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AUTHOR'S VIEW

Crowds and power - coordinated in vitro development of a benign breast lesion

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ABSTRACT
We have discovered an organoid culture approach that recapitulates morphology and coordinated development of a benign breast tumor. This system may be useful to groups investigating normal mammary gland biology and coordination of collective cell behavior in the mammary gland.

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The title of this commentary is a reference to a classic book[1] that Elias Canetti wrote under the traumatizing impression of the totalitarian regimes of the 20th century. It tries to explain why people behave differently as part of a crowd than they do as individuals. If we want to understand how biological organisms – "crowds of cells" – form and function, this is also an essential question about cells. We were reminded of this when we accidentally found that mammary cells can form structures very similar to a benign mammary tumor in tissue culture.[2] A few years ago, we were looking for suitable in vitro models that could serve as normal tissue controls for cancer drug testing experiments. Among others, we tried the HMT-3522 system of related mammary cell lines developed more than 20 years ago in the labs of Ole Petersen and Mina Bissell. They had developed a family of cell lines that is now a very successful 3D culture model and recapitulates aspects of oncogenesis in vitro.[3] The cells were isolated from a woman with a benign mammary lesion and immortalized spontaneously through serial passaging.[4] Albeit immortalized, HMT-3522 S1 cells behave like benign mammary cells: they do not form xenograft tumors, they show contact inhibition of proliferation, and in Matrigel, they form a rudimentary polarized epithelium that can secrete milk proteins. Usually, these cells are plated as uniformly distributed single cells in Matrigel, which contains collagen and laminin and mimics extracellular matrix. Matrigel had been considered necessary for their culture in 3D because it induces cell polarization via integrins. Just out of curiosity, we tried to culture HMT-3522 S1 as "spheroids",[5] floating aggregates that assemble when adherent cells are deposited into non-adhesive plastic culture dishes. We did not expect much in the absence of Matrigel. However, starting as an unordered homogeneous aggregate of cells, the spheroids developed into organized structures of astonishing complexity: Histologic sections looked like the cut surface of an onion – concentric layers of cells separated by basement membrane components (Figure 1). These structures were forming because from the initial spheroid core, cell cords grew out peripherally and were covering the surface of the spheroid, then building the next layer and so on. When we observed spheroid development for a longer time, we realized that we had seen just a snapshot from the middle of a long, coordinated process. If we waited longer, spheroid volume increased suddenly, and their density seemed to change, some of them almost floating in medium, while initially, they had been sinking quite rapidly when perturbed. Looking at a cross section again showed us the reason: between the cell layers, empty spaces, lumina, started to form. It was a strikingly reproducible, regular and beautiful process, but we had no idea what we were looking at.

It was a lucky coincidence that one of us, Stefan Florian, started a residency in pathology and realized that there is a benign neoplasia of the mammary gland that has the same morphological features as our spheroids. It is called usual ductal hyperplasia (UDH) and is a random finding in 25% of breast biopsies – but has no medical significance and does not require treatment[6]

Ductal hyperplasia is neither normal breast tissue nor cancer, but this model has useful lessons for both. Some interesting insights come from how – most likely – UDH develops. In pathology, it has several morphological diagnostic hallmarks whose relationship to each other was unclear, in part because patient biopsies taken at a single time point do not reveal developmental pathways. Our HMT-3522 S1 spheroids allowed longitudinal observation of a growing UDH lesion, and revealed that previously unrelated pathology hallmarks are likely to be sequential steps in an aberrant developmental program. Moreover, both UDH *in situ* and our spheroids feature an interesting twist on the classic principles of lumen formation: rather than forming a lumen in the middle of a cell cord, resulting in a tube, they form the lumen *between* parallel cell cords.

Another interesting observation leads us back to Canetti's book: multicellular systems behave in a fundamentally different way than single cells. If HMT-3522 S1 cells are grown as

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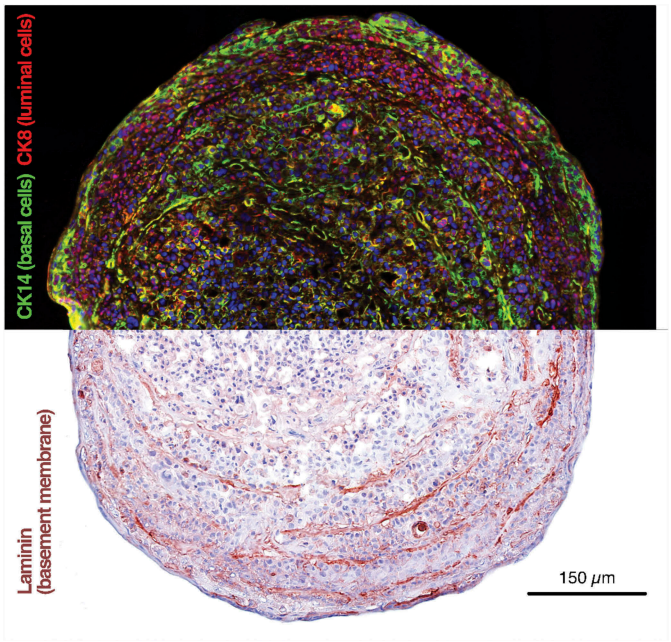

Figure 1. Spheroids form strikingly regular concentric structures.

A composed image of a mammary cell spheroid at the stage when cell cords have formed concentric layers, but lumina have not emerged yet. The upper half is stained for the luminal cell marker cytokeratin 8 (red) and the basal cell marker cytokeratin 14 (green). The proliferation of both cell types is characteristic for the benign lesion UDH, as opposed to malignant tumors. The lower half shows an immunohistochemical staining for the basement membrane component laminin (brown) that separates the concentric cell cords.

single cells in Matrigel, using the classic approach, they form hollow spheres lined by monolayered epithelium, called acini. However, if cells are allowed to assemble into spheroids, they can adopt two different fates depending on their environment: when we transferred spheroids into Matrigel in the first few days after their assembly we observed that at their periphery, cells still form small acini, similar to those grown from single cells. However, after six days floating in medium, a coordinated switch in cellular behavior occurred. Cells in the core of the spheroid stop proliferating while coordinated continued proliferation at the periphery of the spheroid generated erupting cords of cells which grew on the spheroid surface (even in Matrigel). These observations suggest that, similar to other classic examples like slime mold,[7] mammary cells can exist in two states – a collective state where their fate is determined by the role they have to fulfill within the collective and an individual state where all cells show a similar basic behavior. How does this switch to multicellularity occur at the molecular level, how is it coordinated? The answers are highly relevant for our understanding of mammary gland development, collective cell behavior and, indirectly, our understanding of collective cancer invasion. Our system will be useful to study these questions about the relationship between "Crowds and Power".

Disclosure of interest

The authors report no conflict of interest.

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