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A Multisclae Probabilisitc Framework to Model Early Steps in Tumor Metastasis

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Abstract: Tumor metastasis is the leading cause of nearly all cancer related deaths. While several experimental and computational studies have addressed individual stages of the complex metastasis process, a comprehensive systems-biology model that links various stages of metastasis has not been put forth as of yet. In this paper we discuss the formulation and application of such a model that utilizes basic principles of cell biology, physics and mechanics to study the migratory patterns of tumor cells as they move from the parent tumor site to the connective tissue via the basement membrane. The model is first of its kind in capturing the essential early features of metastasis in a single simulation and shows good agreement with recent experimental studies.

1 Introduction

Cancer metastasis, a process by which cancer spreads from one part of the body to another, is responsible for nearly all cancer related fatalities. Metastasis is a multi-step process, starting with cell detachment from parent tumor and movement of the cell in the epithelium. This is followed by invasion of the basement membrane. As the cells pass through the basement membrane, they reach the loosely organized connective tissue, and movement in connective tissue finally leads to penetration of blood capillary and the circulatory system [1]. While only a small number of cells from the parent tumor site are able to survive these multiple steps, the cells that are able to form new colonies and proliferate in environments different than their parent tumors result in cancer progression to advanced and often incurable stages.

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Individual steps of cancer metastasis have been studied in detail through a number of genetic, biochemical, imaging and biophysical methods [2-6]. These studies have shed light on the underlying mechanisms by which a single cell detaches itself from the epithelial layer, migrates towards the basement membrane and penetrates the basal lamina. In addition, in recent years, several groups have also utilized a number of computational methods to study these steps in cancer progression [7-13]. Studies have focused on individual and collective cell migration, angiogenesis and cell-matrix interactions. Novel computational methods have allowed researchers to predict the behavior of *in vivo* tumor cell migration and to study the role of matrix stiffness, cell stiffness, receptor ligand interactions and pore sizes [11, 13]. While these computational and experimental studies have significantly improved our understanding of epithelial-to-mesenchymal transitions, cell-matrix interactions and cell migration in general, so far no attempt has been made to develop a system-wide unifying model that connects various complex steps of tumor metastasis. Such a model would be highly desirable as it will not only provide researchers with a detailed understanding of the transitions at various stages, but will also improve our understanding of the key factors influencing the dynamics and survival of tumor cells at various stages of metastasis. Ultimately, sophisticated models based on first principles and experimental studies, will provide researchers and drug industry with potential targets for improved, customized and efficient therapeutics.

In this study, we report the formulation and applications of a novel system-wide and multiscale, model that addresses various early steps of cancer metastasis, starting from movement of a single

cell in epithelial layer, to basal lamina invasion followed by migration on loose connective tissue. This first of its kind model integrates principles of biophysics, cell biology and mechanics in predicting and explaining various aspects of cell migration, cell matrix interactions and cancer progression. The model described in this paper is scalable and can be customized to specific cancers or may be used to predict underlying features of cancers in general. While several assumptions have been made in developing and implementing this model, we believe that this novel approach in understanding the systems-biology of cancer cell migration will provide researchers with an in depth understanding of tumor cell motility in an effort to control and cure this deadly disease.

2 Methods

A schematic of the multi-step metastasis process leading from migration in an epithelial layer to loose connective tissue is shown in Figure 1.

While metastasis also involves penetration of capillary and eventual detachment and invasion of the target tissue (not shown in Figure 1), the key early steps in metastasis are the migration from the parent tumor site up to the blood stream, as migration through the circulatory system is predominantly carried out by blood flow in the capillaries [1]. Thus, in this study we focus on the first three key steps of metastasis as shown in Figure 1. Our multi-scale model is based on lattice Monte Carlo simulations, which have been previously used to study the effect of MMP activation on persistence and migration in 3D matrices [13].

