Enrichment Map Profiling of Cancer Invasion Front Suggests Regulation of Colorectal Cancer Progression by Bone Morphogenetic Protein Antagonism

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Supplementary Materials and Methods

Generation of protein-protein interaction networks using STRING

The protein dataset of interest (i.e. DPD) was uploaded into the application using gene identifiers and complete lists of human orthologs were included in the network visualization. Protein-protein interaction networks were algorithmically created requesting high confidence scores (>0.7). Proteins are indicated with nodes and protein interactions with connecting lines. Confidence views were requested for the visualization.

Pathway analysis using IPA

The protein dataset of interest (i.e. DPD) was uploaded into the application as standard human gene symbols. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). The IPKB, containing a large network of curated molecular interactions and pathways, was searched to find sub-networks enriched in genes of interest. Graphical representations of these sub-networks, containing direct and indirect molecular relationships, were generated. Genes/proteins are illustrated as nodes and molecular relationships as connecting lines between two nodes (direct relationships as normal lines; indirect relationships as dashed lines). Molecular relationships are supported by at least one literature reference, or by

canonical information stored in the IPKB. Grey nodes represent genes of interest, while white nodes represent hubs that were added by the IPA algorithm to connect a set of genes of interest.

Mathematical model

Parameterization. Let *c* denote the concentration of GREM-1 at any point in the domain; λ, the rate of uptake by tumor cells; λ^*, the rate of decay of GREM-1 in the ECM; *f*, a binary indicator function denoting the location of cancer-associated fibroblasts in the ECM (has value 1 if a CAF is present, 0 otherwise); η, a binary indicator function denoting the location of tumor cells in the ECM; α, the cellular rate of production of GREM-1 by the cancer-associated fibroblasts; c_*, a constant used to scale the equilibrium concentration of GREM-1; and D, the diffusion coefficient of the system.

GREM-1 diffusion gradient model. An accurate reaction-diffusion model of a chemotactic growth factor has been developed previously (1). We have adapted this model to describe the behavior of GREM-1 in the stroma. Several terms account for how the concentration of GREM-1 changes with respect to space and time. GREM-1 is produced by fibroblasts in the stroma at rate α. To describe this behavior, we introduce an indicator function, f, which takes the value 1 where a fibroblast is present and 0 otherwise. Tumor cells consume GREM-1, at rate λ, as a function of the local concentration of GREM-1. Again, this behavior is described using a binary indicator function, η, which is 1 where tumor cells are present and 0, otherwise. Lastly, the phenomenon of diffusion of a dissolved substance is modeled by relating the time derivative of concentration with the spatial laplacian of the concentration; the diffusion term below causes locally high concentrations of GREM-1 to diffuse outward over time, in an attempt to move the system back to a spatial equilibrium of even GREM-1 concentration throughout the stroma.

$$\frac{\partial c}{\partial t} = \overbrace{\nabla \cdot (D \nabla c)}^{\text{Diffusion term}} - \overbrace{\lambda \eta c}^{\text{Consumption term}} - \overbrace{\lambda^* c}^{\text{Decay term}} + \overbrace{f \alpha}^{\text{Production term}}$$

Through the implementation of an individual-cell model, we are looking to observe the effect that discretely positioned CAFs have on discretely positioned tumor cells. We are looking to examine the interaction of individual fibroblasts with growing tumor cells and thereby infer how the position and characteristics of many fibroblasts effect the movement and growth of a centrally-located tumor cell cluster.

Cell Taxis Model. The principle factors influencing tumor cell taxis are chemokinesis, chemotaxis and haptotaxis as described elsewhere (2, 3). Using the principles described by previous models (3-5), we have come up with a sensible model describing the process of cell taxis and how it is influenced by the factors above, namely, the diffusion of GREM-1, cell-cell adhesion, and random motility (haptotactic interactions with fibronectin are not described).

Differences in the order of the relation of state-variables that occur between our model and the model described by (3) are accounted for by observing that Alarcon *et al.* (2003) model cell density, whereas we are, instead, modeling changes in cell position in regards with time. It is easily verified that the underlying physical relations are identical.

