids) from reduction mammoplasty or mastectomy specimens using mechanical and enzymatic dissociation (collagenase ± hyaluronidase) *(25,26)*. The organoids consist of luminal epithelial cells and myoepithelial cells in approximately equal numbers, sometimes with a small number of fibroblasts attached at the periphery. The organoids may be further dissociated by trypsinisation to form a single cell suspension. Although this method avoids culturing the cells, and thus reduces any associated artifacts if the cells are to be analyzed directly, the cells are fragile following the collagenase digestion and, with the added trypsinization damage, a large proportion may be lost. Alternatively the organoids may be plated in culture for 5–10 days, during which time the cells mobilize and spread to form a monolayer that can easily be trypsinized.

Myoepithelial cells rarely divide in the adult breast in vivo whereas luminal epithelial cells go through cycles of division with each menstrual cycle and greatly increase in number during pregnancy *(27,28)*. When the cells are put into culture, however, the myoepithelial cells divide rapidly and the luminal cells more slowly *(29)*. This divergence poses a problem for researchers studying the luminal population, since any residual myoepithelial cells in sorted luminal cultures will gradually increase as a proportion of the cells during time in culture. In contrast, any residual luminal cells in myoepithelial cell populations will be reduced as a proportion over time.

Low calcium medium has been reported to produce pure populations of human luminal cells as a result of their selective detachment from primary cultures, leaving the myoepithelial cells attached to the culture surface *(30,31)*. In our hands this method produces highly enriched cultures of each cell type *(21)*; however, if pure populations of cells are needed then further purification is required.

O'Hare *et al.* separated luminal and myoepithelial cells by fluorescence-activated cell sorting (FACS) using antibodies against epithelial membrane antigen (EMA) and CD10, respectively *(29)*. Although the cell populations are highly purified using this method (>98% by cytokeratin analysis), the number is limited to about 10^5 cells of each type. Several factors contribute to the limited cell numbers. Epithelial cells are difficult to make into a single cell suspension because trypsinization that is adequate to separate the cells (particularly myoepithelial cells) tends to cause some cell damage, resulting in stickiness which causes clumping. This problem can be partly alleviated by including a DNAase step in the cell preparation, but even with this step it is necessary to filter the cells to produce a single cell suspension. A proportion of the cells may be lost during filtration, but the production of a single cell suspension is crucial to all cell sorting techniques and so these losses are unavoidable. For FACS, this step is particularly important to avoid clogging the flow sorter. Doublets and triplets of cells may remain even after filtration, but these are excluded from the flow-sorted populations by gating the light scatter in order to sort only single, viable cells. In addition, cells may be lost due to anti-coincidence gating. Thus, purity is maintained at the expense of high yield.

Immunomagnetic methods have become widely used for cell separation because of their ease of use and relatively low cost. Myoepithelial cells have been successfully separated using Dynabeads combined with anti-CD10 *(21,32)* or anti β4-integrin antibodies

(33). In our hands this method produces 95% pure populations, and these can then be used for a number of studies either with the beads still attached or, by using beads attached via a DNA linker (CELLection beads), the beads can be removed using DNAse. In contrast, luminal cell separation is less reliable using Dynabeads. Using anti-EMA antibodies our group found that antigen stripping occurred from the cell surface and, as a result, very few luminal cells could be separated *(21)*. In contrast, Gomm *et al.* successfully separated luminal cells using anti-EMA antibodies, provided that no mechanical agitation of the system occurred *(32)*.

The MACS separation system (Miltenyi Biotech) uses small iron dextran particles that require a stronger magnetic field created within a MACS column containing a magnetizable matrix that is placed in a permanent magnet to achieve cell separation. In our hands, antigen stripping did not occur with this system, and both luminal and myoepithelial cells could be separated.

