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SUPPLEMENTARY INFORMATION

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Stromal reactivity differentially drives tumour cell evolution and prostate cancer progression

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Supplementary Information

The hybrid discrete-continuum mathematical model

Based on the work of Anderson et al. (2005; 2006) and our previous prostate focused paper (Basanta et al., 2009), we developed a hybrid mathematical model of prostate tumor-stromal interplay to study how growth, evolution and heterogeneity change both spatially and temporally. We consider 6 mathematically abstracted cell types: normal basal (B) and luminal (L) epithelial cells; tumor epithelium (C); native stroma (S); reactive stroma (RS); and motile stroma (I) representing a generic cell with inflammatory properties. The physical microenvironment includes growth factors (G), basement membrane/extracellular matrix (M) and matrix metalloproteinases (E). We use a set of discrete partial differential equations to define migration probabilities for tumor and inflammatory cell types that essentially define a biased-random walk. We then use a set of life-cycle flowcharts to define cellular interactions and properties (Figure 1C). Combined with a system of coupled non-linear partial differential equations that define the microenvironmental variables as chemical densities or concentrations (i.e. for Growth Factor [GF], Matrix Mettaloproteases [MMP] and Extra-Cellular Matrix/Basement Membrane [ECM/BM]) we have the complete hybrid model. To initialize the system we used the normal tissue domain of the peripheral zone and anatomically reconstruct a two-dimensional slice on the lattice.

We generate the probabilities of movement for each cell using the five-point finite-difference approximation of the tumor and inflammatory equations in response to the concentration of local microenvironmental factors. The two dimensional grid coordinates (*i,j*) are used to define cell locations at points on the grid (mesh size h), at discrete time intervals k (with $x = ih$, $y = jh$ and $t = qk$). The partial differential equation governing tumor migration (in the absence of cell proliferation) is:

$$\frac{\partial C}{\partial t} = D_C \nabla^2 - \rho \nabla . (\mathrm{C} \nabla \mathrm{M})$$

After discretization (using central finite difference approximation):

$$C_{i,j}^{q+1} = C_{i,j}^{q} PM_{C0} + C_{i+1,j}^{q} PM_{C1} + C_{i-1,j}^{q} PM_{C2} + C_{i,j+1}^{q} PM_{C3} + C_{i,j-1}^{q} PM_{C4}$$

PM_{C0} represents the probability of a tumor cell (*C*) remaining stationary:

$$PM_{C0} = 1 - 4\beta - 4\psi\left(M_{i+1,j}^{q} + M_{i-1,j}^{q} - 4M_{i,j}^{q} + M_{i,j+1}^{q} + M_{i,j-1}^{q}\right)$$

The four equations $PM_{C1\text{-}4}$ represent the probability of a single tumor cell located at the lattice coordinates (*i,j*) migrating to one it four orthogonal neighbors:

$$PM_{C1} = \beta - \psi\left(M_{i+1,j}^{q} - M_{i-1,j}^{q}\right)$$
$$PM_{C2} = \beta + \psi\left(M_{i+1,j}^{q} - M_{i-1,j}^{q}\right)$$
$$PM_{C3} = \beta - \psi\left(M_{i,j+1}^{q} - M_{i,j-1}^{q}\right)$$
$$PM_{C4} = \beta + \psi\left(M_{i,j+1}^{q} - M_{i,j-1}^{q}\right)$$

where β represents the unbiased migration coefficient ($\beta = kD_C/h^2$, where D_C is the random motility coefficient) and ψ represents the biased migration coefficient ($\psi = \rho k/4h^2$) up gradients of

ECM/BM (*M*). These movement probabilities are then scaled to such that they sum to 1 and now define the probability of movement to each of a cells orthogonal neighbors or remaining stationary ($PM_{0\text{-}4}$, Figure S1) each time step (*k*).

The partial differential equation governing inflammatory cell motion (in the absence of cell proliferation) is:

$$\frac{\partial I}{\partial t} = D_I \nabla^2 I - \xi \nabla . (\mathrm{I} \nabla \mathrm{G})$$

After discretization (using central finite difference approximation):

$$I_{i,j}^{q+1} = I_{i,j}^{q} PM_{I0} + I_{i+1,j}^{q} PM_{I1} + I_{i-1,j}^{q} PM_{I2} + I_{i,j+1}^{q} PM_{I3} + I_{i,j-1}^{q} PM_{I4}$$

