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Supplementary information

Mechanics of a multilayer epithelium instruct tumour architecture and function

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Supplementary Information

Mechanics of a multilayer epithelium instruct tumor architecture and function

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Video S1: Computational simulation of a multilayer epithelium with differential junctional tensions. Shape evolution of the model multilayer tissue in the absence of the basement membrane at three values of differential tension ($\gamma = 0$, 0.163, and 1.057). All simulations start from a flat cross-sectional tissue and result in budded morphologies.

Video S2: Computational simulation of a multilayer epithelium with basement membrane mechanics. Shape evolution of the model multilayer tissue attached to basement membrane. All simulations start from a flat cross-sectional tissue and result in budded, folded, and pearled morphologies [$(B, \tau_a) = (1.47876, 0.158489)$, $(41.117, 0.158489)$, and $(41.117, 398.107)$, respectively].

Video S3: Computational simulation of a stratified epithelium with basement membrane and suprabasal cell mechanics. Shape evolution of the model multilayer tissue attached to the basement membrane in the presence of the suprabasal stiffness gradient. All simulations start from a flat cross-sectional tissue and result in budded, folded, and budded morphologies [$(B, \tau_a) = (1.47876, 0.158489)$, $(41.117, 0.158489)$, and $(41.117, 398.107)$, respectively].

Supplementary Notes

Supplementary Note 1: The model

In the *monolayer model*, the tissue is described in cross section as a one-dimensional (1D) chain of quadrilateral cell cross sections, attached to the basement membrane (BM) at the basal side. The basement membrane is represented by a two-dimensional (2D) curve, discretized into a chain of linear spring-like elements that carry the local stretching and bending energies. In contrast, the *multilayer model* considers tissue in cross section as a 2D polygonal tiling comprised of 5 cell layers (1 basal layer and 4 suprabasal layers). Similarly to the monolayer model, here the bottom (basal) layer of cells is attached to BM.

The dynamics. In both the monolayer- and multilayer model, shapes of individual cells are parametrized by positions of their vertices $\boldsymbol{r}_i = (x_i, y_i)$, which undergo friction-dominated dynamics, described by the overdamped equation of motion:

$$\eta \frac{\partial \boldsymbol{r}_i}{\partial t} = -\nabla_i W(\underline{\boldsymbol{r}}) = -\left(\frac{\partial W}{\partial x_i}, \frac{\partial W}{\partial y_i} \right) , \tag{1}$$

where η is the friction coefficient. The forces $\boldsymbol{F}_i = -\nabla_i W$ are assumed conservative, i.e. they drive the system towards the nearest local minimum of the overall potential energy W, which in dimensionless form reads

$$w = \frac{W}{\Gamma_0 A_0} = \sum_k K_A \left(a_k - a_k^{(0)} \right)^2 + \sum_j \gamma_j l_j + \sum_{j \in \mathrm{BM}} K_S \left(l_j - l_j^{(0)} \right)^2 + \sum_{i \in \mathrm{BM}} B_i c_i^2 \Delta l_i \, . \tag{2}$$

Here Γ_0 and A_0 are the effective surface tension of the basal cells and the target cell cross-section area, respectively; $\lambda_0 = \sqrt{A_0}$ is hereafer used as the unit of length, whereas Γ_0 is used as the unit of tension. The variables a_k, l_j, and c_i are the cross-section area of cell k, length of cell edge j, and the local curvature of the basement membrane, evaluated at basal vertex i. The characteristic time scale of the relaxation towards the local minimum of the potential energy w is determined by the competition between tension and friction. In particular, the unit of time in our model $\tau_0 = \eta/\Gamma_0$. The time scale τ_0 is of the order of $\sim 1 - 10$ min.

Force contributions. The first energy term describes cell compressibility with K_A and $a_k^{(0)}$ being the dimensionless cell bulk modulus and target cell cross-section area, respectively. For simplicity, cells are assumed nearly incompressible, which is ensured by setting $K_A = 100$. The second term describes the line energy of cell-cell junctions due to tension at the level of the actomyosin cortex and cell-cell adhesion, which are jointly captured by effective line tensions at cell edges $\gamma_j = \gamma_j(t) = \gamma_0 + \left(1 - \delta_{[j_1],[j_2]}\right) \gamma_{\mathrm{diff}} + \Delta\gamma_j(t)$. Here γ_0 is the average line tension on junctions between same-type cells, whereas tensions on junctions between different-type cells are increased by γ_{diff}. Indices j_1 and j_2 denote identification numbers of cells that meet at the junction j, whereas $[j_1]$ and $[j_2]$ denote their types; δ is the Kronecker delta. The time-depending part $\Delta\gamma_j(t)$ describes tension fluctuations due to stochastic turnover of force producing molecules, contributing the time scale τ_m, and obeys the Ornstein-Uhlenbeck process $\Delta\dot{\gamma}_j = -(1/\tau_m)\Delta\gamma_j + \sqrt{2\sigma^2/\tau_m}\, \xi_j(t)$; $\xi_j(t)$ describes the white noise. In all our simulations, tissues behave like solids, which is ensured by assuming only subtle fluctuations with $\sigma = 0.15$ and $\tau_m = 0.5$ (Ref. 45). This process provides enough fluctuations to prevent unrealistic cell shapes by driving cell rearrangements that include very short junctions, however, it does not affect the overall shapes in any significant way.

