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Commentary

Polarity determination in breast tissue: desmosomal adhesion, myoepithelial cells, and laminin 1

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Abstract

In all epithelial organs, apicobasal polarity determines functional integrity and contributes to the maintenance of tissue and organ specificity. In the breast, the functional unit is a polar double-layered tube consisting of luminal epithelial cells surrounded by myoepithelial cells and a basement membrane. It is far from clear how this double-layered structure is established and how polarity is maintained. Two recent papers have shed some light onto this intriguing problem in mammary gland biology. The results point to desmosomes and laminin 1 as having crucial roles. However, some questions remain.

Keywords: breast acini, desmosomes, epithelial positioning, luminal and myoepithelial cells, polarity, laminin 1

Introduction

Every cell in every tissue and organ *in vivo* is polar, in that it is organized asymmetrically. Even when tissues seem to lack morphological asymmetry, the cells within the tissues can still be polarized. Homeostasis itself is dependent on the maintenance of polarity. This is particularly true in the secretory epithelium, where without correct polarity, fluids would not be transported to the appropriate compartments. Yet we know little about what determines epithelial polarity in organs and how it is established and maintained *in vivo*.

In the past decade, the power of genetics in *Drosophila* and *Caenorhabditis elegans* has allowed the elucidation of some of the molecules and the molecular mechanisms involved in establishing polarity during embryonic development (reviewed in [1,2]). The task has been more difficult in mammals, in which tissue structure can be much more complex and genetic manipulations time-consuming and expensive. In the absence of suitable animal models, many studies on the establishment of polarity have been performed with cells in culture. Much useful information on junctional complexes, cell–cell and cell–extracellular matrix (ECM) interactions has been derived from these studies (reviewed in [3–7]). However, cultured cells, particularly epithelial cells, are known to lose their tissue-specific functions in culture (reviewed in [8]). Even in instances where apicobasal epithelial polarity is maintained on two-dimensional substrata, the manifestation of polarity on plastic and *in vivo* is markedly different. The existing three-dimensional culture models of epithelial cells partly fulfill a gap between these two extremes [9,10].

Three-dimensional models to study the interaction of epithelial and myoepithelial cells in the breast

Organs are made of multiple cell types. One of the best-studied organs, the mammary gland, contains two epithelial cell types, called luminal epithelial and myoepithelial cells, that together form the acini, the functional unit of the gland. Nevertheless, studies in three-dimensional cultures using both rodent and human mammary luminal epithelial cells indicate that these cells can establish apicobasal polarity and cell junctions even in the absence of myoepithelial cells, if cells are cultivated in the presence of an exogenous basement membrane, Matrigel (a reconstituted basement membrane gel) [11–13]. Apicobasal polarity can also be established on a flexible collagen 1 gel; under

ECM = extracellular matrix.

these conditions, as cell–cell contact is increased, the cells can make and deposit their own basement membrane and become functionally differentiated [14]. Since there is neither Matrigel nor floating collagen gels *in vivo*, what then determines acinar polarity in the mammary gland, and what molecules and forces help to maintain it?

This commentary is focused on two recent papers [15,16] that used similar human breast epithelial cells, but different approaches, to arrive at complementary conclusions about the requirements for the formation of the acini-like 'double-layered tube'. Both papers took advantage of techniques allowing the isolation of pure populations of luminal epithelial and myoepithelial cells from human breast, using tissues obtained from reduction mammoplasties [17,18].

Adhesion, mediated through either cell–cell or cell–ECM interactions, is clearly important in setting up epithelial polarity. But can one of these kinds of adhesion be excluded in favor of the other? Is there a hierarchy, or are both needed simultaneously? In a comprehensive and informative review, Yeaman and colleagues [3] discuss in detail the literature on polarized epithelial cells and postulate that polarity is established initially by extrinsic cues (namely cell–cell and cell–ECM adhesions) leading to asymmetry in the membranes at the site of the cue that is then transmitted throughout the rest of the cell. These 'symmetry-breaking' adhesive cues could involve any member of the cellular junction complexes. At the most apical part of the lateral membrane, these include cell–cell junctions such as tight junctions, adherens junctions and desmosomes (reviewed in [5]). Basally, hemidesmosomes that mediate cell–ECM interactions are the primary candidates [19]. In theory, any or all of these could be required for setting correct polarity, or, conversely, disruption of any one could lead to loss of polarity.