It follows that the equation describing cell taxis is given by:

$$\frac{\partial \mathbf{x}}{\partial t} = \mathbf{R}(\theta) \left(k_c \nabla c + k_h \Delta n \right),$$

where it makes sense for the velocity of the cells to be proportional to the gradient of the concentration since cells will always move to highest density of GREM-1.

$\mathbf{R}(\theta)$ corresponds to a stochastic function, the mean of which is given by its argument. The magnitude of any image vector is equal to the magnitude of the pre-image vector; however, the angle from the horizontal axis (given by $\arctan(v_y/v_x)$, where v_y and v_x are the y and x components of the vector, respectively), is altered by the amount given by a normal distribution

with mean in the direction of the argument (as stated). The von Mises distribution is also coined the "circular normal distribution". It is easily verified that this method is near-equivalent to the method of random movement described by Sun *et al*. (2005) (1).

Cell division. Cell division is modeled very simplistically, merely for the purpose of allowing the number of cells to increase over time. The model describing the number of cells in each grid square that divide at each time step is given by a Binomial distribution. Cells divide with probability/rate $1\ \mathrm{hr}^{-1}$ (chosen conservatively). Thus, the probability of a cell dividing at each point follows a Bernoulli distribution with: $p = 1\ \mathrm{hr}^{-1}$.

Discretization. The Alternating-Direction Implicit (ADI) method was used to solve numerically the system modeling the GREM-1 diffusion process at each time step. The Crank-Nicolson method was used to difference the reaction terms in the reaction-diffusion system.

Proportionality to cell density. We have observed that the equation describing cell movement by Anderson (2005) (4) relates the derivative of cell density with respect to time to the laplacian of cell density. Indeed, this relation is conserved implicitly when the flux of cell density is instead related to the change in cell position with respect to time (as in our model). Cell density describes the amount/concentration of cells per unit area. Cell position describes the exact location of individual cells. After the discretization of our model, cell positions within some unit area (each grid square in the lattice) are treated identically. Thus,

$$\sum_{pointwise} \mathbf{x} = n,$$

where n represents cell density as in previous studies (3-5), and **x** represents the two-dimensional position vector corresponding to the spatial location of each cell. The point-wise sum can be thought of as matrix addition, where corresponding row-column elements are summed, thereby

yielding a number of cells per unit grid square that is “density”. Since derivatives are linear operators, it follows immediately that:

$$\frac{\partial}{\partial t}\left(\sum \mathbf{x}\right) = \sum \frac{\partial \mathbf{x}}{\partial t} = \frac{\partial n}{\partial t}$$

Thus, $\sum \frac{\partial \mathbf{x}}{\partial t}$ indeed holds the same proportionality to Δ n, as desired.

Differencing. Note that after discretization, η, f represent matrices, with a number of rows and columns corresponding to the width and height of the domain **L**, respectively. In this way, an element of η has value 1 if that element is occupied by a tumour cell; similarly, if unoccupied, that element of η has value 0. Likewise for f and cancer-associated fibroblasts. D, λ, λ^*, c_*, α remain constants.

By ignoring the particularities of the boundary conditions at the moment, the continuous model becomes the following after differencing and multiplying by Δ t:

$$\begin{cases} 2\left(c^{n+1/2} - c^{n}\right) & = \psi\left(c_{j+1}^{n+1/2} - 2c^{n+1/2} + c_{j-1}^{n+1/2} + c_{l+1}^{n} - 2c^{n} + c_{l-1}^{n}\right) \\ & \quad -\xi\left(c^{n+1/2} + c^{n}\right) + f\alpha\Delta t \\ 2\left(c^{n+1} - c^{n+1/2}\right) & = \psi\left(c_{j+1}^{n+1/2} - 2c^{n+1/2} + c_{j-1}^{n+1/2} + c_{l+1}^{n+1} - 2c^{n+1} + c_{l-1}^{n+1}\right) \\ & \quad -\xi\left(c^{n+1} + c^{n+1/2}\right) + f\alpha\Delta t \end{cases}$$

$$\psi = \frac{D\Delta t}{(\Delta x)^2}, \qquad \xi = \frac{\Delta t}{2}\left(\lambda\eta + \lambda^*\right)$$

where, for each (j,l) in the square domain

$$\mathbf{L} = \{(j,l) \mid j = 2,3,\ldots,\mathrm{nRows}-1, l = 2,3,\ldots,\mathrm{nCols}-1\}$$

(i.e., not including points along the boundary of the domain), a subscripted j+1 denotes that c is to be evaluated at (j +1, l) [similarly for l+1 and (j,l+1), etc.] and no subscript means that c is evaluated at the current point [i.e., (j,l)]. Superscripts denote discrete time steps, where $n \in [0,\tau] \cap \mathbb{N}$ for some maximum time step, τ.

