Commentary

The mammary myoepithelial cell – Cinderella or ugly sister?

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Abstract

The breast myoepithelial cell is the Cinderella of mammary biology. Although its contribution to benign and some malignant pathologies is recognised, it has been largely neglected in molecular and biological studies. The reason for this has been the perception that its role in normal physiology is confined to lactation and the belief that most breast cancers arise from luminal epithelial cells. This review presents our perspective on its broader biological significance and its potential use as a model system for understanding breast carcinogenesis.

Keywords: breast, breast cancer, cell biology, myoepithelial, pathology

Introduction

Two epithelial cell types, present in roughly equal numbers, line the entire normal duct and lobular system of the human breast. There is an inner 'luminal' or 'secretory' cell layer and an incomplete outer myoepithelial cell layer. Since the myoepithelium is incomplete, some luminal cells reach the basement membrane. The myoepithelial cells, in contrast, do not reach the luminal surface. The myoepithelial cells are attached to the basement membrane by hemidesmosomes and to the adjacent luminal cells by desmosomes. The cells have pinocytic vesicles, containing microfilaments and dense bodies resembling smooth muscle. The myofilaments are not as well developed in the cells lining the acini compared with those lining the terminal ducts and the interlobular ducts. The myoepithelial cell is, in some respects, the Cinderella of the breast. It has been largely ignored in the context of breast cancer. Although the cell's auxiliary role in lactational physiology is well recognised, this is a minor component of the process and is limited to assisting milk ejection during suckling in response of oxytocin. What, however, of the other 99% of time? Those with a more philosophical bent have found it hard to believe that the contractile function represents the be all and end all of myoepithelial cell function.

Its position, interposed between stroma and lumen, places it in an ideal situation to control many aspects of luminal function. It could regulate polarity, electrolyte and fluid flow, filter and process signals of endocrine or paracrine nature, and perhaps act as an intermediary in such signalling processes by passing information both inwards and outwards in a paracrine fashion or via intra-epithelial gap-junctions [1]. One school of thought has indeed invested the myoepithelium with great significance as a paracrine inhibitor of invasion and thus an inhibitor of tumour progression [2]. Other workers have proposed that, in the absence of fully differentiated myoepithelial cells, a failure to sequester local growth factor such as β-fibroblast growth factor may contribute to uncontrolled growth of malignant breast cells [3]. That said, there is only limited direct experimental information that addresses these hypotheses [2,4–6]. Perhaps the most convincing experiment would

NOS = no special type.

be removal of the myoepithelium either by gene knockout or by targeted chemotherapeutic ablation, in a manner similar to that described for the luminal compartment [7]. Many key genes undoubtedly impact on myoepithelial function but, if deletions are lethal at early stages of embryonic or postpartum life, then their effects on mature myoepithelial function cannot be assessed. In this context, the transgenic fat-pad model [8,9] and the rarely used xenograft model [10] could be useful experimental tools if combined with appropriate cell separation techniques.

We and other workers have developed methods to separate pure populations of luminal and myoepithelial cells from the mouse [11], the rat [12,13] and, most importantly, from humans [14–16]. These methods give us, in principle, the means to analyse the role of the myoepithelial cell in detail, at least *in vitro*. In practice, no group has exploited this with vigour, due in part to an emphasis on comparing luminal cells with invasive cancer, discarding the myoepithelial cells as an unwanted contaminant. This is also due to the fact that the essential contractile aspect of myoepithelial function, in the form of organised myofilaments, is progressively lost under conventional culture conditions in human, rat and mouse cells [11,12,14]. The human myoepithelial cells do, however, retain a basal phenotype that is stable, retained during cloning, and that allows them to be readily distinguished from luminal cells. The use of complex culture environments such as extracellular matrix-containing substrates can delay or reduce this 'de-differentiation' to some extent [17]. However, there is no disguising the fact that, once they have been separated and introduced into long-term culture, myoepithelial cells are but a shadow of their former selves. It is perhaps understandable under these circumstances that no great effort has been made in analysing their detailed physiology. There is an urgent need for more short-term culture experiments involving separation and controlled recombination of the major breast cell types in order to reveal their functions and interactions in the non-lactating mammary gland. As a step in this direction, a highly detailed analysis of the patterns of protein expression from freshly separated human luminal and myoepithelial cells has been completed recently using proteomic technology [18].