Both types of immunomagnetic systems have the disadvantage that doublets and triplets of cells will be retained if either/any of the cells carry beads. This can result in an increased yield if each of the cells is in fact positive, however the purity can be reduced if a negative cell is attached to a positive one. By combining the two systems, however, the purity can be increased while retaining any positive clusters. For luminal cells, we found the main contaminant to be myoepithelial cells, which can be removed with Dynabeads following a MACS luminal cell separation, because the MACS beads are too small to allow the positive luminal cells to be retained in the Dynal magnet. Although luminal cells are more difficult to remove from myoepithelial cell preparations because of the difficulty of separating them with Dynabeads, their lower proliferative potential compared to myoepithelial cells means that they will become a smaller proportion of the population with time in culture. If CD10 antibodies are used to separate myoepithelial cells, a number of fibroblasts may also be positively sorted, since they are frequently found in primary breast cell cultures and because they gradually switch on expression of CD10 in culture. Although the majority of the fibroblasts can be removed by using a Dynabead step with anti-fibroblast activation protein antibodies, this difficulty can be avoided by using anti-β4 integrin antibodies. Furthermore, by combining low calcium medium incubation as described earlier with immunomagnetic methods, the purity of the cell preparations can be further increased to reach >98% purity for each cell type.

ANALYSIS OF SORTED LUMINAL AND MYOEPITHELIAL CELLS

The development of methods to separate large numbers of luminal and myoepithelial cells has allowed a number of experiments to be undertaken that were previously difficult or, in some cases, unfeasible. The studies include the production of *(34)* and response to *(35,36)* growth factors by breast cells, the study of potential stem cell populations *(37)*, the development of a cell line with stem-like properties *(38)* and an analysis of the contribution of different cell types and extracellular matrix to breast epithelial morphogenesis *(39)*. Importantly, the availability of sufficient cells has also allowed the luminal and myoepithelial cells to be compared using differential display *(40)* proteomics *(22)* and expression profiling *(19)*, in order to identify signatures for each cell type.

The use of sorted luminal and myoepithelial cells in differential display experiments represented an important improvement over previous experiments. A number of studies had previously used mixed populations of normal breast epithelial cells and compared these to tumor-derived cells or tumor cell lines. Since the majority of the tumor cells are of a luminal phenotype, the comparison with a population that includes a large proportion of myoepithelial cells will generate spurious results. In contrast, Martin *et al. (40)* used the separated populations to identify genes that were altered in tumors compared to normal luminal cells. They identified four clusters of genes that had expression patterns associated with parameters used clinically to characterize breast tumors: ER status, tumor stage, and tumor size. The tumor size-associated cluster included a reduced expression of some markers of myoepithelial cells, such as CK14 and CK5. The authors postulated that the reduction in CK14 in larger tumors may reflect a reduced proportion of normal cells in these large specimens.

A proteomic study of separated luminal and myoepithelial cells identified 33 proteins in the luminal population and 18 proteins in myoepithelial cells that were elevated by two-fold or more compared to the other cell type *(22)*, including a range of markers that were already identified as being differentially expressed, such as some cytokeratins (e.g., CKs14, 18, 19), and others that had not been previously identified, such as a 27 kD heat shock protein in myoepithelial cells.

We have used cDNA microarrays to compare the expression profiles of immunomagnetically sorted luminal and myoepithelial cells *(19)*. The

study had several objectives: to provide a baseline reference dataset to help understand tumor expression profiles, to establish whether novel cell-type specific markers can be used for tumor subclassification and differential diagnosis, and to identify new predictive and prognostic markers. Differential expression was confirmed by semiquantitative PCR for 56 genes. Where antibodies were available, the expression of a number of these genes was examined in paraffin-embedded archival samples by immunohistochemistry on tissue microarrays. Differential luminal expression in normal breast lobules was found for claudin 4 (CLDN4), CD24, and galectin 3 (LGALS3). CLDN4, a tight junction adhesion protein, strongly labeled luminal cell membranes. CD24 labeled the cytoplasmic compartment and apical cell membrane of normal luminal cells. Both of these markers were lost in a proportion of tumors examined (26% for CLDN4 and 70.4% for CD24), but neither marker provided any independent prognostic information. LGALS3 labeled the nucleus and cytoplasm of luminal cells and also labeled intralobular fibroblasts. This marker was lost in almost half of the tumor samples studied but its expression did not correlate with prognostic outcome except in those tumors where it was localized to the nucleus (2.1%), in which case it correlated with a poorer overall survival.

