PNAS COMMENTARY

On the control of cell proliferation

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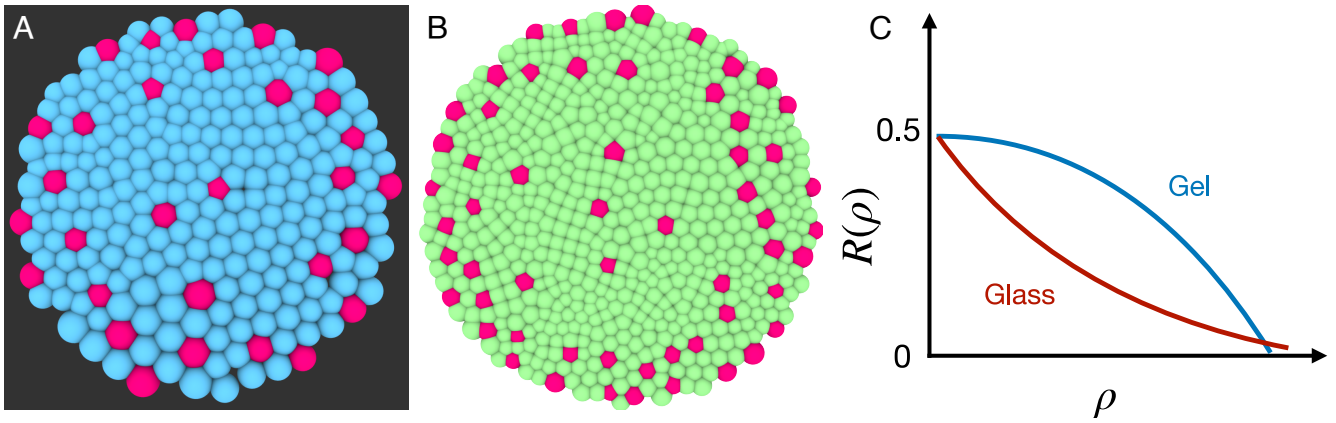

Fig. 1. Schematic of a growing tissue. Cell monolayer grown on (*A*) rigid glass, (*B*) soft gel substrate. The cells in red indicate dividing cells. (*C*) The probability of cell proliferation, *R* (the ratio of the number of dividing cells to the total number of cells) as a function of cell density, ρ, for tissues grown on glass (red) and a gel (blue).

Regulation of cell proliferation plays an integral part in tissue maintenance, embryogenesis, and tumorigenesis. It has been known for a long time that cell proliferation is controlled by contacts between cells, a mechanism that is referred to as contact inhibition of cell proliferation (CIP) (1, 2). Although the CIP mechanism has been known for decades, a clear understanding of the interplay of the factors that control intercellular contacts resulting in cell proliferation across various tissues has not emerged. Contacts between cells are influenced not only by biochemical signaling pathways (3) but also by the mechanical forces arising from interactions with the environment (4). An intuitively appealing concept one that is not unrelated to CIP but is broader in scope, is that biomechanical feedback between cadherin-mediated cell–cell adhesion molecules and mechanical stresses could explain the limits on cell proliferation (5, 6). Since the origin of CIP is linked to contacts between cells, it follows that cell number density (number of cells per unit area in two dimensions) must be a relevant controlling factor. However, as shown in an insightful article by Höllring et al. (7), density itself depends on cell growth, division, and the stiffness of the surrounding substrate on which the tissue grown, which makes it difficult to disentangle the contribution of various factors to the regulation of cell proliferation (8, 9).

By combining experiments probing the unconstrained growth of epithelium from Madin-Darby Canine Kidney II (MDCK-II) cells in collagen-coated glass and a soft deformable polyacrylamide (PA) gel, accompanied by theoretical and computational models, Höllring et al. (7) provide a novel mechanism of cell proliferation dynamics in epithelial tissues. Because proliferation depends on the probability of cell division, they identified those cells that cross the S phase in the cell cycle and hence are poised to divide. Using fluorescence imaging to detect EdU-stained nuclei (the cells in red in Fig. 1 *A* and *B*), which mark the cells in the S phase, the authors measured the fraction of dividing cells, *R*, for tissues grown on the stiff glass and a soft polyacrylamide (PA) gel. The two key experimental findings are the following: 1) *R*, which depends sensitively on the substrate stiffness, is solely a function of the cell density, ρ. 2) Strikingly, the ρ dependence of *R* exhibits positive (convex) curvature for tissues grown on the glass substrate, whereas the curvature is negative (concave) when the substrate is a gel, as shown schematically in Fig. 1*C*.

In tissues grown on both glass and gel, the probability of cell proliferation decreases sharply as the density increases, approaching a homeostatic value where cell division essentially ceases. This finding is consistent with the hypothesis that mechanical compression from neighboring cells inhibits cell division at high cell densities (5). Interestingly, cell growth on the softer PA gel has a higher proliferation probability (blue concave curve in Fig. 1*C*) across the entire density range compared to growth on the rigid glass substrate (red convex curve in Fig. 1*C*).