2.1 Mimicking the 3D in vivo environment

As a starting point, we divide the sample space of interest into three-dimensional cubic lattices. Depending upon the environment (e.g. parent tumor, basal lamina or loose connective tissue) these lattice sites are filled with other deformable cells, binding ligands or empty space. In case of a parent tumor in the epithelial layer, the lattice sites are filled with deformable yet immobile cells. The migration of a given cell therefore depends on its ability to squeeze through these steric obstacles. The basement membrane is characterized by a dense network of extracellular matrix comprising of a number of cell-adhesion ligand proteins. We capture the essential features of the basement membrane by filling the lattice sites with high concentration of protein ligands such as collagen, laminin or fibronectin, which provide traction to the migrating cells, have known mechanical properties and are also degradable by matrix metalloproteases. The final stage of our model deals with cell-matrix interactions and migration in connective tissue. Connective tissue environments are a loose network of extracellular matrix protein with pore sizes on the order of a few cell-diameters. The matrix fiber thickness is more or less uniform and the pore sizes are also consistent [14-16]. To model this kind of environment, we fill a smaller number of lattice sites with protein ligands, but the pore sizes are bigger and a number of lattice sites are kept empty to recreate the loose connective tissue environment. The computational rendering of the environments in shown in Figure 2.

2.2 Monte Carlo Routine

A schematic of the Monte Carlo routine is shown in Figure 3.

The Monte Carlo routine is based on migration of cells from one lattice site to another. Our model assumes that in each time step (Δt) a cell can move only one lattice space. The cell can move into any of its six neighboring lattice spacings. Diagonal moves would correspond to movement by $\sqrt{2}x$ lattice spacing and are hence disallowed.

The first step in our simulation is creating the appropriate ECM environment, which may include filling the lattice sites with other cells, extracellular protein or creation of a model connective tissue.

The cell's initial lattice site is chosen randomly in the first time step. In the subsequent time steps, a cell can go to any neighboring lattice based on its "ψ value" for each of its neighboring lattice sites. The ψ value for any given lattice site is defined as:

$$\psi = \frac{\text{Cell cross section}}{\text{Pore size}} \tag{1}$$

A low ψ value implies that the neighboring site

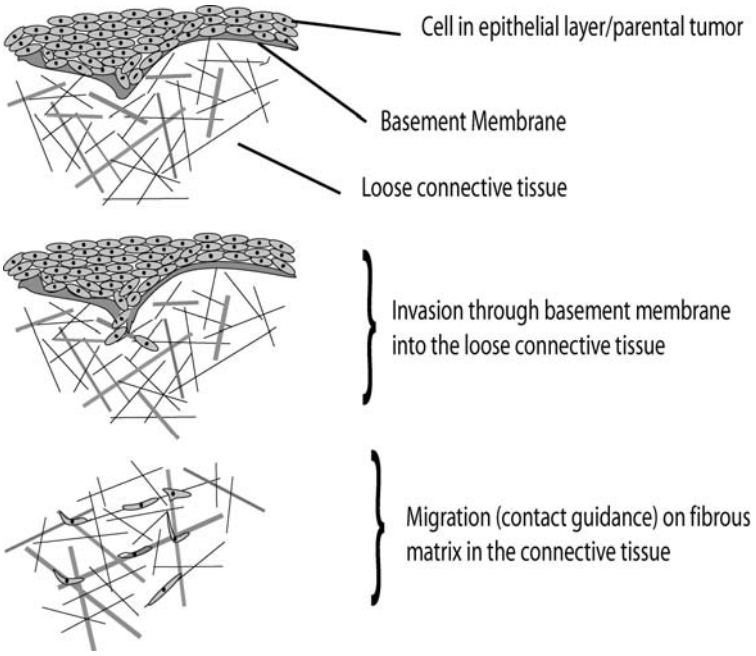

Figure 1: A schematic of the three key stages of metastasis. Cells move in the parent epithelial layer towards the basement membrane, followed by invasion of the membrane and reach the connective tissue where they migrate on loosely organized extracellular matrix.

