Autologous Chemotaxis of Tumor Cells: a Novel Homing Mechanism to Lymphatics

Mark E. Fleury[1], Jacqueline D. Shields[1], Alice A. Tomei, Melody A. Swartz[1,2]

1. *Integrative Biosciences Institute, École Polytechnique Fédérale de Lausanne,*
2. *Department of Chemical Engineering, Northwestern University, Evanston, IL, 60208 USA,*

Introduction

Cell response to extracellular cues is often driven by gradients of morphogenetic and chemotactic proteins. Many of these proteins are secreted in extracellular matrix(ECM)-binding form to be subsequently released proteolytically, and here we explore how this feature, along with interstitial flow, which is present in all tissues, can affect pericellular morphogen gradients. We created an in silico model of mass transport for cells in a 3D porous ECM and examined the roles of flow, blocking antibodies, and paracrine signaling on calculated pericellular morphogen gradients. We also created an in vitro model of tumor cell metastasis using 3D tissue equivalents, tumor cells and lymphatic endothelial cells (LECs) to mimic conditions of the in silico model. The results of both models revealed the influence of interstitial flow, lending support to the hypothesis that interstitial flow can affect tumor migration through the spatial modification of biochemical cues.

Methods

Mass transport is modeled using a coupled convection-diffusion mass transport equation and solved numerically:

$$\frac{\partial v}{\partial t} + v\nabla C = \nabla \bullet (D\nabla C) + R_v \quad ,$$

where C=concentration, v=fluid velocity, D=diffusion coefficient, and R_v= generation (e.g., morphogen release from the matrix).

The mass transfer problem was solved for two species, cell-released proteases and CCL21, an ECM-binding protein that is secreted by both tumor cells and LECs is known to act as a chemoattractant. The cell in its extracellular matrix was modeled as a sphere in a porous media, and the Brinkman equation was used to solve for the flow profile around the cell. Previous work had shown the possibility of autologous gradient formation in simple models [1,2], and here we more thoroughly investigated the phenomenon using the specific CCL21 chemokine in a 3D geometry under both transient and steady-state assumptions.

In vitro experiments were performed using MDA-MB 435s tumor cells seeded into 3-D collagen and proteoglycan-based tissue equivalents which were seeded into Boyden chambers [3]. The constructs were then either subjected to slow interstitial flow, (approximately 0.1um/s) or to static conditions with and without LECs on the bottom side of the membrane. Net migration of cells across the membrane was measured and compared between conditions. To differentiate between physical effects of the flow on the cell versus effects of flow on the pericellular distribution of CCL21 we repeated the experiments utilizing antibodies against the chemokine CCL21.

Results

The computed ECM-released gradients of enzymes and CCL21 showed trends that matched in vitro migration results quite well. Figure 1 shows both the migration results for each of the experimental conditions as well as the results of the mass transfer calculations for each of the conditions. The computational model was used to determine transcellular gradients of both cell-

secreted enzymes and soluble CCL21. Transcellular gradients were measured as the difference in concentration 1 um above and below the cell divided by the average of these two values. We found that in static conditions the presence of LECs increased the sensed transcellular gradients of tumor cells located in the tissue equivalent, but that a similar gradient could be achieved without the LECs by introducing interstitial flow (Fig 1A). Interestingly the in vitro migration assay showed similar results (Fig. 1C). In both the static LEC case and the flow with tumor cells alone, the increased migration was abolished by CCL21 blocking antibodies, indicating the CCL21 dependence of this migration (Fig. 1C). Adding flow to the simulation with a LEC monolayer on the bottom of the membrane increased the computed transcellular gradient approximately 3-fold and the percentage of cells crossing the membrane in the corresponding migration assay also roughly tripled, again a phenomenon that was nearly abolished by using an antibody for CCL21. This cancellation of flow-enhanced migration indicates a greater role for the biochemical environment in terms of CCL21 distribution rather than through biophysical effects on the cells such as fluid "pushing" the cells.

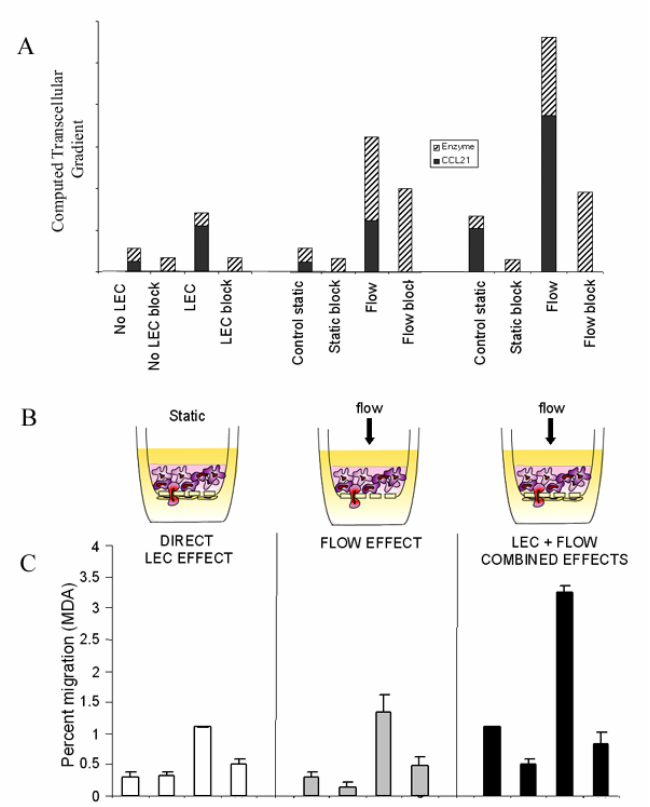

Figure 1: Computational pericellular gradients around tumor cells qualitatively correspond to in vitro migration results. (A) Computational results of transcellular gradients of CCL21 (solid bars) and cell-released enzyme (crosshatched bars) predict that the presence of LECs and of flow both increased CCL21 gradients, a result that is negated by CCL2. (B) Tumor cells suspended in a 3D matrix were subjected to transwell migration studies under static and flow conditions with and without LECs on the bottom side of the transwell membrane. (C) Migration results mimic the computational results from (A).

Conclusion

Here we have shown a newfound importance of the biophysical environment in influencing chemotactic gradients, and additionally introduced the idea of autologous chemotaxis. We have shown computationally that low but physiological levels of convection can lead to chemotactic

gradients produced and sensed by the same cell (hence the term 'autologous'), and furthermore shown using an in vitro cancer model that this flow enhanced gradient does indeed increase directed tumor cell migration in the direction of flow. Given that, physiologically, interstitial flow drains into the lymphatic system leads us to hypothesize that this autologous gradient formation mechanism may explain the metastasis of tumor cells through the lymphatic system.

References

1. Helm CLE, ME Fleury, AH Zisch, F Boschetti, MA Swartz. 2005. Synergy between interstitial flow and VEGF directs capillary morphogenesis in vitro through a gradient amplification mechanism. Proc. Nat. Acad. of Sci. U.S.A. 102:15779-15784

2. Fleury, M. E., Boardman, K. C. & Swartz, M. A. (2006) Autologous morphogen gradients by subtle interstitial flow and matrix interactions. Biophys. J. **91,** 113-121.

3. Shields JD, Fleury ME, Young C, Tomei AA, Randolph GJ, Swartz MA. Autologous Chemotaxis as a Mechanism of Tumor Cell Homing to Lymphatics via Interstitial Flow and Autocrine CCR7 Signaling. Submitted