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Supplemental Information

Physical Mechanisms of Cancer in the Transition to Metastasis

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Supplemental Text

Text S1. Mathematical model for epithelial dynamics

Force balance in an epithelial layer. Our model for the dynamics of epithelial tissues is based off the model we developed previously (14,15). This model considers a continuous epithelium made up of motile cells adhered to each other and to the extracellular matrix (ECM). We begin by considering the physics of a single motile cell and then, by averaging over the cell-cell adhesion forces, we construct a continuum level model for the epithelial tissue.

In wound healing assays, the cells are constrained to move along the surface of the substrate. Therefore, we can consider a two-dimensional model for the cellular motions. Intracellular cytoskeletal flow interact with the substrate through adhesion proteins, such as integrins. It is reasonable to treat the force per area on the cell that arises from this interaction as being a resistive drag proportional to the cytoskeletal velocity (14). In 2D, stresses inside the cell, defined by the stress tensor σ_{in}, lead to cytoskeletal actin flows $\mathbf{V}$. Since the resistive forces within a cell are large compared to the inertia of the cell, the sum of all the forces acting at a point within the cell sum to approximately zero. It can be shown (14) that the correct 2D approximation for force balance on the cell is then given by

$$\nabla \cdot \sigma_{\text{in}} = \zeta \mathbf{V} \tag{1}$$

where ζ is the drag coefficient.

The cytoskeletal velocity is spatially-dependent, with most crawling cells having a retrograde flow of actin at the leading edge and flow at the rear of the cell approximately equal to the velocity of the cell. Therefore, we decompose the cytoskeletal velocity using the net cell velocity $\mathbf{V}_0$ and a deviation velocity $\delta\mathbf{v}$; $\mathbf{V} = \mathbf{V}_0 + \delta\mathbf{v}$. We assume that the velocity of the cell is in the direction of the polarization of the cell, $\mathbf{V}_0 = V_0\mathbf{d}$. The thrust force that the cell exerts on the substrate is $\mathbf{F} = \zeta V_0 A\mathbf{d} = Af_p\mathbf{d}$, where A is the area of the cell, and f_p is the propulsive force per area.

From our definitions, the deviation velocity $\delta\mathbf{v}$ is zero at the rear of the cell and negative at the front of the cell. The internal cellular stress must generate this flow. In general, we can write the internal stress as a function of position as

$$\sigma_{in} = f(x,y)\mathbf{I} + g(x,y)\mathbf{dd} + h(x,y)\mathbf{de}, \tag{2}$$

where **I** is the identity matrix, and **e** is the unit vector perpendicular to **d**. For highly polarized cells, we choose set f(x,y) and h(x,y) equal to zero. In addition, in the absence of other external forces applied to the cell, the two-dimensional internal stress must equal zero at the edges of the cell. These conditions imply that the average internal stress is positive and aligned along the dyadic **dd.** We define the average intracellular contractile stress as f_d, which then gives that $\sigma_{in} = f_d\mathbf{dd}$. From our definitions, the deviation velocity is

$$\delta\mathbf{v} = \frac{1}{\zeta}\nabla\cdot\sigma_{in} - \frac{f_p}{\zeta}\mathbf{d}. \tag{3}$$

We can now use these results to write an equation for the force balance inside an epithelial cell monolayer. Treating the monolayer as a continuous medium, we define the stress σ_m in the monolayer. Averaging this stress over the height of the cell layer and assuming that the substrate produces a resistive drag against the cells, the equation for the monolayer is analogous to (Eq. 1):

$$\nabla\cdot\sigma_m = \zeta\mathbf{V}, \tag{4}$$

where v is the local velocity of the actin flow inside the monolayer. Once again, we decompose this velocity into an average velocity of the cells centered at the location x, **v**(x), and the internal variations in the cytoskeletal velocities, $\delta\mathbf{v}$. If we assume that the intracellular cytoskeletal flows remain roughly the same, then we can re-write Eq. 4 as

$$\nabla\cdot\sigma_m = \zeta\mathbf{v} + \nabla\cdot(f_d\mathbf{dd}) - f_p\mathbf{d} \tag{5}$$

A constitutive relation is required to define the monolayer stress. For this, we define two types of forces that act between the cells. First, we assume that the cells are elastic objects that have a preferred spread area. Motion of the cells can change the density that the cells

are packed in the monolayer. If we define the density as c and the preferred density as c_0, then we expect that there will be an isotropic pressure that is proportional to $c - c_0$ that tries to maintain the preferred cell density. Second, there is a stress between cells, σ_c that arises from cell-cell adhesion, which is predominantly mediated by cadherin bonds. Since cadherin molecules turnover, the cells can slip with respect to each other. As we describe below, this dynamics can be approximated by the Maxwell model for viscoelastic fluids.