The myoepithelial markers identified by expression profiling were also used for immunohistochemical labeling of the tumor microarrays. Osteonectin (SPARC) was found to be positive in 4.9% of the cases, and Kaplan-Meier survival curves demonstrated a clear poor prognosis for SPARC-positive tumors. Furthermore, SPARC was found to be an independent prognostic factor with the highest relative risk of all factors fitted. Other myoepithelial markers were also positive in a proportion of tumors. S100A2 was positive in 1.8% of cases, but gave no independent prognostic information. Maspin (SERPINB5) was positive in 32.4% of cases and appeared to indicate a better overall survival, however, this did not reach statistical significance.

Another study using one of the myoepithelial cell markers identified by Jones *et al.* further highlights the problem of using unsorted breast epithelial cells to compare with tumor cells. 14-3-3σ (HME1/stratifin) is one of seven mammalian 14-3-3 isoforms that constitute a family of highly conserved proteins. This family functions in several regulatory processes including intracellular signaling, apoptosis, and cell-cycle control *(41,42)*. 14-3-3σ is associated with G1/S and G2/M cell-cycle arrest through its p53-dependent transactivation in response to DNA damage. It was identified as a myoepithelial cell marker in the expression profiling study, and yet it had previously been identified as a tumor suppressor gene *(43–46)*, a role that might be thought to be consistent with its function. In the studies that identified 14-3-3σ as a potential tumor suppressor, mixed populations of breast epithelial cells were used as the control, and thus a large proportion of myoepithelial cells would have been present. Molecules preferentially expressed in myoepithelial cells may appear to be down-regulated in breast carcinomas when compared with these cell populations. Our studies demonstrated by immunohistochemistry that 14-3-3σ is expressed by myoepithelial cells in normal breast tissue *(47)*. Luminal cells in some benign lesions showed occasional weak/moderate labeling for 14-3-3σ. Weak labeling was also observed in 27.7% of ductal carcinomas in situ with only one case in which the labeling was as intense as the reactivity observed in myoepithelial cells. Positivity for 14-3-3σ was also observed in foci of squamous metaplasia in malignant phyllodes tumors and myoepithelial neoplasms. The majority (81.1%) of invasive ductal carcinomas did not express this protein, however on whole tissue sections, 10/12 tumors with a basal-like or malignant myoepithelial phenotype (seven basal-like carcinomas and three malignant myoepitheliomas) expressed 14-3-3σ protein. Those tumors in which 14-3-3σ was localized in the cytoplasmic compartment had a shorter overall survival, and this association was even stronger in ER- and/or PR-positive tumors. Recent studies have shown that 14-3-3σ can regulate the activity of steroid receptors by determining their subcellular localization *(48)*, suggesting that 14-3-3σ may confer a distinct biological behavior in breast tumors.

Interestingly, unlike many other myoepithelial markers, such as smooth muscle actin, calponin, and smooth muscle myosin, which are related to the smooth muscle function of myoepithelial cells, 14-3-3σ showed focal positivity in fibroblasts and myofibroblasts in only 0.2% of the cases. This suggests that 14-3-3σ may aid in the diagnosis of hyalinized papillary lesions and complex sclerosing lesions, in which it is important to be able to identify myoepithelial cells surrounding entrapped ducts to exclude the possibility of invasion.