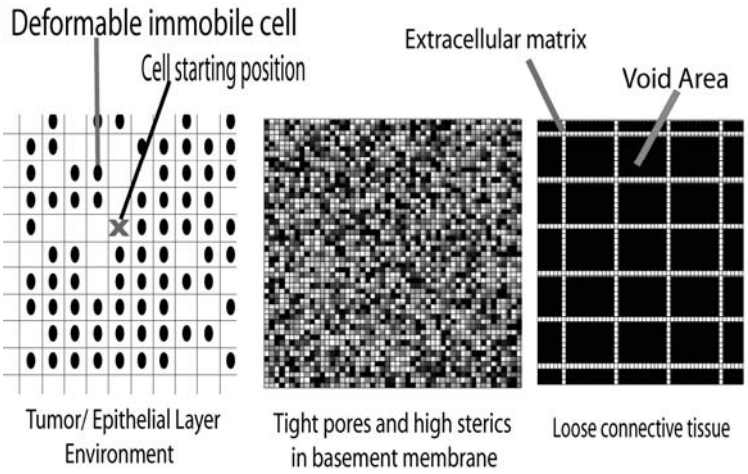

Figure 2: Computer rendition of the three key environments. (Left) A mature tumor with lower packing density and deformable cells where individual cells can migrate. (Center) A tightly organized basement membrane. (Right) Loose connective tissue where pores are bigger and the void areas occupy most of the volume.

does not enough protein ligands to provide the necessary binding and traction for migration. This is especially true in many cases in connective tissue environment where the fibrous extracellular matrix occupies a smaller portion of the overall space as compared to open voids [14-16].

A cell can move into any of its neighboring lattice sites if $\psi < 1$. If there are more than one neighbors that fulfill this criteria, the cell chooses its neighbor randomly. If the ψ value for all the six neighbors is greater than 1, the cell can choose one of the two options: 1) The cell can decrease its surface area and deform. This would increase