The last two energy terms describe stretching and bending elasticities of the basement membrane, respectively, with the corresponding elastic moduli K_S and B_i. The bending energy per vertex i is computed as

$$c_i^2 \Delta l_i = \left| \frac{\Delta \boldsymbol{t}_i}{\Delta l_i} \right|^2 \Delta l_i = \frac{2}{l_j + l_{j+1}} \left(\boldsymbol{t}_{j+1} - \boldsymbol{t}_j \right)^2 = \frac{4\left(1 - \cos\theta_i\right)}{l_j + l_{j+1}} \, , \tag{3}$$

where j and $j+1$ are the ids of two adjacent BM edges (described by unit vectors $\boldsymbol{t}_j$ and $\boldsymbol{t}_{j+1}$, respectively) that meet at vertex i. The external angle at vertex i, θ_i, is the angle between

vectors $\boldsymbol{t}_j$ and $\boldsymbol{t}_{j+1}$. Since the basement membrane is a thin elastic sheet, its stretching modulus dominates over the bending modulus. Therefore, we assume $K_S = 100$ for all BM springs. In contrast, the bending modulus B_i at vertices adjacent to cancerous cells are generally different from the vertices adjacent to normal basal cells.

Cell growth and division. In our model, cells transition form the rest phase, in which the preferred cell area $a_k^{(0)} = \text{const.}$, to the growth phase, with probability $\mathrm{d}P/\mathrm{d}t = 1/\tau_d$, where $\tau_d = 100$ is the time scale of cell division. During the growth phase, $a_k^{(0)}$ grows linearly with time as

$$\frac{\partial a_k^{(0)}}{\partial t} = \frac{1}{\tau_g}\,, \tag{4}$$

where $\tau_g = 10$ is the time scale of cell growth. When $a_k^{(0)}$ reaches 2, the cell is divided into two daughter cells and the preferred areas of both cells are reset to $a_k^{(0)} = 1$. In the monolayer model, cells always divide along the apico-basal axis, preserving the monolayer structure of the tissue, whereas in the multilayer model, they divide along their short axes. Cell's short axis is calculated by diagonalizing the Gyration tensor of the point distribution of cell's vertices. For simplicity, only the mutant basal cells are allowed to divide, since their observed division rates are much higher than those of the normal basal cells.

Basement membrane assembly. Due to the finite time scale of turnover of BM components, BM does not instantaneously adapt to cell deformations, e.g., due to growth. In our model, this is taken into account by the relaxation dynamics of the local BM rest length. In particular, the preferred lengths $l_j^{(0)}$ evolve in time as

$$\frac{\partial l_j^{(0)}}{\partial t} = -\frac{1}{\tau_a}\left(l_j^{(0)} - \Lambda\sqrt{a_k^{(0)}}\right)\,, \tag{5}$$

where $\Lambda\sqrt{a_k^{(0)}}$ is the target preferred length, set by the area of adjacent cell k and a dimensionless parameter $\Lambda = L_x/N_x = 1.07457$, where $L_x = 107.457$ is the width of the simulation box and $N_x = 100$ is the initial number of basal cells. The parameter τ_a is the relaxation time scale of the extracellular matrix, i.e. the inverse assembly rate of the BM, that describes how quickly the BM locally adapts its preferred length to changes of cell's linear dimension due to growth.

Supplementary Note 2: Estimation of bending and stretching moduli

Hooke's law for an isotropic elastic body relates stress and strain by

$$\sigma_{ik} = \frac{Y}{1+\nu}\left(u_{ik} + \frac{\nu}{1-2\nu}u_{ll}\delta_{ik}\right)\,, \tag{6}$$

where Y and ν are the Young's modulus and the Poisson ratio of the material, respectively. In equilibrium $\partial\sigma_{ik}/\partial x_k = 0$, whereas at the boundaries, $\sigma_{ik}n_k = P_i$, where n_k is the k-th component of the normal to the surface and P_i is the i-th component of the surface stress.