CONCLUSION

The development of immunomagnetic separation systems for the isolation of large numbers of

luminal and myoepithelial cells has led to the identification of a new range of luminal and myoepithelial-specific markers which are currently being used in studies of the basal phenotype in breast carcinomas. It has been increasingly recognized that the spectrum of tumors that exhibit a basal/myoepithelial phenotype is larger than traditionally appreciated by pathologists. These studies have the potential to lead to an understanding of the biological and clinical behavior of the subtypes of "basal" cancers and eventually to the identification of new targets for therapy. This is particularly important for basal tumors, since not only are many of the tumors aggressive, the majority are also ER negative and do not over-express ErbB2/HER2/Neu. They will therefore not respond to anti-estrogen therapy such tamoxifen or the new aromatase inhibitors currently completing clinical trials, nor will they be suitable candidates for the antibody therapy, trastuzumab (Herceptin), targeted to ErbB2. Furthermore, if markers can be established that distinguish between the basal tumors that have a bad prognosis and those that have a better prognosis, it will be possible to ensure that patients are neither under-treated nor over-treated.

Whether this category of tumors is really derived from the myoepithelial cell or is the result of luminal cell plasticity or stem cell transformation, there is little doubt that the myoepithelial cell has been the Cinderella of breast pathology and biology. It is clear that it has been misunderstood and is slowly but surely being put into its rightful place.

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REFERENCES

(1) Wellings SR. A hypothesis of the origin of human breast cancer from the terminal ductal lobular unit. Pathol Res Pract 1980;166:515–35.

(2) Lakhani SR, O'Hare MJ. The mammary myoepithelial cell—Cinderella or ugly sister? Breast Cancer Res 2001;3: 1–4.

(3) Murad TM, Scharpelli DG. The ultrastructure of medullary and scirrhous mammary duct carcinoma. Am J Pathol 1967;50:335–60.

(4) Eusebi V, Casadei GP, Bussolati G, Azzopardi JG. Adenomyoepithelioma of the breast with a distinctive type of apocrine adenosis. Histopathology 1987;11:305–15.

(5) Foschini MP, Eusebi V. Carcinomas of the breast showing myoepithelial cell differentiation. A review of the literature. Virchows Arch 1998;432:303–10.

(6) Lakhani SR, O'Hare MJ, Monaghan P, Winehouse J, Gazet JC, Sloane JP. Malignant myoepithelioma (myoepithelial carcinoma) of the breast: a detailed cytokeratin study. J Clin Pathol 1995;48:164–7.

(7) Thorner PS, Kahn HJ, Baumal R, Lee K, Moffatt W. Malignant myoepithelioma of the breast. An immunohistochemical study by light and electron microscopy. Cancer 1986;57:745–50.

(8) Jones C, Foschini MP, Chaggar R, Lu YJ, Wells D, Shipley JM, *et al.* Comparative genomic hybridization analysis of myoepithelial carcinoma of the breast. Lab Invest 2000;80:831–6.

(9) Hungermann D, Roeser K, Buerger H, Jakel T, Loning T, Herbst H. Relative paucity of gross genetic alterations in myoepitheliomas and myoepithelial carcinomas of salivary glands. J Pathol 2002;198:487–94.

(10) Gusterson BA, Warburton MJ, Mitchell D, Ellison M, Neville AM, Rudland PS. Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases. Cancer Res 1982;42:4763–70.

(11) Jones C, Nonni AV, Fulford L, Merrett S, Chaggar R, Eusebi V, *et al.* CGH analysis of ductal carcinoma of the breast with basaloid/myoepithelial cell differentiation. Br J Cancer 2001;85:422–7.

(12) Malzahn K, Mitze M, Thoenes M, Moll R. Biological and prognostic significance of stratified epithelial cytokeratins in infiltrating ductal breast carcinomas. Virchows Arch 1998;433:119–29.

(13) Tsuda H, Takarabe T, Hasegawa F, Fukutomi T, Hirohashi S. Large, central acellular zones indicating myoepithelial tumor differentiation in high-grade invasive ductal carcinomas as markers of predisposition to lung and brain metastases. Am J Surg Pathol 2000;24:197–202.