Transition from elasticity to viscosity. Cell-cell adhesion is mediated by cadherin molecules (19, 20). Fluorescent recovery after photobleaching (FRAP) experiments show that cadherin turnover in adhesions has a characteristic rate of around 3.9 hr^{-1} (20). Therefore, we expect that on timescales less than 15 minutes, that nearby cells remain well adhered, and that the cell monolayer will behave elastically for times less than this. However, for times longer than 15 minutes, the adhesions can break, and the cells can slip. Therefore, we expect viscous behavior on longer timescales. The simplest model that reflects this behavior is the Maxwell model for a viscoelastic fluid, which relates the stress in the material to the velocity. Therefore, to model cell-cell adhesion, we use the Maxwell model to describe the intercellular stress σ_m:

$$\tau \frac{\partial \sigma_m}{\partial t} + \sigma_m = \eta(\nabla \mathbf{v} + (\nabla \mathbf{v})^T) + (\lambda - \eta)(\nabla \cdot \mathbf{v})\mathbf{I} - \sigma_0 \left(c - c_0\right)\mathbf{I} - \beta\left(\frac{V_{\text{tumor}}^0 - V_{\text{tumor}}}{V_{\text{tumor}}^0}\right)\mathbf{I}_{\text{tumor}} \tag{8}$$

In this model, τ is the cadherin turnover timescale, and η is a cell-cell viscosity that depends on the average number of bound cadherin molecules and the strength of the cadherin bond. In addition, there is resistance to compression or extension that is defined by λ. The identity matrix is defined as $\mathbf{I}$. For our monolayer tumor model, we also add in an additional pressure to preserve the area of mutated region. The strength of this term is given by β and this term is only used within the tumor region.

Equations (6-9) are a closed set of equations that describe the dynamics of the epithelial tissues that we investigate. We non-dimensionalize these equations using the following parameters and state variables:

$$\tilde{b}=\frac{f_{\mathrm{d}}}{\zeta V_0 L},\quad \tilde{\eta}=\frac{\eta}{\zeta L^2},\quad \tilde{\lambda}=\frac{\lambda}{\zeta L^2},\quad \tilde{K}=\frac{K}{\zeta_{\mathrm{r}} V_0 L},\quad \tilde{\tau}=\frac{\tau V_0}{L},\quad \tilde{f}_{\mathrm{p}}=\frac{f_{\mathrm{p}}}{\zeta V_0},\quad \tilde{D}=\frac{D}{V_0 L}$$

(5)

$$\tilde{\sigma}=\frac{\sigma A}{\zeta V_0},\quad \tilde{\mathbf{v}}=\frac{\mathbf{v}}{V_0},\quad \tilde{c}=\frac{c}{c_0} \qquad (9)$$

where L is the initial thickness of the strip in our wound healing simulations, or the circular diameter of the mutated region in our cancer metastasis simulations.

Numerical method

Our numerical procedure for solving the wound healing assay simulations and cancer metastasis model follow the methodology that we have used previously and is described in detail in (14). In what follows, we describe the numerical procedure briefly for the cancer metastasis model and the wound healing simulations.

The procedure for the monolayer tumor simulations

The initial cell orientation is distributed randomly with a uniform distribution in orientation angle. To define the random cellular polarization at the initial time, we assume the initial orientation $\theta \in [-\pi, \pi)$. We choose our time step such that the maximum velocity times the time step size is less than the grid spacing.

The time-stepping algorithm for our simulations is composed of the following steps:

(1) Time step the cell density dynamics (Eq. 6).
(2) Time step the cellular orientation dynamics (Eq. 7).
(3) Time step the cell-cell adhesion stress dynamics (Eq. 8).
(4) Time step the border location.

For simplicity, we apply the following notations for the advection and curl operators A^n and ω^n with the finite volume ΔV^n at time step n:

$$A^n = \Delta V^n \mathbf{v}^n (\cdot\nabla)^n$$
$$\omega^n = \nabla^n \times \mathbf{v}^n$$

At the boundary between the benign tissue and the biophysically modified cells, the stress is continuous. To handle this, we compute the total stress $\sigma_{\mathrm{T}} = \sigma_c - f_{\mathrm{d}}\mathbf{dd}$ at the boundary rather than the cell-cell adhesion stress.