In an elegant study, Runswick and colleagues [15] cultured mouse mammary cell lines in Matrigel, and incubated purified human luminal epithelial and myoepithelial cells in rotary cultures in suspension. The latter form the double-layered tube in rotary cultures and resemble the mammary gland acini *in vivo*, although with a somewhat smaller acinus size. Using these systems, the authors showed that desmosomes were crucial in establishing polarized structures under both conditions. Immunofluorescence studies suggested that ECM components were not found in association with these double-layered tubes. However, the addition of specific peptides that block the adhesion of E-cadherin and desmocollins (desmosomal cadherins) resulted in disturbances in polarity, and the structures in Matrigel or the double-layered tubes in solution failed to organize. The authors concluded that the desmosomes are crucial for the formation of the double-layered acini, and that the ECM molecules in general and laminin specifically need not be involved.

Using the same cell types but a different assay, Gudjonsson and colleagues [16] came to a different conclusion. Petersen and colleagues [12] had previously shown that luminal epithelial cells in three-dimensional Matrigel make organized, polar acini, but that the same cells grown in collagen I gels express different integrins on their basal surface [20] and do not have correct polarity [21]. Gudjonsson and colleagues [16] took advantage of these findings and used the behavior of human luminal cells in collagen I as a means of assaying the function of myoepithelial cells in lumen formation. They showed that the addition of myoepithelial cells to luminal cells in collagen I corrected the polarity of luminal cells, and that this function was dependent on the production of laminin 1 but was not reproduced by laminin 5 and laminin 10/11, two prominent laminins expressed in the breast. They also found that 75% of breast cancer-derived myoepithelial cells tested did not produce laminin 1 and were unable to reverse the polarity of epithelial cells in collagen I gels. These studies therefore concluded that an important requirement for the polarity and formation of the double-layered tubes was the ability of myoepithelial cells to synthesize laminin 1.

Thus, these two studies identify essential and distinct determinants of mammary acini polarity. It would be interesting now for Runswick and colleagues to test myoepithelial cells that can not produce laminin 1 to see whether they can still form the double-layered tubes in the absence of this molecule when the desmosomes are intact. Similarly, Gudjonsson and colleagues [16] did not address the requirement for desmosomes, and they should now do so in their model system. However, it is likely that both laminin 1 and desmosomes would be necessary *in vivo*. It should be pointed out that the interpretation of experiments conducted by Gudjonsson and colleagues [16] with purified laminins *ex vivo* might also be complicated by the fact that laminins are never presented to cells in isolation *in vivo*; rather, these proteins are usually organized into a three-dimensional multi-protein polymerized structure. When studies are conducted *ex vivo* with purified laminins, results must be interpreted to include the possibility that only laminin-1 maintains the capacity to polymerize by itself, whereas laminin 5 and laminin 10/11 do not.

Another intriguing area to investigate is the inability of tumor myoepithelial cells to produce laminin 1, which seems to render them unable to signal for apicobasal polarity. Why are tumor myoepithelial cells unable to make laminin 1? Is this due to mutations, silencing, or post-transcriptional regulation? The evidence in Gudjonsson and colleagues [16] points to regulation at the level of mRNA. Evidence for links between a loss of epithelial polarity and tumor-like growth and invasiveness also exist in *Drosophila* mutants [1,22]. The laminin 1-impaired human

tumor cells can thus be used as functional 'mutants' in signaling studies.

These experiments have provided new insight into the contributions of cell–cell and cell–ECM interactions to the formation of acinar polarity in culture. The molecular mechanisms for correct cell–cell interactions and positioning remain to be determined both for cells in solution and in collagen gels. Future work will also need to address how these interactions at the cell surface are coupled to the intracellular pathways that actually execute polarity by trafficking proteins (including junctional adhesion receptors) to specific sites on the membrane. Evidence derived from invertebrate genetics can provide insight, yet invertebrates have distinctly different junctional structures [2], and *Drosophila* even seems to lack intermediate filaments. Moreover, evidence for cell–ECM interactions in invertebrate epithelial polarity is lacking [23]. The availability of RNA technologies to disrupt specific gene function, which can be applied to vertebrate cell cultures, might aid in distinguishing the functional requirement for signaling pathways that connect two different cell types as well as the relative contributions of E-cadherin, desmocollin, and integrin-based junctions to the polarization of mammary epithelia.

One important task now is to determine what molecules are involved in the formation and maintenance of these double-layered tubes *in vivo* so that more accurate and physiological models can be created in culture.

Competing interests

MJB is a co-author on the article from Gudjonsson and colleagues [16].

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