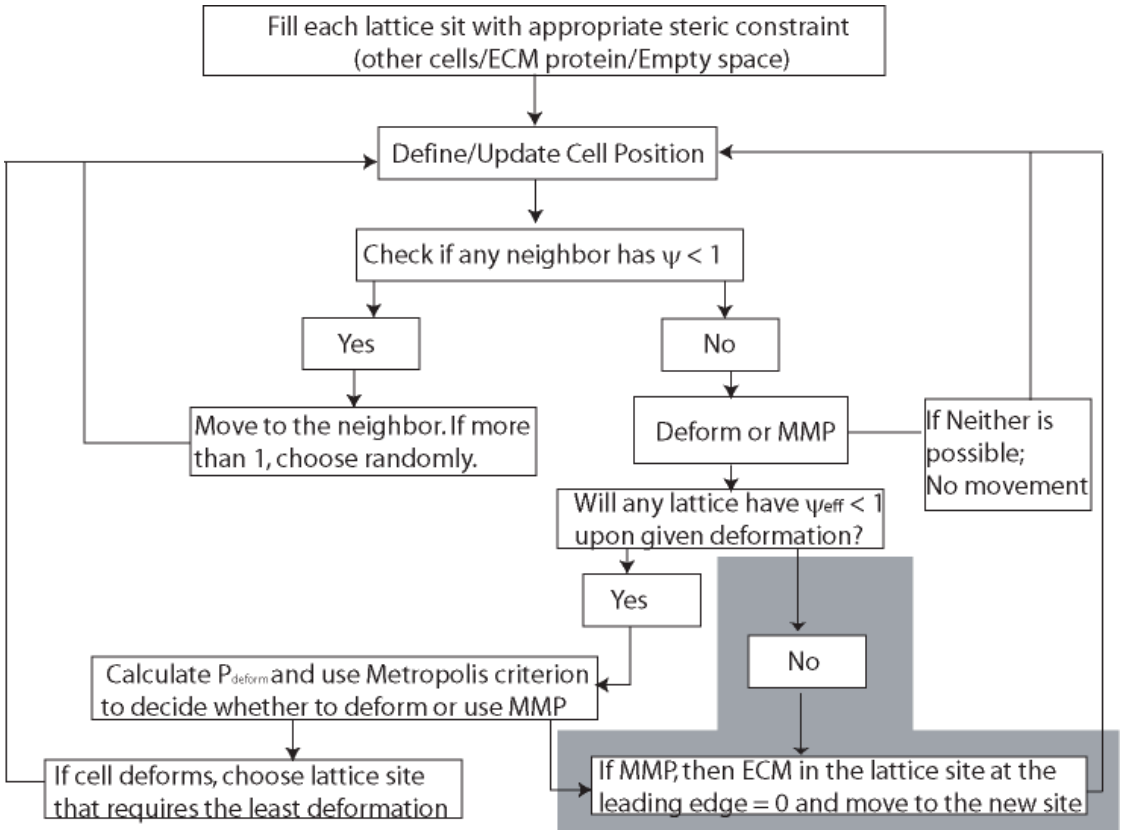

Figure 3: A schematic of the general Monte Carlo routine. The shaded area is relevant only for situations where proteolysis enhances migration such as movement through basement membrane.

the effective ψ value by decreasing the numerator in equation 1 above. 2) The cell can also utilize matrix proteases and deplete the neighboring lattice of sterically constraining protein. This would result in increasing the pore size and decrease the effective ψ value. Based on these two scenarios, we define effective ψ value as:

$$\psi_{eff} = \frac{\text{Cell cross section} - \begin{array}{c}\text{decrease in cross}\\ \text{section due to de-}\\ \text{formation}\end{array}}{\text{Pore size}} \tag{2}$$

When $\psi \geq 1$ and $\psi_{eff} < 1$, the cell decides to move to a neighboring lattice by calculating P_{deform}, which depends upon the number of neighbors where given percent deformation would lead to $\psi_{eff} < 1$. Mathematically, for a given cell type P_{deform} can be written as:

$$P_{deform} = \delta x \frac{m}{6} \tag{3}$$

Where δ has a value of one if the cell can deform at all and zero otherwise. "m" is the number of neighboring lattice site that will result in $\psi_{eff} < 1$ at a given deformation (maximum of six). The cell's choice to deform or not is calculated through Metropolis Monte Carlo method. A random number, **R**, is chosen between 0 and 1. If **R** is greater than P_{deform}, the cell does not deform and relies on its available preoteolysis machinery (i.e. if there is active proteolysis machinery inside the cell or if proteolysis will affect the ψ_{eff} value). On the other hand, if **R** is less than P_{deform} the cell deforms and goes to the neighbor with that will result in $\psi_{eff} < 1$. In case there are multiple neighboring sites that meet this criterion, the cell chooses the site that would require the least deformation to get $\psi_{eff} < 1$. In situations where the cell lacks any proteases or proteolysis has no effect, such as the case of movement in the parental tumor, and P_{deform} is less than **R**, the cell does not move in that time step. The process is then

repeated until P_{deform} is greater than **R**.

For situations where proteolysis can enhance migration, MMP activity is additional available option for the cell (Figure 3). For simplicity, we assume that proteolysis activity is non-isotropic. In other words, our model assumes that once the cell decides to use its proteolysis machinery, it digests protein in only one neighbor. As the time scale of proteolysis is much shorter than that of migration, we can safely assume that the proteolysis of matrix proteins at the leading edge is complete in the time scale of migration.

We calculate mean-squared displacement, speed and velocity from our lattice Monte Carlo simulations. The mean squared displacement is given by:

$$\langle d^2(t)\rangle = \langle x(t_0+t)-x(t_0)\rangle^2 + \langle y(t_0+t)-y(t_0)\rangle + \langle z(t_0+t)-z(t_0)\rangle \quad (4)$$

$x_i(t_0+t)$ is the x-coordinate of the cell at time t, $y_i(t_0+t)$ is the y-coordinate of the cell at time t and $z_i(t_0+t)$ is the z-coordinate of the cell at time t. The probability to migrate is calculated by histogramming the mean-squared displacement over all simulations.

Our model allows us to look at various stages of the metastasis process for given cell or populations of cells in a single simulation, or we can separate individual biological processes and study the effect of key factors such as effect of stiffness, proteolysis, pore size etc. In the present study, we present results of complete simulations studying cellular migratory behavior from the parent tumor in the epithelial layer through the basal lamina to the connective tissue. The simulations were performed in a 3D cubic lattice with periodic boundary conditions and the results discussed are averaged over 100,000 independent simulations.