(14) Tsuda H, Takarabe T, Hasegawa T, Murata T, Hirohashi S. Myoepithelial differentiation in high-grade invasive ductal carcinomas with large central acellular zones. Hum Pathol 1999;30:1134–9.

(15) Lakhani SR, Van De Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, *et al.* The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002;20:2310–8.

(16) Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, *et al.* Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418–23.

(17) Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.

(18) van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J, *et al.* Expression of cytokeratins 17 and 5

identifies a group of breast carcinomas with poor clinical outcome. Am J Pathol 2002;161:1991–6.
(19) Jones C, Mackay A, Grigoriadis A, Cossu A, Reis-Filho JS, Fulford L, *et al.* Expression profiling of purified normal human luminal and myoepithelial breast cells: identification of novel prognostic markers for breast cancer. Cancer Res 2004;64:3037–45.
(20) Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, *et al.* Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A 2003;100:10393–8.
(21) Clarke C, Titley J, Davies S, O'Hare MJ. An immunomagnetic separation method using superparamagnetic (MACS) beads for large-scale purification of human mammary luminal and myoepithelial cells. Epithelial Cell Biol 1994;3: 38–46.
(22) Page MJ, Amess B, Townsend RR, Parekh R, Herath A, Brusten L, *et al.* Proteomic definition of normal human luminal and myoepithelial breast cells purified from reduction mammoplasties. Proc Natl Acad Sci U S A 1999;96:12589–94.
(23) Hewett PW, Murray JC, Price EA, Watts ME, Woodcock M. Isolation and characterization of microvessel endothelial cells from human mammary adipose tissue. In Vitro Cell Dev Biol Anim 1993;29A:325–31.
(24) Atherton AJ, O'Hare MJ, Buluwela L, Titley J, Monaghan P, Paterson HF, *et al.* Ectoenzyme regulation by phenotypically distinct fibroblast sub-populations isolated from the human mammary gland. J Cell Sci 1994;107:2931–9.
(25) Soule HD, McGrath CM. A simplified method for passage and long-term growth of human mammary epithelial cells. In Vitro Cell Dev Biol 1986;22:6–12.
(26) Stampfer M, Hallowes RC, Hackett AJ. Growth of normal human mammary cells in culture. In Vitro 1980;16:415–25.
(27) Joshi K, Smith JA, Perusinghe N, Monoghan P. Cell proliferation in the human mammary epithelium. Differential contribution by epithelial and myoepithelial cells. Am J Pathol 1986;124:199–206.
(28) Suzuki R, Atherton AJ, O'Hare MJ, Entwistle A, Lakhani SR, Clarke C. Proliferation and differentiation in the human breast during pregnancy. Differentiation 2000;66: 106–15.
(29) O'Hare MJ, Ormerod MG, Monaghan P, Lane EB, Gusterson BA. Characterization in vitro of luminal and myoepithelial cells isolated from the human mammary gland by cell sorting. Differentiation 1991;46:209–21.
(30) Berthon P, Pancino G, de Cremoux P, Roseto A, Gespach C, Calvo F. Characterization of normal breast epithelial cells in primary cultures: differentiation and growth factor receptors studies. In Vitro Cell Dev Biol 1992;28A:716–24.
(31) Colomb E, Berthon P, Dussert C, Calvo F, Martin PM. Estradiol and EGF requirements for cell-cycle progression of normal human mammary epithelial cells in culture. Int J Cancer 1991;49:932–7.