This Inflammatory cell (*I*) equation allows us to define the movement probabilities that consider both unbiased migration (of rate $\omega = kD_I/h^2$, where D_I is the *I* random motility coefficient) and biased migration (driven by chemotaxis, at a rate of $o = \xi k/4h^2$) towards higher GF concentrations (*G*). The equation PM_{I0} represents the probability of no movement:

$$PM_{I0} = 1 - 4\omega - 4o\left(G_{i+1,j}^{q} + G_{i-1,j}^{q} - 4G_{i,j}^{q} + G_{i,j+1}^{q} + G_{i,j-1}^{q}\right)$$

The four equations $PM_{I1\text{-}4}$ represent the probability of a single inflammatory cell located at the lattice coordinate (*i,j*) moving to one it four orthogonal neighbors:

$$PM_{I1} = \omega - o\left(G_{i+1,j}^{q} - G_{i-1,j}^{q}\right)$$

$$PM_{I2} = \omega + o\left(G_{i+1,j}^{q} - G_{i-1,j}^{q}\right)$$

$$PM_{I3} = \omega - o\left(G_{i,j+1}^{q} - G_{i,j-1}^{q}\right)$$

$$PM_{I4} = \omega + o\left(G_{i,j+1}^{q} - G_{i,j-1}^{q}\right)$$

These probabilities enable us to describe individual cell movements, but cells also interact and have other phenotypic behaviors such as mitosis, apoptosis, production and consumption of environmental factors, which define the cells life cycle (Figure 1C).

Discretization of the continuous microenvironmental variables:

See Experimental Procedures for description of the partial differential equations for the continuous variables: GF, MMP and ECM/BM. The discretized form of the GF equation is defined as:

$$G_{i,j}^{q+1} = G_{i,j}^{q} + \delta_G \left\{ \left(G_{i+1,j}^{q} + G_{i-1,j}^{q} - 4G_{i,j}^{q} + G_{i,j+1}^{q} + G_{i,j-1}^{q}\right)\left(m_0 - M_{i,j}^{q}\right) \right.$$

$$\left. - \frac{\delta_G}{4}\left\{\left(M_{i+1,j}^{q} - M_{i-1,j}^{q}\right)\left(G_{i+1,j}^{q} - G_{i-1,j}^{q}\right) + \left(M_{i,j+1}^{q} - M_{i,j-1}^{q}\right)\left(G_{i,j+1}^{q} - G_{i,j-1}^{q}\right)\right\} + k\alpha_B B_{i,j}^{q} \right.$$

$$+ k\gamma C_{i,j}^{q} + k\chi_{RS} RS_{i,j}^{q} G_{i,j}^{q} - k\rho_{RS} RS_{i,j}^{q} G_{i,j}^{q} - k\beta_S S_{i,j}^{q} G_{i,j}^{q} - k\mu_E M_{i,j}^{q} G_{i,j}^{q} - k\eta_L L_{i,j}^{q} G_{i,j}^{q} - k\varphi G_{i,j}^{q}$$

Where the subscripts indicate the concentration at the specified lattice coordinate. $\delta_G = kD_G/h^2$ is the GF diffusion coefficient modulated by the ECM/BM concentration i.e. no diffusion occurs when $M=m_0$, where m0 is the highest concentration of M. Also, α_B, γ, χ_{RS}, ρ_{RS}, β_S , μ_E, η_L, and φ are positive constants. χ_{RS} is the reactive stroma growth factor production rate and is different in low and high reactive stroma (see below).

The discretized form of the MMP equation is defined as:

$$E_{i,j}^{q+1} = E_{i,j}^{q} + \delta_E\left(E_{i+1,j}^{q} + E_{i-1,j}^{q} - 4E_{i,j}^{q} + E_{i,j+1}^{q} + E_{i,j-1}^{q}\right) + k\xi C_{i,j}^{q} - k\kappa E_{i,j}^{q}$$

where $\delta_E = kD_E/h^2$ is the GF diffusion coefficient and ζ, κ are positive constants.

The discretized form of the ECM/BM equation is defined as:

$$M_{i,j}^{q+1} = M_{i,j}^{q} + kB_{i,j}^{q}\nu_B\left(m_0 - M_{i,j}^{q}\right) + k\tau_I I_{i,j}^{q} G_{i,j}^{q} - k\sigma M_{i,j}^{q} E_{i,j}^{q}$$

where ν_B, τ_I and σ are positive constants. See Basanta et al. (2009) for more details regarding these equations and their parameterization.