3 Results

Using multi-scale lattice Monte Carlo simulations, we study the migration of individual tumor cells from the parent tumor site to the loose connective tissue via basement membrane. The first step in this multi-step migration is movement in the parent epithelial environment. Figure 4a shows the most probable distance sampled by a given cell. Migration in parent epithelial layer is primarily driven by finding open spaces and deformable cells which can be pushed so the cell can squeeze through till it reaches the site of the basement membrane. In the absence of an external chemical gradient the movement is primarily stochastic and leads to relatively lower displacement. In a non-deformable environment, where cells present significant steric hindrance to the motile cell, the probability to cover larger distances is very small, hence only cells that are closer to basal lamina are able to reach the site of the membrane and move on.

Cells that are farther from the site of the basement membrane will take much longer or will never reach the site of the basement membrane. As the environment flexibility increases, a given cell, with given stiffness, is able to "push" the non-motile cells, squeeze through and move forward. Thus with increasing flexibility of the surrounding cells the average distance covered by a given cell increases. Our simulations suggest that tumor stiffness and flexibility play an important role in controlling a cell's ability to move greater distances and reach the site of basement membrane. Another interesting feature that emerges out of our simulations is the effect of cell packing density (Figure 4b). Epithelial layers and parent tumors that are tightly packed present a bigger steric obstacle and hence lead to fewer cells reaching the basement membrane than cells in environments where the packing density is lower. This result is in accordance with the experimental observation that mature tumors, which are larger in size, and have more "open spaces" are more likely to metastasize than younger tumors which are smaller and more tightly packed .....[1].

After a given cell reaches the basement membrane, the invasion through basement membrane requires either deformation through the pores, proteolysis, or a combination of both. Figure 5 shows the behavior of a given cell in four different situations, namely a non-deformable cell without an active proteolysis machinery, a non-deformable cell with an active MMP machinery, a cell that can deform up to 30% but is unable

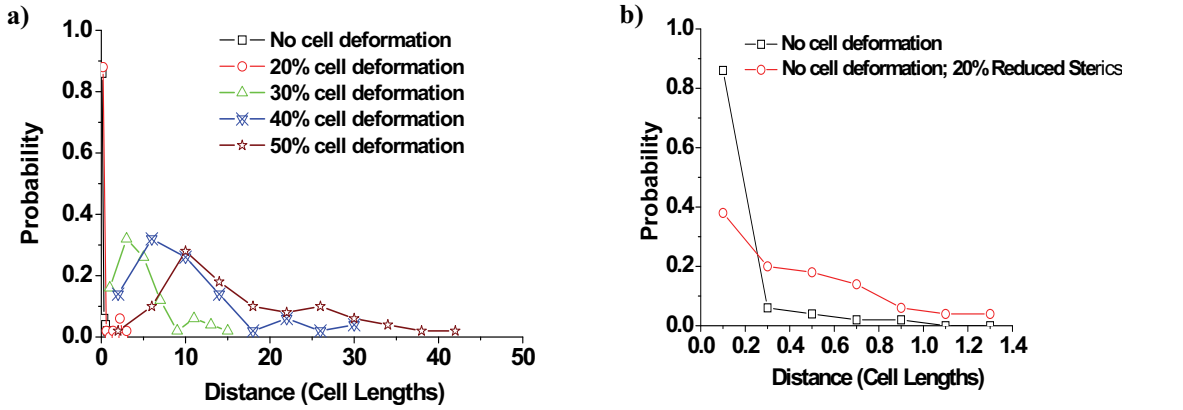

Figure 4: a) Probability of a given cell to migrate in the epithelial layer and reach the basal lamina plotted as a function of the environment stiffness. Cells are able to migrate significantly if they are able to push and squeeze flexible cells in their surroundings. b) Probability of a given cell to migrate and reach the basement membrane at greater distances increases with decrease in packing density of the epithelial layer.

to proteolyse the matrix and a cell that can deform by 30% and is able to proteolyze the matrix. Cells that are able to proteolyse the matrix are able to move much farther, as the basement membrane pores are much smaller than cellular dimensions. In addition, the matrix itself is quite flexible and therefore the ability of the cell to deform also plays an important role.