(32) Gomm JJ, Browne PJ, Coope RC, Liu QY, Buluwela L, Coombes RC. Isolation of pure populations of epithelial and myoepithelial cells from the normal human mammary gland using immunomagnetic separation with Dynabeads. Anal Biochem 1995;226:91–9.
(33) Jones JL, Shaw JA, Pringle JH, Walker RA. Primary breast myoepithelial cells exert an invasion-suppressor effect on breast cancer cells via paracrine down-regulation of MMP expression in fibroblasts and tumor cells. J Pathol 2003;201:562–72.
(34) Gomm JJ, Browne PJ, Coope RC, Bansal GS, Yiangou C, Johnston CL, *et al.* A paracrine role for myoepithelial cell-derived FGF2 in the normal human breast. Exp Cell Res 1997;234:165–73.
(35) Gomm JJ, Coope RC, Browne PJ, Coombes RC. Separated human breast epithelial and myoepithelial cells have different growth factor requirements in vitro but can reconstitute normal breast lobuloalveolar structure. J Cell Physiol 1997;171:11–9.
(36) Palmieri C, Roberts-Clark D, Assadi-Sabet A, Coope RC, O'Hare M, Sunters A, *et al.* Fibroblast growth factor 7, secreted by breast fibroblasts, is an interleukin-1beta-induced paracrine growth factor for human breast cells. J Endocrinol 2003;177:65–81.
(37) Pechoux C, Gudjonsson T, Ronnov-Jessen L, Bissell MJ, Petersen OW. Human mammary luminal epithelial cells contain progenitors to myoepithelial cells. Dev Biol 1999;206:88–99.
(38) Gudjonsson T, Villadsen R, Nielsen HL, Ronnov-Jessen L, Bissell MJ, Petersen OW. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. Genes Dev 2002;16:693–706.
(39) Gudjonsson T, Ronnov-Jessen L, Villadsen R, Bissell MJ, Petersen OW. To create the correct microenvironment: three-dimensional heterotypic collagen assays for human breast epithelial morphogenesis and neoplasia. Methods 2003;30:247–55.
(40) Martin KJ, Kritzman BM, Price LM, Koh B, Kwan CP, Zhang X, *et al.* Linking gene expression patterns to therapeutic groups in breast cancer. Cancer Res 2000;60:2232–8.
(41) Tzivion G, Shen YH, Zhu J. 14-3-3 proteins; bringing new definitions to scaffolding. Oncogene 2001;20:6331–8.
(42) van Hemert MJ, Steensma HY, van Heusden GP. 14-3-3 proteins: key regulators of cell division, signalling and apoptosis. Bioessays 2001;23:936–46.
(43) Ferguson AT, Evron E, Umbricht CB, Pandita TK, Chan TA, Hermeking H, *et al.* High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer. Proc Natl Acad Sci U S A 2000;97:6049–54.
(44) Prasad GL, Valverius EM, McDuffie E, Cooper HL. Complementary DNA cloning of a novel epithelial cell marker protein, HME1, that may be down-regulated in neoplastic mammary cells. Cell Growth Differ 1992;3:507–13.
(45) Umbricht CB, Evron E, Gabrielson E, Ferguson A, Marks J, Sukumar S. Hypermethylation of 14-3-3 sigma (stratifin) is an early event in breast cancer. Oncogene 2001;20:3348–53.
(46) Vercoutter-Edouart AS, Lemoine J, Le Bourhis X, Louis H, Boilly B, Nurcombe V, *et al.* Proteomic analysis reveals that 14-3-3sigma is down-regulated in human breast cancer cells. Cancer Res 2001;61:76–80.
(47) Simpson PT, Gale T, Reis-Filho JS, Jones C, Parry S, Steele D, *et al.* Distribution and significance of 14-3-3sigma, a novel myoepithelial marker, in normal, benign, and malignant breast tissue. J Pathol 2004;202:274–85.
(48) Kino T, Souvatzoglou E, De Martino MU, Tsopanomihalu M, Wan Y, Chrousos GP. Protein 14-3-3sigma interacts with and favors cytoplasmic subcellular localization of the glucocorticoid receptor, acting as a negative regulator of the glucocorticoid signaling pathway. J Biol Chem 2003;278:25651–6.