A major enigma in myoepithelial biology and pathobiology is the apparent infrequency with which this cell type gives rise to tumours. The cells are an integral part of benign lesions such as sclerosing adenosis and papillomata. Their contribution to breast malignancy has been unclear. Tumours showing myoepithelial differentiation have been reported more frequently in the past 20 years and include adenoid cystic carcinoma, adenosquamous carcinoma, adenomyoepithelioma, pure myoepithelial carcinoma and poorly differentiated myoepithelial rich carcinoma [19–21]. These tumours appear to be rare in clinical practice [22] and reports in the literature are generally of small series or isolated case reports [20,22–24]. The contribution of myoepithelial cells to ordinary ductal carcinomas is also unclear and reports suggest that 2–18% of so-called ductal carcinomas–no special type (NOS) show focal or diffuse myoepithelial differentiation by immunohistochemical criteria (eg basal cytokeratins, actin, calponin, caldesmon, S100 protein) [25–27].

As part of our investigation of genetic changes in pre-invasive breast disease, we have shown that loss of heterozygosity identified in invasive cancer is already present independently in 'normal' luminal and myoepithelial cells [28]. This suggests that there was a common precursor cell that acquired the mutation prior to differentiation into the two epithelial cell types. Some cells within the lobules are difficult to categorise, morphologically and ultrastructurally, into luminal or myoepithelial and it has been suggested that these 'intermediate' or 'indeterminate' cells are the precursors of the two epithelial cell types. It therefore seems odd that tumours exhibiting myoepithelial differentiation should be so rare in clinical practice; in contrast, for example, to the salivary gland.

The morphology of myoepithelial carcinomas is different to tumours derived from luminal cells. The myoepithelial carcinomas resemble sarcomas by having a predominantly spindle cell growth pattern [20,23]. Although there are only a few reports in the literature, approximately 50% of the published cases followed an aggressive course [20]. We carried out comparative genomic hybridisation analysis on 10 pure myoepithelial carcinomas [29] and compared the data with that of ductal carcinomas–NOS. The most striking observation was the paucity of alterations in myoepithelial carcinomas (mean, 2.1) compared with 'ordinary' breast carcinomas with luminal differentiation (mean, 5.4 in Grade I; mean, 11.7 in Grade III) [30–34]. The data are especially surprising in view of the aggressive morphology and behaviour of myoepithelial carcinomas. Seven out of 10 tumours were larger than 2 cm in size and four out of seven patients for whom follow-up information was available had died within 6 years of diagnosis. None of the alterations identified in myoepithelial carcinomas were unique, and they have been previously described in invasive breast carcinomas with luminal phenotype [30–34]. Although there was considerable overlap in the genetic profile of the two distinct epithelial tumour types, some of the most common alterations described in ductal carcinomas, such as gains of 1q, 8q and 20q, and losses of 1p, 8p and 13q [30–34], were not identified in myoepithelial carcinomas. On the assumption that the two cell types are derived from a common precursor cell [28], genetic alterations that overlap between luminal and myoepithelial tumours must have occurred within precursor cells prior to differentiation into the two epithelial cell types. Those alterations, which do not overlap, would have to occur subsequent to differentiation. We had, in one of our cases, the opportunity to

investigate this fact, as ductal carcinoma and myoepithelial carcinoma was present within the same tissue block. Loss at 17p was seen in both tumour types. Only one other alteration was seen in the myoepithelial tumour, while the ductal carcinoma showed 13 other alterations. We would therefore hypothesise that loss at 17p occurred within a 'stem cell/precursor cell', while alterations at other loci occurred subsequent to differentiation into the two epithelial cell types. We cannot formally exclude the possibility that the myoepithelial cell with 17p loss was derived from the luminal cell, as has been suggested recently [35]. However, unlike some other laboratories [36], we interpret our own extensive studies of separated luminal and myoepithelial cells *in vitro* as showing no evidence of any lability or interchangeability between the two basic epithelial cell types, either in bulk culture or as clones. We believe that the enhanced proliferative potential of the cultured human myoepithelial cell is the likely source of apparent heterogeneity in established cultures.