Time-stepping from t^n to t^{n+1} uses a Crank-Nicolson second order method, which is described in the sub-steps (A) and (B) below.

At the boundary of the computational domain, we use periodic boundary condition for the velocity, orientation, and stress variables.

A. Intermediate time stepping: $(\tilde{\mathbf{v}}^n, \mathbf{d}^n, \tilde{\sigma}_{\mathrm{T}}^n, \phi^n, \tilde{c}^n) \rightarrow (\tilde{\mathbf{v}}^{n+1/2}, \mathbf{d}^{n+1/2}, \tilde{\sigma}_{\mathrm{T}}^{n+1/2}, \phi^{n+1/2}, \tilde{c}^{n+1/2})$

1*A*) $(\Delta V^{n+1/2}\mathbf{I} - \frac{\tilde{D}\Delta t}{2}\mathbf{L}^{n+1/2})\tilde{c}^{n+1/2} = \Delta V^n \tilde{c}^n - A^n \tilde{c}^n \frac{\Delta t}{2}$

1*B*) $(\frac{2\Delta V^{n+1/2}}{\Delta t}\mathbf{I} - \tilde{K}\mathbf{L}^{n+1/2})\mathbf{d}^{n+1/2} = \frac{2\Delta V^n}{\Delta t}\mathbf{d}^n - A^n\mathbf{d}^n + \frac{1}{2}\omega^n \times \mathbf{d}^n \Delta V^n$

1*C*) $(1+\frac{2\tilde{\tau}}{\Delta t})\Delta V^{n+1/2}\tilde{\sigma}_{\mathrm{T}}^{n+1/2} = \frac{2\tilde{\tau}\Delta V^n}{\Delta t}\tilde{\sigma}_{\mathrm{T}}^n + \frac{\tilde{\eta}_i}{2}(D^n\tilde{\mathbf{v}}^n + (D^n\tilde{\mathbf{v}}^n)^T) + (\tilde{\lambda} - \frac{\tilde{\eta}_i}{2})D^n \cdot \tilde{\mathbf{v}}^n$

$$+ \frac{2\tilde{\tau}\tilde{b}_i}{\Delta t}\{\Delta V^{n+1/2}(\mathbf{d}^{n+1/2}\mathbf{d}^{n+1/2}) - \Delta V^n(\mathbf{d}^n\mathbf{d}^n)\} + \tilde{b}_i\Delta V^n(\mathbf{d}^n\mathbf{d}^n)$$

$$- \beta(\frac{V_{\mathrm{tumor}}^0 - V_{\mathrm{tumor}}^n}{V_{\mathrm{tumor}}^0})\Delta V^n$$

1*D*) $\Delta V^{n+1/2}\tilde{\mathbf{v}}^{n+1/2} = D^{n+1/2}\cdot\tilde{\sigma}_{\mathrm{T}}^{n+1/2} + \tilde{f}_{\mathrm{p}}^i\mathbf{d}^{n+1/2}\Delta V^{n+1/2} - D^{n+1/2}(\tilde{\sigma}_0(\tilde{c}^{n+1/2} - \tilde{c}_0))$

B. Full time stepping: $(\tilde{\mathbf{v}}^n, \mathbf{d}^n, \tilde{\sigma}_{\mathrm{T}}^n, \phi^n, \tilde{\mathbf{v}}^{n+1/2}, \mathbf{d}^{n+1/2}, \tilde{\sigma}_{\mathrm{T}}^{n+1/2}, \phi^{n+1/2}) \rightarrow (\tilde{\mathbf{v}}^{n+1}, \mathbf{d}^{n+1}, \tilde{\sigma}_{\mathrm{T}}^{n+1}, \phi^{n+1})$

$$2A)\quad (\Delta V^{n+1}\mathbf{I} - \frac{\tilde{D}\Delta t}{2}\mathbf{L}^{n+1})\tilde{c}^{n+1} = \Delta V^{n}\tilde{c}^{n} - A^{n+1/2}\tilde{c}^{n+1/2}\Delta t + \frac{\tilde{D}\Delta t}{2}\mathbf{L}^{n}\tilde{c}^{n}$$