Rearranging the system above, we obtain the following pair of tridiagonal systems with corresponding boundary conditions:

$$\begin{cases} (2+2\psi+\xi)\,c^{n+1/2} - \psi\left(c_{j+1}^{n+1/2} + c_{j-1}^{n+1/2}\right) & \text{for } (j,l) \in \mathbf{L}, n \in [0,\tau] \\ \quad = (2-2\psi-\xi)\,c^{n} + \psi\left(c_{l+1}^{n} + c_{l-1}^{n}\right) + f\alpha\Delta t & \\ (2+2\psi+\xi)\,c^{n+1} - \psi\left(c_{l+1}^{n+1} + c_{l-1}^{n+1}\right) & \text{for } (j,l) \in \mathbf{L}, n \in [0,\tau] \\ \quad = (2-2\psi-\xi)\,c^{n+1/2} + \psi\left(c_{j+1}^{n+1/2} + c_{j-1}^{n+1/2}\right) + f\alpha\Delta t & \\ \begin{cases} c_{(1,l)}^{n} = c_{(2,l)}^{n} & \text{for } l \in 2,3,\ldots,\mathrm{nCols}-1, n \in [0,\tau] \\ c_{(\mathrm{nRows},l)}^{n} = c_{(\mathrm{nRows}-1,l)}^{n} & \text{for } l \in 2,3,\ldots,\mathrm{nCols}-1, n \in [0,\tau] \\ c_{(j,1)}^{n} = c_{(j,2)}^{n} & \text{for } j \in 2,3,\ldots,\mathrm{nRows}-1, n \in [0,\tau] \\ c_{(j,\mathrm{nCols})}^{n} = c_{(j,\mathrm{nCols}-1)}^{n} & \text{for } j \in 2,3,\ldots,\mathrm{nRows}-1, n \in [0,\tau] \end{cases} \end{cases}$$

Under-the-agarose cell migration assays

Experimental setup. Petri dishes were coated with fibronectin for 1 h at 37 ^{o}C. 1-cm-thick agarose gels [2% low melt agarose in Dulbecco's modified Eagle's medium (DMEM)] were cast into each dish. After the gel was solidified, one large (~1 cm) well in the center, surrounded by four smaller (~0.5 cm) wells in the periphery of Petri dishes were punched, according to the original protocol (6). SW480/SW620 cells were stained with crystal violet (Primurlyn) fluorescent dye according to the manufacturer's recommendations. The cells were treated for 24 h with serum-free DMEM and then 500 μL of 5,000 cells/100 μl of serum-free DMEM were plated into the central cavity at the center of gel, and the surrounding holes were each filled with 200 μL of DMEM containing 10% fetal bovine serum (FBS) to create a chemotactic gradient. Specific factors, such as GREM1 rhGREM1 and rhBMP7 were then supplemented to the surrounding wells to create the experimental conditions under investigation. Then the dishes were incubated for 48 h in a cell culture incubator under standard conditions to allow cells to migrate and the cells were fixed in 4% paraformaldehyde.

Quantification of cell migration. Fixed cells on Petri dishes were scanned and the obtained images were uploaded into ImageJ software for quantification of migratory behavior and were processed as follows. First, a dashed circle was drawn in the periphery of the central cavity, to exclude cells that did not migrate from the central hole underneath the agarose. Second, the entire Petri dish surface was divided in four distinct quadrants, each one referring to the area of the chemotactic gradient created by the corresponding surrounding well. ImageJ then calculated the total crystal violet intensity in the Petri dish, excluding fluorescence in the central cavity. This score was determined as the "baseline intensity" for each image. To determine cell migration towards specific gradients, ImageJ was requested to provide the percentage of the baseline intensity that corresponded to the surface of each individual quadrant on one image at a time.

References

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