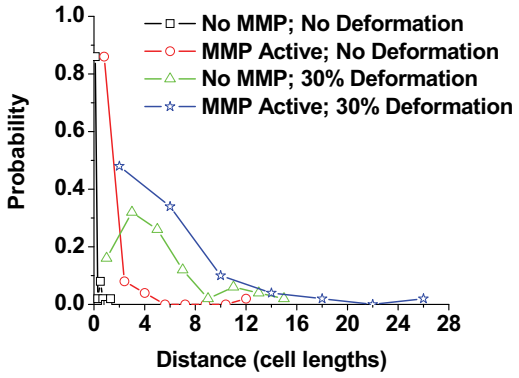

Figure 5: Probability of tumor cell migration through the tight pores of basement membrane as a function of cell deformation ability and proteolytic activity. Cells that are able to deform and secrete MMPs are able to migrate over long distances, however, blocking MMP alone is not sufficient since cells lacking MMP activity also migrate significantly.

We note that maximum migration correlates with cells that are able to deform and use MMPs, and blocking MMP alone is not sufficient for cells to move. While cells, as expected, move maximum distances when they have the ability to deform and secrete MMPs, they still move significant distance when they are devoid of MMP activity. This result is potentially very useful as traditionally MMP activity has been the key target for blocking tumor cell motility, yet our computational results, as well as recent experimental studies suggest that blocking MMPs may not be sufficient in ceasing all cancer cell movement [5, 17].

The final stage modeled by our multi-scale model is movement at the connective tissue level. We model the connective tissue as a loose network of fibrous extracellular matrix as has been shown in a number of experimental studies [5, 14, 17]. The primary mechanism of motility in this step is contact guidance, where cells travel "on the fibers" rather than degrading the matrix and invading through the membrane. Our simulations capture the experimental behavior of cells showing independence to stiffness and MMP activity (Figure 6).

The most probable distance covered remains the same whether or not the cell has the ability to proteolyse the environment or deform. However, our simulations suggest that the ability of a cell to move appreciable distance is closely related to its adhesion to the matrix (Figure 6 inset). Cells that

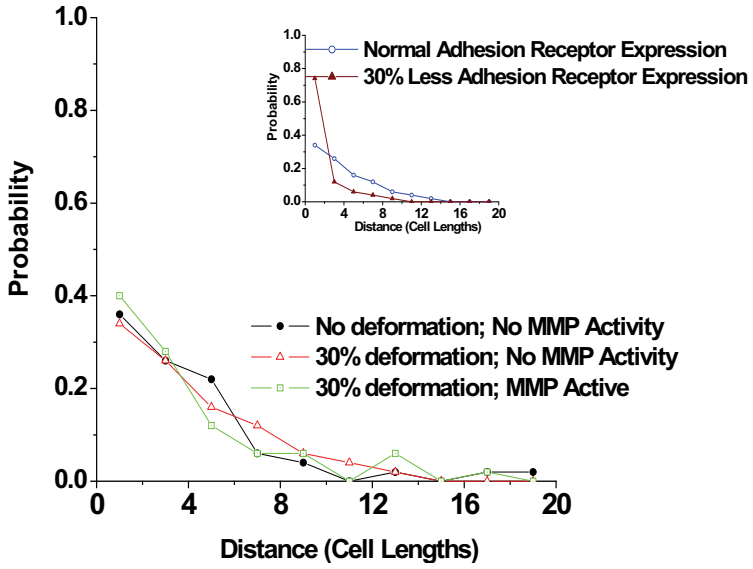

Figure 6: Probability of migration in loose connective tissue is independent of MMP activity or deformation as the cells move "on top" (i.e. contact guidance) rather than invade through the matrix. The migration ability, however, is affected by integrin activity (Figure 6 inset).

have normal integrin expression, show migration over greater distances than cells that are lacking adhesion machinery. This is primarily due to the fact that contact guidance is very similar to 2D movement where cells are more or less moving in one plane, which is significantly different from invasion through the basal lamina. Our simulations suggest that cell deformation and MMP activity are relatively minor effects as compared to cell adhesion that characterizes movement through the loose connective tissue.