$$2B)\quad (\frac{\Delta V^{n+1}}{\Delta t}\mathbf{I} - \frac{1}{2}\tilde{K}\mathbf{L}^{n+1})\mathbf{d}^{n+1}$$
$$= \frac{\Delta V^{n}}{\Delta t}\mathbf{d}^{n} - A^{n+1/2}\mathbf{d}^{n+1/2} + \frac{1}{2}\omega^{n+1/2}\times\mathbf{d}^{n+1/2}\Delta V^{n+1/2}$$

$$2C)\quad (\frac{1}{2}+\frac{\tilde{\tau}}{\Delta t})\Delta V^{n+1}\tilde{\sigma}_{\mathrm{T}}^{n+1} = (-\frac{1}{2}+\frac{\tilde{\tau}}{\Delta t})\Delta V^{n}\tilde{\sigma}_{\mathrm{T}}^{n} + \frac{\tilde{\eta}_i}{2}(D^{n+1/2}\tilde{\mathbf{v}}^{n+1/2} + (D^{n+1/2}\tilde{\mathbf{v}}^{n+1/2})^{T}) + (\tilde{\lambda} - \frac{\tilde{\eta}_i}{2})D^{n+1/2}\cdot\tilde{\mathbf{v}}^{n+1/2}$$
$$+ \frac{\tilde{\tau}\tilde{b}_i}{\Delta t}\{\Delta V^{n+1}(\mathbf{d}^{n+1}\mathbf{d}^{n+1}) - \Delta V^{n}(\mathbf{d}^{n}\mathbf{d}^{n})\} + \frac{\tilde{b}_i}{2}\Delta V^{n+1/2}(\mathbf{d}^{n+1/2}\mathbf{d}^{n+1/2})$$
$$-\frac{1}{2}\ \beta(\frac{V_{\mathrm{tumor}}^{0} - V_{\mathrm{tumor}}^{n+1/2}}{V_{\mathrm{tumor}}^{0}})\Delta V^{n+1/2}$$

$$2D)\quad \Delta V^{n+1}\tilde{\mathbf{v}}^{n+1} = D^{n+1}\cdot\tilde{\sigma}_{\mathrm{T}}^{n+1} + \tilde{f}_{\mathrm{p}}^{i}\mathbf{d}^{n+1}\Delta V^{n+1} - D^{n+1}(\tilde{\sigma}_{0}(\tilde{c}^{n+1} - \tilde{c}_{0}))$$

The procedure for the wound healing assay

The algorithm is similar to the cancer metastasis simulations except that we also need to solve for the motion of the free boundary at the wound edge. At this boundary, we apply the left-handed and right-handed projections of the intermediate boundary stress tensor $\tilde{\sigma}_{c}^{*}$ in the tangential direction $\mathbf{t}$ and $\mathbf{n}$ as follows:

$$\mathbf{n}\cdot\tilde{\sigma}^{n+1}\cdot\mathbf{n} = \mathbf{n}\cdot(\tilde{b}\mathbf{n}\mathbf{n})\cdot\mathbf{n}$$
$$\mathbf{t}\cdot\tilde{\sigma}^{n+1}\cdot\mathbf{n} = \mathbf{t}\cdot(\tilde{b}\mathbf{n}\mathbf{n})\cdot\mathbf{n}$$
$$\mathbf{t}\cdot\tilde{\sigma}^{n+1}\cdot\mathbf{t} = \mathbf{t}\cdot\tilde{\sigma}^{*}\cdot\mathbf{t}$$

After algebraic rearrangement,

$$P\tilde{\sigma}^{n+1} = \tilde{\sigma}^{P}$$

where

$$\tilde{\sigma}^{P} = \begin{pmatrix} \mathbf{t}\cdot\tilde{\sigma}^{*}\cdot\mathbf{t} \\ 0 \\ \tilde{b} \end{pmatrix}$$

and the projection matrix is constituted in the following:

$$P = \begin{pmatrix} n_y^2 & -2n_x n_y & n_x^2 \\ n_x^2 & 2n_x n_y & n_y^2 \\ n_x n_y & n_x^2 - n_y^2 & n_x n_y \end{pmatrix}$$

where n_x and n_y are the x and y components of the normal vector of the boundary. The normal vector is computed using the gradient of the level set function.

The procedure for the circular tumor model

Our circular tumor model is defined with an initial condition that includes three regions, a core region, a cortex and an exterior region. The core and the cortex can have different values for the biophysical parameters. In addition, because the initial tumor region is considered to be primarily cell-filled, the cell-ECM drag coefficient (ζ) is low within the initial tumor region and higher outside it. In order to delineate these regions, we define two signed distance maps, ψ_1 and ψ_2. The zero contour of the first distance map is used to identify the boundary of the tumor, and the zero contour of the second distance map defines the boundary between the core and the cortex. The initial condition for the tumor geometry is a circle of diameter of 400 μm and the core is a circle with diameter 300 μm.