Since invasive ductal carcinomas exhibit a large number of alterations, it has been difficult to differentiate between pathogenic mutations and non-specific mutations due to genomic instability. Myoepithelial cells, in contrast, appear resistant to transformation, and myoepithelial carcinomas exhibit few genetic alterations. Since the alterations identified in myoepithelial carcinomas are also those seen in ductal carcinomas, these alterations are likely to be pathogenetically significant in breast carcinogenesis. The relatively few genetic alterations in otherwise aggressive neoplasms lead us to propose that myoepithelial tumours may be a better model than ductal carcinomas–NOS for the delineation of genes important in breast tumorigenesis. These data also provide a tool with which to investigate the significance of apparent myoepithelial differentiation in morphologically ductal carcinoma–NOS. The presence of specific genetic changes should discriminate between entrapped non-malignant cells and true tumour cells showing myoepithelial differentiation. Furthermore, the patterns of such changes, if present, could throw light on the clonal evolution within such cancers.

There is no doubt, even allowing for under-recognition of the contribution of myoepithelial cells to breast cancer, that the cells represent less than one-fifth of all cancers. The possible reasons for the resistance to transformation of this cell range from the simplistic to the untestable. As a relatively mitotically quiescent cell [37], it may simply not be as susceptible to tumorigenesis as the cycling luminal cell. Alternatively, or in addition, it could possess a greater DNA repair capacity, a hypothesis that is directly testable using separated cells. Another possibility is that myoepithelial cells express a greater range of tumour suppressor functions that need to be inactivated before a malignant phenotype is acquired. However, this seems unlikely in light of the data from our laboratory [29] that myoepithelial carcinomas, which are generally aggressive, show fewer genetic alterations. The low proliferation index of the myoepithelium in adult humans highlights another extraordinary feature of its behaviour. Studies with separated human cells have shown that, *in vitro*, myoepithelial cells can re-enter the cell cycle very rapidly and proliferate very fast [14]. As clones can be obtained from at least 50% of such separated cells, this is clearly not a property of rare stem cells, but extends to the majority of the functionally differentiated cells. This potential proliferative capacity emphasises the paradox of their failure to transform because they are clearly not a permanently postmitotic population. The presence of cycling cells of this phenotype in the pregnant human breast [38] further attests to their innate proliferative potential. Their proliferation *in vitro* has, in a practical context, caused much grief in studies of unseparated breast epithelial cells, the culture of tumour samples, many of which are still surrounded by myoepithelial cells, and in the spontaneous or engineered establishment of cell strains and lines. Some of the latter, which purport to be the appropriate controls for breast malignancy, express basal rather than luminal characteristics, including basal cytokeratins and specialised junctional proteins such as hemidesmosome components. It is fascinating but appalling to observe such differences being highlighted in complex transcriptional profiling experiments [39] without the penny dropping! The advent of improved methods of human cell immortalisation, combined with the use of pre-separated characterised cells, does enable more appropriate cell line models to be developed [40]. Matched pairs of luminal and myoepithelially derived lines can also be developed for further investigation of normal functional interactions. The reproducible development of similar lines from malignant breast epithelial cells, be they luminal or myoepithelial in phenotype, remains a major challenge of breast tumour biology and pathology.

Conclusion

The myoepithelial cell is the Cinderella rather than the ugly sister of mammary cell biology. Its role and potential have been under-recognised. We believe that it will play an increasingly important part in delineating and understanding the events in breast physiology and pathology.

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