To explain the experimental findings, Höllring et al. developed a theoretical framework that captures the role of

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mechanical forces, such as those generated by cell–cell and cell–substrate interactions, in regulating cell proliferation. In the Cell-level model for Divisions in Tissue Environment (CDTE) model, the cell cycle is divided into two distinct phases: a stochastic growth phase, during which the cell size increases, and a division phase of fixed duration after which two daughter cells are created. In the CDTE model the growth and the division phases take place in succession. Furthermore, the model does not explicitly consider cell interactions with the substrate but introduces mechanosensitivity into the growth phase of the cell cycle by assuming that the time spent in this phase depends on the cell density. The calculations based on the model quantitatively account for the experimental measurements of R as a function of ρ on both glass and PA substrates using three adjustable parameters. It is worth noting that a recent study suggests that after reaching confluency the cells can only divide implying that cell division and growth are uncoupled (8). Thus, a tidy partitioning of cell growth and division phases, as assumed in the CTDE model, may not always hold.

By combining experiments probing the unconstrained growth of epithelium from Madin-Darby Canine Kidney II (MDCK-II) cells in collagen-coated glass and a soft deformable polyacrylamide (PA) gel, accompanied by theoretical and computational models, Hoellring et al. provide a novel mechanism of cell proliferation dynamics in epithelial tissues.

The CDTE model assumes implicitly that mechanical pressure arising from neighboring cells increases linearly with local cell density. As a result, the cell growth phase slows down and the probability of cell division is suppressed at high densities as observed in experiments. Without explicitly modeling the substrate, they explain the differences in the density-dependent cell proliferation behavior on soft and stiff substrates (Fig. 1*C*) by adjusting a length scale, which is the ratio of cell growth rate and the rate of division. The model also captures effect of substrate stiffness on the cell size: Cells are smaller on soft substrates due to their rapid proliferation rate (Fig. 1 *A* and *B*), which is consistent with experimental observations (10).

To extract spatial dependency of proliferation, which cannot be obtained using the CDTE model, Höllring et al. used simulations of particle-based Dissipative Particle Dynamics (DPD) and nonlinear Delayed Fisher–Kolmogorov (DFK) partial differential equation used to model the evolution of the density profile. Unlike in the CDTE model, the DPD simulations explicitly consider forces between cells. The transition from the growth to division is assumed to be stochastic whereas cell division is instantaneous. Utilizing both DPD simulations and the numerical solution of the DFK equations, they showed that the fraction of dividing cells is greater at the periphery of the tissue than at the center. The density profile is nonuniform across the tissue, shown schematically in Fig. 1 *A* and *B*. Similar spatial heterogeneity was reported in previous studies (11, 12) using agent-based simulations in three-dimensional tissues [see video 2 in (11)]. Taken together, the current and previous studies provide a compelling demonstration of the role of mechanosensitive proliferation in driving spatial organization of tissues (6).

The Höllring et al. study is a significant advance because it elucidates the role of mechanical signals from the environment in regulating cell proliferation and tissue growth. The combination of experiments and theoretical model is a powerful approach to dissecting the complexities of tissue mechanosensitivity. The findings could be relevant in understanding mechanical regulation of cell behavior in tissue development, repair, and disease. Mechanosensitive cell proliferation is critical for maintaining tissue homeostasis, allowing tissues to adapt to their mechanical environment (13). A mechanism that prevents overgrowth by automatically reducing cell proliferation when the cell density and mechanical pressure increase seems to be general and possibly universal. This self-regulatory mechanism is essential for maintaining tissue architecture, ensuring self-healing, and preventing abnormal growth that could lead to pathological conditions like cancer. Mechanosensitive mechanisms may help explain the cell competition between normal and mutant cells, which contributes to tissue homeostasis and cancer development (14).

Like all thought-provoking studies, the current work raises several questions that warrant additional scrutiny. 1) The finding that proliferation is reduced as the substrate stiffness increases appear to be at variance with previous studies which reach the opposite conclusion (15, 16). We hasten to add that hydrogel stiffness was varied from ∼(8 to 24) kPa (16) whereas there are a several orders of magnitude difference in the stiffness between a glass (∼GPa) and the PA gel (∼kPa). It would be interesting to test whether the predicted transition from concave to convex behavior (7) is observable by changing the stiffness of the PA gel in the physiological range. Only by exploring different mechanical environments one can fully understand the impact of mechanosensitivity on tissue growth (17). 2) The CDTE model assumes that the cell size ($\sigma(t)$) increases linearly with time (t) until it reaches a maximum value. Subsequently, the cells stochastically enter the division phase at a rate that also changes linearly with time provided the cell size is greater than a value in the homeostatic state. In contrast, in previous agent-based simulations, the cell size increases nonlinearly ($\sigma(t) \sim t^{\frac{1}{3}}$) provided the local pressure is less than a critical value (11, 12), thus coupling cell growth to local stresses through a feedback mechanism (5, 6). The robustness of proliferation mechanism could be established by using the agent-based models (11, 12) to recapitulate the experimental findings (7). 3) Ultimately, the gap between tissue level growth on soft versus rigid substrates and the underlying molecular mechanism related to the regulation of extracellular matrix receptors such as integrin (18) or nuclear Yes-associated protein (8, 19, 20) has to be filled. This would require connecting subcellular process to tissue level dynamics, which is nontrivial. 4) Finally, this study focuses exclusively on MDCK-II epithelial cells. Future research should extend these findings by exploring a broader range

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of cell types that may exhibit different mechanosensitive behavior (15, 16).

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