Cell death probabilities:

Tumor cell death is modeled as a simple switch dependent on the level of GF ($G_{i,j}^{q}$) that the current cell ($C_{i,j}^{q}$) under consideration perceives, so the probability of apoptosis is:

$$PA_c = \begin{cases} 1, \text{ if } G_{i,j}^{q} < G_A \\ 0, \text{ otherwise} \end{cases}$$

Where G_A is the critical level of GF at which death occurs. Whereas for basal cells, we assume that the concentration of MMP (E) enzymatic activity is directly proportional to cell death (scaled by ψ),

$$PA_B = \psi E_{i,j}^{q}.$$

Finally, for the luminal cells apoptosis is driven more by stress from neighboring tumor cells (scaled by υ), so for a luminal cell $L_{i,j}^{q}$ at location (i,j) then,

$$PA_L = \upsilon\left(C_{i+1,j}^{q} + C_{i-1,j}^{q} + C_{i,j+1}^{q} + C_{i,j-1}^{q}\right).$$

Proliferation probabilities:

Proliferation of tumor cells is dependent on their age (T), the level of GF (G) they perceive and the amount of ECM/BM (M) they experience, scaled with the positive constant (ς). For simplicity we assume this probability increases with T and G but decreases with M, the reasoning being that GF are known to stimulate proliferation and high ECM/BM densities can be inhibitive. So the probability of tumor cell $C_{i,j}^{q}$ at location (i,j) dividing is given by,

$$PC_{i,j}^{q}(Division) = \varsigma(m_0 - M_{i,j}^{q})T_{i,j}^{q} G_{i,j}^{q}.$$

The tumor cell proliferation probability is set to 0, when no empty orthogonal grid spaces are available for a daughter cell to divide into.

For simplicity we assume that proliferation of basal and luminal cells is space and phenotype dependent. Specifically, proliferation only occurs if an empty orthogonal position appears (e.g. due to cell death). If that position was previously occupied by a basal (*B*) or luminal (*L*) cell, then it will be replaced with a new daughter cell of that phenotype. This maintains the integrity of the epithelial compartment. See Basanta et al. (2009) for more details regarding these rules and their parameterization.

Reactive Stromal activation:

The switch between stroma (*S*) and reactive stroma (*RS*) phenotypes is driven by GF stimulus. Reactive stromal cells are activated if the level of GF (*G*) is above the threshold G_{RS}, and are deactivated if the growth factor level *G* falls below the threshold G_{RS}. Initially, all stromal cells are assumed non reactive (*S*), i.e. have no GF production. After activation, the cell produces GF dependent upon the degree of stromal reactivity. For simplicity we consider two reactive phenotypes: low stromal reactivity (low SR; stromal cells that upon activation produce low amounts of GF and have the baseline production rate of χ_{RS}) and high stromal reactivity (high SR; stromal cells that upon activation produce high amounts of GF and produce twice the baseline rate, $2\chi_{RS}$).

Tumor phenotype variation and selection

Simulation of PCa pathogenesis is achieved by allowing a single normal luminal cell in a duct near the center of the lattice to mutate. Mutation is achieved through modification of two variable phenotypes as described in the main text: tumor cell GF production (parameter γ), and MMP production (parameter ζ). When a tumor cell in the HCA model divides, the phenotypes of each daughter cell are varied by a small random additive factor as follows. For the GF phenotype, a random number is chosen between ($-\Delta\varepsilon_G$, $\Delta\varepsilon_G$) and is added to the GF phenotype parameter of the parent cell, and assigned to both daughter cells. The same process is used for the MMP phenotype, using $\Delta\varepsilon_E$ as the phenotypic variation parameter, operating on the MMP phenotype parameter. The evolution and selection of these phenotypes in time and space is an important consideration of this work and significantly differentiate the current model from the original (Basanta et al., 2009).

Simulation process for the hybrid discrete-continuum model

All the 2D simulations of the HCA model were carried out on a 1000×1000 grid, which is a discretization of a unit square, $[0,1] \times [0,1]$, with a space step of h = 0.0084mm (approximated from the diameter of selected duct since different types of cells have different diameters). Given a unit of length of 8.4mm. Wherever possible parameters have biologically significant values as based on (Basanta et al., 2009). Each iteration of the simulation process involves solving numerically the non-linear partial differential equations representing the microenvironmental variables (GF, MMP, ECM/BM) until dynamic equilibrium of the diffusible molecules is reached. The probabilities of movement for tumor and inflammatory cells are then calculated taking into account changes in the microenvironmental concentrations. The magnitude of these probabilities dictates which movement direction is selected, therefore each cell is restricted to migrate to one of its four orthogonal neighboring coordinates or remain stationary (when probabilities of movement are equal or the probability of no movement is largest, an unbiased random movement will be

produced). After updating all cells positions, then the individual-based processes for all the cells phenotypes (proliferation, apoptosis, mutation, etc.) is updated. This entire process is repeated until a tumor cell reaches one of the edges of the lattice.

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