Assuming there is no strain along the direction perpendicular to the cross section and no stress along the direction perpendicular to the membrane's surface, $u_{xx} = 0$ and $\sigma_{yy} = 0$, at the respective boundaries. The solution of the equations of equilibrium satisfying the boundary conditions is a homogeneous deformation described by a constant stress tensor. The free-energy density of such deformation reads

$$\frac{\partial \mathcal{F}}{\partial V} = \frac{1}{2}\sigma_{ik}u_{ik} = \frac{1}{2}\sigma_{zz}u_{zz}\,. \tag{7}$$

From the Hooke's law, the stress along the direction of stretching

$$\sigma_{zz} = \frac{Y\nu}{(1+\nu)(1-2\nu)}\left[(1-\nu)u_{zz} + \nu u_{yy}\right] \tag{8}$$

and the strain along the BM thickness $u_{yy} = -\nu u_{zz}/(1-\nu)$. Combining these equations gives

$$\frac{\partial \mathcal{F}}{\partial V} = \frac{Y}{2(1-\nu^2)} u_{zz}^2 \,. \tag{9}$$

We are left with integration over the basal area of a single basal cell, which yields the energy per unit length:

$$\frac{\mathcal{F}}{l_0} = \int \frac{\partial \mathcal{F}}{\partial V} \, \mathrm{d}A = \frac{Yhd}{2(1-\nu^2)} \epsilon^2 = \frac{K_S}{2} \epsilon^2 \,, \tag{10}$$

where $\epsilon = \Delta l / l_0$ is the strain along the BM in the cross-section plane. The stretching modulus

$$K_S = \frac{Yhd}{(1-\nu^2)} \tag{11}$$

differs by a factor of $1/(1-\nu^2)$ from a known result corresponding to the case where instead of zero strain, zero stress is assumed in the direction perpendicular to the cross section (i.e. Hooke's law for uniaxial expansion/compression of an isotropic elastic material).

To estimate the bending modulus of the basement membrane, we consider pure bending of a thin elastic plate into a cylindrical configuration with constant radius of curvature in the cross-section plane, $1/C$, and zero curvature in the direction perpendicular to the cross section. Energy per unit area of the basement membrane reads $\partial F_b / \partial A = (D/2)C^2$, where

$$D = \frac{Yh^3}{12(1-\nu^2)} \tag{12}$$

is the flexural rigidity or the bending modulus of the plate.

The total dimensionless elastic energy of the basement membrane can therefore be approximated in discrete form by

$$w_{\mathrm{BM}} = \frac{W_{\mathrm{BM}}}{\Gamma_0 d \lambda_0} = \sum_{j \in \mathrm{BM}} \frac{Yh}{2\Gamma_0(1-\nu^2)} \left(l_j - l_j^{(0)} \right)^2 + \sum_{i \in \mathrm{BM}} \frac{Yh^3}{24\lambda_0^2 \Gamma_0 (1-\nu^2)} c_i^2 \Delta l_i \,. \tag{13}$$

Comparing Eq. (13) with Eq. (2) yields the dimensionless elastic parameters of the vertex model:

$$K_S = \frac{Yh}{\Gamma_0(1-\nu^2)} \tag{14}$$

and

$$B = \frac{Yh^3}{12\lambda_0^2 \Gamma_0 (1-\nu^2)} \,. \tag{15}$$

The ratio of B and K_S gives an estimate of contribution of bending and stretching to the overall elastic energy. Taking $h = 0.5\mu$m and $\lambda_0 = 8\mu$m, it immediately follows

$$\frac{B}{K_S} \approx \frac{1}{24} \left(\frac{h}{\lambda_0} \right)^2 \approx 10^{-3} \,, \tag{16}$$

which shows that stretching dominates over bending and justifies the thin-plate assumption for the basement membrane. Finally, assuming basement membrane incompressibility ($\nu = 1/2$), we see that Y is approximately equal to B.

Supplementary Note 3: Effective cell Young's modulus and stiffness gradient

Together with the fixed-cell-volume constraint, cells' surface tensions determine the minimal-energy cell shape. Any deformation from this shape costs surface energy, which, to the lowest order, is expected to scale quadratically with the strain. Therefore, an effective Young's modulus could be related to the cell's surface tension, even though in our model, the cell-interior is fluid- rather than elastic-like.

