Tumor proliferation and diffusion on percolation clusters

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Abstract We study in silico the influence of host tissue inhomogeneity on tumor cell proliferation and diffusion by simulating the mobility of a tumor on percolation clusters with different homogeneities of surrounding tissues. The proliferation and diffusion of a tumor in an inhomogeneous tissue could be characterized in the framework of the percolation theory, which displays similar thresholds (0.54, 0.44, and 0.37, respectively) for tumor proliferation and diffusion in three kinds of lattices with 4, 6, and 8 connecting near neighbors. Our study reveals the existence of a critical transition concerning the survival and diffusion of tumor cells with leaping metastatic diffusion movement in the host tissues. Tumor cells usually flow in the direction of greater pressure variation during their diffusing and infiltrating to a further location in the host tissue. Some *specific sites* suitable for tumor invasion were observed on the percolation cluster and around these *specific sites* a tumor can develop into scattered tumors linked by some advantage tunnels that facilitate tumor invasion. We also investigate the manner that tissue inhomogeneity surrounding a tumor may influence the velocity of tumor diffusion and invasion. Our simulation suggested that invasion of a tumor is controlled by the homogeneity of the tumor microenvironment, which is basically consistent with the experimental report by Riching et al. as well as our clinical observation of medical imaging. Both simulation and clinical observation proved that tumor diffusion and invasion into the surrounding host tissue is positively correlated with the homogeneity of the tissue.

Chongming Jiang and Chunyan Cui contributed equally to this work.

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1 Introduction

Cancer invasion is a cell- and tissue-driven process that includes physical, cellular, and molecular determinants to adapt and react throughout its progression [1]. More than 80% of clinical tumor patients die from tumor invasion and metastasis [2]. Tumor metastasis is a multistage process in which malignant cells spread from the original tumor to colonize distant organs [3–5]. A very important feature of tumor metastases is the ability of different tumor types to metastasize frequently to a similar range of organ sites [6–8] and different types of tumors have different ranges of target tissues. For example, the metastasis of prostate cancer is largely confined to bone [9] while that of ocular melanoma is almost exclusively limited to the liver [3]. Adenocarcinomas of the breast and lung typically relapse within the bone, lung, liver, and brain [8].

It is of both theoretical and clinical significance to evaluate the ability of disseminated cancer cells (DTCs) to invade and colonize a host organ. What enables these cells to survive as latent infiltrates and to diffuse into these target organs? Some researchers believe that some genes, microRNAs, and proteins support tumor metastasis to particular organs [10–12]. Ewald and colleagues found that tumor cells including cytokeratin 14 had greater invasive ability [13]. Guo and colleagues found that Exo70 affected cell structure and movement by bending the cell membrane, which then impacted the cell migration and invasion of a tumor [14]. However, these studies did not incorporate the effects of the surrounding host tissue. Otey et al. confirmed the existence of a new protein in pancreatic surrounding cells (named palladin) that promoted the cancer-associated fibroblast assembly of invasive pseudopodia to break down the barriers between cells. A strong correlation exists between the invasiveness of tumor progression and the expression of palladin [15]. The common target organs invaded, such as the lung and liver, are highly vascular. Tumor cells are more likely to diffuse along the vascular distribution. Cao and colleagues found that the interaction between the vascular endothelial growth factor and the tumor necrosis factor in the microenvironment could affect tissue angiogenesis and promote tumor diffusion and metastasis in mice [16]. Ruemann et al. found that tumor cells secreted angiotensin receptor 1, which could improve the proliferation of tumor vessels and promote tumor movement and then facilitated tumor metastasis and invasion [17]. The process that tumor cells can successfully transfer to an organ and form metastases is divided into two stages [8]; (1) the cancer cells are transferred to and captured by the target organ and (2) the cells adapt to the target tissue microenvironment and gain the ability to form colonies. The second stage plays a crucial role in successful tumor metastasis. A growing number of researchers believe that oncogenic transformation is not sufficient for metastatic competence, because so many oncogene-driven mouse models of cancer do not automatically establish distant metastases [8]. Repasky and colleagues found that thermoneutral conditions reduced tumor formation and metastasis [18]. Transformed cells must therefore acquire additional abilities to overcome natural barriers and survive in order to metastasize [19, 20].