Rather than solving the system of equations for our epithelial model (Eqs. 5-8) as written, we note that if we take the time derivative of Eq. 5, multiply it by τ, and then add it back to Eq. 5, we can remove the monolayer stress from the equations of motion:

$$\tau \frac{\partial}{\partial t}\left[\nabla \cdot \sigma_m - \zeta \mathbf{v} - \nabla \cdot \left(f_d \mathbf{dd}\right) + f_p \mathbf{d}\right] + \nabla \cdot \sigma_m - \zeta \mathbf{v} - \nabla \cdot \left(f_d \mathbf{dd}\right) + f_p \mathbf{d} = 0$$

$$\nabla \cdot \left(\tau \frac{\partial \sigma_m}{\partial t} + \sigma_m\right) - \zeta \left(\tau \frac{\partial \mathbf{v}}{\partial t} + \mathbf{v}\right) - \nabla \cdot \left(\tau \frac{\partial \left(f_d \mathbf{dd}\right)}{\partial t} + f_d \mathbf{dd}\right) + f_p \left(\tau \frac{\partial \mathbf{d}}{\partial t} + \mathbf{d}\right) = 0$$

We then use Eq. 8 to rewrite the first term:

$$\begin{aligned} \zeta \left(\tau \frac{\partial \mathbf{v}}{\partial t} + \mathbf{v}\right) = {} & \nabla \cdot \left(\eta(\nabla \mathbf{v} + (\nabla \mathbf{v})^T) + (\lambda - \eta)(\nabla \cdot \mathbf{v})\mathbf{I}\right) \\ & - \nabla \cdot \left(\tau \frac{\partial \left(f_d \mathbf{dd}\right)}{\partial t} + f_d \mathbf{dd}\right) + f_p \left(\tau \frac{\partial \mathbf{d}}{\partial t} + \mathbf{d}\right) - \sigma_0 \nabla c \end{aligned} \qquad (10)$$

We then solve Eqs. 6, 7, and 10 to determine the dynamics of the tumor. Because the core and the cortex can have different values for the parameters, the viscosity η, dipole stress f_d and propulsive force f_p are functions of space. We define the values of these parameters in the cortex as η_1, f_{d1} and f_{p1} and in the core as η_2, f_{d2} and f_{p2}. For numerical stability, we smooth out the variation in these biophysical parameters over approximately two nodes using a hyperbolic tangent function that depends on the second distance map:

$$\eta(\mathbf{x}) = \eta_2 + \frac{1}{2}(\eta_1 - \eta_2)\left(1 + \tanh\left(\frac{\psi_2}{\Delta x}\right)\right)$$

$$f_d(\mathbf{x}) = \eta_2 + \frac{1}{2}(f_{d1} - f_{d2})\left(1 + \tanh\left(\frac{\psi_2}{\Delta x}\right)\right)$$

$$f_p(\mathbf{x}) = \eta_2 + \frac{1}{2}(f_{p1} - f_{p2})\left(1 + \tanh\left(\frac{\psi_2}{\Delta x}\right)\right)$$

where Δx is the grid spacing.

The boundary conditions at the edge of the tumor are that there is no flux for the cell density and the stress and orientation are given by

$$\sigma_m \cdot \mathbf{n} = f_d \mathbf{d}(\mathbf{d} \cdot \mathbf{n})$$
$$(\mathbf{n} \cdot \nabla)\mathbf{d} = 0$$

The stress boundary condition is used to define that the flux due to the divergence terms in Eq. 10 are zero at the tumor boundary.

We use the Boundary Node Method (47) to solve the equations on an 80 × 80 grid of size 600 μm × 600 μm. The time-stepping algorithm we use is as follows:

1. Time step the density equation (Eq. 6) a half time-step using a backward Euler method.
2. Time step the orientation equation (Eq. 7) a half time-step using a backward Euler method.
3. Time step the velocity (Eq. 10) a half time-step using backward Euler. The time derivative terms of the orientation vector are computed numerically using the results of step 2.
4. Using the velocity at the half time computed in step 3, time step both distance maps a full time step using the level set method.

5. Using geometric information from both time t and time $t + \Delta t$ (as is described in (49)) time step the Eqs. 6, 7 and 10 a full time step using a semi-implicit Crank-Nicolson routine.