A new 'rotational' twist in an old tale: mammary epithelial cells 'weave' a laminin matrix as they rotate during acinar morphogenesis.

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Abstract : Single mammary epithelial cells are capable of regenerating complete mammary gland structures when given the right stimuli. Mammary tumor cells however, are incapable of this. In a successful attempt to understand some of the underlying morphogenetic differences between non-malignant and malignant mammary epithelial cells, Wang et al discovered a novel phenomenon that is unique to non-malignant cells. Their observations and the potential therapeutic relevance of their findings are discussed in this comment.

Keywords: mammary gland, acinar morphogenesis, rotational motion, apico-basal polarity, laminin, basement membrane, breast cancer

That single adult mammary epithelial cells are capable of recapitulating entire mammary gland structure and function when grown in three-dimensional laminin-rich basement membrane (3D lrBM) matrices is a phenomenon that was observed over two decades ago [1]. However, the underlying mechanisms still remain elusive. Moreover, why malignant cells are incapable of this behavior is unknown [2-4]. Clearly, mammary epithelial cells must have a morphogenetic program that is lost during malignant transformation. To understand this better, Wang *et al* utilized real-time imaging techniques to visualize the behavior of single non-malignant and malignant mammary epithelial cells as they formed spheroids or acini in 3D lrBM matrices. Their unexpected findings were recently reported in PNAS [5].

Using the non-malignant human mammary epithelial cell line, MCF10A, engineered to express fluorescent protein reporters, Wang *et al* performed live imaging of acinar-morphogenesis in 3D lrBM over a period of 9 days. They found that the acinar morphogenetic program was highly dynamic during the first 4 days, with individual cells undergoing division and rotational motion along the periphery of the multicellular structure whilst constantly maintaining apico-basal polarity. Following day 4, cells became less motile. Rotational motion was completely dependent on the establishment of apical polarity, a feature that is usually disrupted in malignant cells [6]. Dynein-based microtubule motors drove this novel cell motility program. That mammary epithelial cells undergo coordinated rotational motion during morphogenesis is not a novel observation [7]. However, what makes Wang *et al*'s study noteworthy is their discovery of the functional contribution of rotational behavior to the morphogenetic program. In addition to being required for the formation of spheroid-shaped acini, rotational motion was found to be crucial for the assembly of basement membrane (BM) components such as laminins around the acini. Cessation of rotational motion after day 4 was dependent on completion of BM assembly, since enzymatic disruption of the BM restored rotational movement and re-initiated BM organization. Cancer cells, which are unpolarized, had disorganized microtubules

and were unable to undergo rotational motion or to organize the BM around their irregular-shaped structures.

The rotational behavior observed by Wang *et al* may not be unique to mammary epithelial cells. Similar cell movements have been observed during embryogenesis in several organisms [8-10], suggesting that within the context of 3D lrBM matrices, adult mammary epithelial cells may utilize evolutionarily conserved, embryonic morphogenetic programs to regenerate normal tissue architecture and function. This re-engagement of an embryonic program in an adult tissue is highly relevant in the case of the mammary gland which is the only adult gland that undergoes multiple rounds of reorganization during a woman's reproductive life [11].

Wang *et al*'s novel observations leave us with many questions. How does rotational motion drive BM organization? Do BM receptors such as integrins or discoidin domain receptors play a role? Is rotation within the BM matrix facilitated by matrix degradation? The presence of intact, organized BM surrounding epithelial cells, separating them from the surrounding tissue stroma, is crucial for epithelial differentiation and function. Indeed, one of the hallmarks of epithelial cancers is a breach in the BM, resulting in invasion of malignant cells into the surrounding stroma [12, 13]. In light of this, it is interesting to note that cancer cells were unable to organize BM around them due to their inability to rotate. From a therapeutic standpoint, it would be worth investigating whether upon phenotypic restoration of rotational motion in breast cancer cells as described previously [7] an intact and organized BM can be re-established. If this is so, finding pharmacological ways to restore rotational behavior in cancer cells would be of immense therapeutic interest.

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