4 Discussion

Using lattice Monte Carlo simulations, we have developed a multi-scale, system-wide approach, that is able to capture the essential biophysical aspects of tumor metastasis. While previous computational studies have focused on a single step [11, 13], our approach allows us to simultaneously model various steps using the unifying approach discussed in the methods section.

Using this system-wide approach, we have modeled and studied migration of a given cell through the parent tumor environment to the loose connective tissue via the basement membrane. Our results show that the probability of a given cell to migrate significant distances varies depending upon the stage of tumor metastasis. In the earlier stages, we note that tumor sterics play a significant role in determining the ability of a given cell to reach the basal lamina. Stiffer environments and parent tumors with high packing density provide a tougher environment than mature and more flexible tumors. Once a given cell reaches the basement membrane, the ability of the cell to invade depends simultaneously on its ability to proteolyse the matrix as well as to deform. The results show that blocking MMP itself is not sufficient as cells that are flexible and can deform through the pores also migrate significantly. Finally, cells reaching the connective tissue need a strong adhesion machinery to migrate and do not depend on their MMP machinery or deformation ability to migrate significantly.

4.1 Comparison with experiments

Our results show excellent agreement with a number of recent experimental findings. First and foremost, our model is able to capture the multiscale *in vivo* behavior of a tumor cell migrating through various environments [4, 5, 10]. Our simulations show that tumor cells are much more likely to reach the basement membrane site in

flexible and mature tumors, than in younger tumors which are sterically constrained [1]. Our results agree very well with those of Wolf et al who recently showed that migration of HT-1080 cells is more or less unaffected by MMP inhibition [17]. The simulations suggest that MMP inhibition may have an effect, but for smaller distances that are relevant for basement membrane invasion, MMP inhibition alone may not be sufficient in blocking cells from migrating. Finally, our results are in agreement with those of Gibson and co-workers as well as those of Friedl and coworkers who note that in loose connective tissue environments, lack of adhesion significantly affects a cell's ability to migrate [5, 15-19]. While there are several limitations in our current model (e.g. ignoring sub-cellular events, implicit modeling of cell-matrix interactions, no explicit treatment of cellular forces and morphology and ignoring matrix laid out by cells themselves), our ability to capture the underlying mechanism of tumor metastasis along with good comparison with experimental results shows broad applicability of our method.

5 Conclusions: Implications for cancer research and drug design

Our multiscale framework allows us to identify the underlying mechanism for cell migration in a variety of extracellular environments. In addition, we are able to predict the key factors governing migration in these changing environments. While the results of our model are primarily qualitative at this stage, good comparison with experimental results suggest our ability to capture the complexity of cancer metastasis. Our results show that for each stage of tumor progression and metastasis, an entirely different set of factors would determine the ability or inability of a cell to migrate. For example, while MMP activity is one of the key aspects of enhanced migration, blocking MMPs may not be sufficient in stopping all migration through the basement membrane. Similarly, cell matrix adhesions and integrin expression may be the limiting factor in the connective tissue environment, but it does not affect cell motility in the parent tumor and epithelial tissue. Among the novel findings of our simulations is the role of matrix and cell flexibility that allows for cells to control and overcome steric factors, and hence regulate motility. This area has been largely overlooked by pharmaceutical industry, and may present a very fertile ground for drug development. At the systems biology level, our method is the first of its kind to map the complex landscape of metastasis, and accounts for integration and interaction of multiple key biochemical, mechanical and biophysical factors. While we are able to predict several novel aspects of tumor metastasis using our scalable approach, the real test of our theoretical results lies in experimental studies that simultaneously probe the mechanical, biophysical and biochemical aspects of tumor cell migration and metastasis. We hope that advancements in high resolution imaging, rheological measurements and genetic perturbation of tumor cells will rigorously test our predictions. Such experiments, along with modeling strategies similar to the one discussed in this paper will contribute significantly towards a thorough understanding of cancer progression and will lead to design and development of more effective therapeutics.

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