To find the relation between the two, we need to first relate the deformation energy to the strain, while viewing the cell as a body comprised of an incompressible fluid on one hand, and as a solid elastic body on the other hand. For simplicity, we consider the resting cell shape to be a cube with linear dimension λ_0 and thus the surface energy $W_0 = 6\Gamma\lambda_0^2$, where Γ is the cell's surface tension. Let us assume a deformation that changes 2 cell dimensions as $\lambda_0 \to y = \lambda_0 + \Delta y$ and $\lambda_0 \to z = \lambda_0 + \Delta z$, keeping the third dimension intact ($\lambda_0 \to \lambda_0$). The surface energy of the deformed cell reads

$$W = 2\Gamma\left(yz + \lambda_0 y + \lambda_0 z\right) . \tag{17}$$

Assuming that the cell is incompressible, $yz\lambda_0 = \lambda_0^3 =$ fixed. Inserting $y = \lambda_0^2/z$ into the energy gives

$$W = 2\Gamma\lambda_0^2\left(1 + \frac{\lambda_0}{z} + \frac{z}{\lambda_0}\right) . \tag{18}$$

As expected, in the stress-free state, the condition $\partial W/\partial z = 0$ yields $z = \lambda_0$. The energy can then be expanded around this stress-free state. In particular, for small strain [$\epsilon = \Delta z/\lambda_0 \ll 1$], $W = W_0 + \Delta W = 6\Gamma\lambda_0^2 + 2\Gamma\lambda_0^2\left(\Delta z/\lambda_0\right)^2$, where the deformation energy

$$\Delta W = 2\Gamma\lambda_0^2\left(\frac{\Delta z}{\lambda_0}\right)^2 . \tag{19}$$

Now we need to compare this result to the energy of an isotropic elastic body under stress along the z-direction and deformed both along the y- and z-directions. The deformation energy per unit length in such a case reads

$$\frac{\Delta W}{\lambda_0} = \frac{E\lambda_0^2}{2(1-\nu^2)}\epsilon^2 , \tag{20}$$

where $\epsilon = \Delta z/\lambda_0$ is the strain along the BM in the cross-section plane. In turn, this yields

$$\Delta W = \frac{E\lambda_0^3}{2(1-\nu^2)}\left(\frac{\Delta z}{\lambda_0}\right)^2 . \tag{21}$$

Equating deformation energies given by Eq. (19) and Eq. (21) and assuming cell incompressibility ($\nu = 1/2$), yields the effective cell's Young's modulus:

$$E = \frac{3\Gamma}{\lambda_0} . \tag{22}$$

Stiffness gradient. Equation 22 shows that the relative cell stiffnesses of differentiated cell populations can be directly set by assuming differential surface tensions. In our model, this is done by treating tensions as cell properties. In particular, the interfacial tensions γ_j acting at cell-cell interfaces are calculated as $\gamma_j = (1/2)(\psi_k + \psi_m)$, where cells k and m are cells sharing the junction j, whereas ψ_k and ψ_m are their respective tensions. As suggested by Eq. 22, these tensions are proportional to cells' effective Young's moduli. In the multilayer model *without stiffness gradient*, all cells are prescribed unit tension, i.e. $\psi_k = 1$ for all cells k. In contrast, in the multilayer model *with stiffness gradient*, only the basal cells are prescribed unit tension, whereas others are prescribed tensions based on their distance from the basal layer. In particular, the apical suprabasal cells (i.e. cells that are adjacent to the lumen) are prescribed tension $\psi = 5$, corresponding to approximately 5-times higher stiffness compared to the basal cells, whereas the remaining suprabasal cell layers following the linear tension profile between the minimum (unit) tension and the maximum tension $\psi = 5$.

Supplementary Note 4: Sensitivity of shape factor, S, on the cutting angle

To quantify the sensitivity of the measured morphometric parameters, namely the shape factor, S, on varied cutting angles, we created a 3D reconstruction of the tissue based on simulated 2D outlines. In particular, let $A(x)$ and $B(x)$ be the interpolating functions describing

the apical and the basal sides of the tissue such that in parametric form, the apical and the basal contours $\boldsymbol{r}_a = (x, y, A(x))^T$ and $\boldsymbol{r}_b = (x, y, B(x))^T$, respectively.

In an ideal case, the tissue is imaged at $y = y_0 = \text{const.}$, i.e., in the x-z plane, yielding the exact value of the shape parameter. However, when the image is taken in the plane that is rotated by an angle X about the x-axis and by an angle Z about the z-axis, the measured shape parameter corresponds to the rotated tissue, whose basal side is described by the vector

$$\boldsymbol{r}'_b = \begin{pmatrix} x/\cos Z - [y_0 + B(x)\sin X]\tan Z/\cos X \\ y_0 \\ [B(x) + y_0 \sin X]/\cos X \end{pmatrix} . \tag{23}$$

Supplementary references

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