Different dynamics of tumor metastasis relate to the questions above. Ewing et al. proposed a purely mechanical model of tumor metastases, namely the ‘mechanical-circulatory’ hypothesis [21]. They believed that the anatomy of the vascular system determined tumor metastasis and that the differences in the organ anatomy of a vascular system made it easier for tumors to metastasize to certain organs. Although brain capillaries are more difficult to penetrate because of the unique nature of the hematoencephalic barrier, the brain is still one of the main target organs of tumor metastases. Paget proposed the ‘seed and soil’ hypothesis that tumor metastasis is the result of the diffusion and growth of specific tumor cells (seeds) in a suitable environment (the soil) [22]. Many kinds of tumor cells spread randomly in the circulatory system but only those that fall into the appropriate organs can survive and form both micrometastases and macrometastases. Hoshino et al.’s latest research proved that the ‘soil’ acts as an important role as the ‘seed’ during a tumor metastasis [23]. The tissue microenvironment, which includes the organ structure and function, the local matrix, and the immunological properties, strongly affects the colonization and growth of tumor cells. The transfer characteristic of human lung adenocarcinoma cells is insignificant when they are transplanted into the subcutaneous tissue of nude mice but very significant when the cells are transplanted into their abdominal muscles [24]. Furthermore, it is difficult to observe the epithelial–mesenchymal transition [24–31] in some organs, for example, the heart. Even when disseminated tumor cells (DTCs) metastasize to those organs, the cells will fail to proliferate, due to an inability to reproduce or the limitations of the environment, such as the inhomogeneity of the surrounding tissues or the presence of transforming growth factor β . Therefore, not all of the DTCs can eventually infiltrate an organ, depending on the host tissue microenvironment, especially its inhomogeneous surrounding matrix distribution. A suitable tissue microenvironment serves as a vital ‘soil’ for tumor growth and invasion. Pattabiraman and Weinberg found that mesenchymal stem cells and their derived cell types created a tissue microenvironment that was conducive to the survival of cancer stem cells through the release of prostaglandin E2 and cytokines [32]. Wei et al. recently reported in *Nature Cell Biology* that the stiffness and rigidity of the surrounding matrix can regulate tumor cellular behavior and correlate to tumor survival [33]. Riching et al. observed that there exist advantage tracks in the matrix, which facilitate tumor invasion and cells migrating out of the tumor; they found that the matrix topography, in addition to stiffness, is the dominant feature by which an aligned matrix can enhance invasion [34]. However, the role of the inhomogeneity of the tissue microenvironment in selection of suitable targets for the infiltration process is still not well known.

Cancer can be regarded as the result of dysregulation in the context of the macro- and micro-environment. It can also be viewed and explored as a complex adaptive system [1, 35, 36]. Another important variable for tumor metastasis is the spatiotemporal characteristic, because a tumor’s metastasis and diffusion velocities are significantly varied for different tissues and progression stages. Young studied the invasive growth of malignant tumors based on elastic-jelly models and found that pressure may play an important role in tumor invasion [37]. Mechanical stress, as suggested by Montel et al. [38] and Guo and Levine [39], also has significant influence on tumor progression. Huang et al. found that endothelial cells exert mechanical stress on a tumor; endothelial cells were seeded on reduced adherent surfaces to increase their cytoskeletal tension [40]. Delarue et al. found that the surrounding matrix behaves as a compressive stress and causes a reduction of the volume of multicellular tumor spheroids (MTS), leading to a reduction of the cell volume in the core of MTS; the surrounding tissue can be viewed as a restraint environment for tumor progression [41, 42]. Cheng et al. [43], Desmaison et al. [44], and Deisboeck et al. [45] also observed that the surrounding extracellular matrix

(ECM) possesses a specific external pressure as well as tumors' interstitial pressure on a solid tumor, affecting considerably the migration of tumor cells. Hughes et al. posited that the migration of tumor cells can be simplified into two types of mobility, random movement, and directional taxis [46]. This mobility underlies the current study, in which the spatiotemporal characteristic of tumor migration within a surrounding tissue can be explored in a percolating cluster [47, 48].

The percolation theory describes the permeability of a substance diffusing at random through porous materials and it is also the simplest model to display a state transition phenomenon [47, 49]. Theoretical knowledge of percolation is of immense importance in such diverse fields as biology and physics [50]. Lee et al. remodeled a tumor growth in a dynamical evolving blood vessel network by adapting the percolation method; but their study did not include any inhomogeneous surrounding matrix [48]. Amyot et al. used percolation to depict tumor vascularization while taking into account the inhomogeneous nature of ECM [51]. Percolation has also been used to represent the vascular network of tumors and normal tissues. Baish et al. [52] and Welter and Rieger [53] found that when percolation was incorporated, the delivery of blood-borne molecules and nanoparticles in the tumor and tissue were dependent on the differences in their vascular architectures. However, they did not compare the differences in the homogeneity of the tissues surrounding a tumor. Wendykier et al. [54], Lipowski et al. [55], Ferreira et al. [56], and Moglia et al. [57] used percolation to examine the critical behavior of avascular tumor growth, in which the survival and reproduction of tumor cells depended on the supplied nutrients. However, these studies did not systematically investigate the influence of the inhomogeneity of the surrounding tissue on tumor proliferation and diffusion. It is necessary to adopt percolation to investigate the influence of the inhomogeneity of different tissues surrounding tumors on their proliferation and diffusion during the process of tumor migration.

In this study, we employed the Monte Carlo method to produce a percolation cluster to emulate the inhomogeneous tissue microenvironment. A partial differential equation system was used to describe the spatiotemporal proliferation and diffusion of a tumor on the percolation cluster. We concentrated on the effects of tissue inhomogeneity on the velocity of tumor proliferation and invasion. Some clinical images were also included for comparison with the simulation. The remainder of the paper is organized as follows. In Section 2, we describe the mathematical model and percolation clusters. The typical simulation and clinical results are presented in Section 3. The discussion and concluding remarks are given in Section 4. The paper also incorporates some supplemental materials, including some simulation results and original clinical images used for quantitative data processing.

2 Description of the model

2.1 Mathematical model

Based on a mathematical model of the proliferation and diffusion of tumor cells [58–60], we assumed that the 'inhomogeneity' of surrounding tissues represents the degree or status of the surrounding matrix (or tissue cells) distribution. A highly inhomogeneous surrounding tissue indicates that the distribution of the surrounding matrix or tissue cells is of significant

inhomogeneity in the microenvironment. We addressed the change in tumor cell density (denoted by n) that quantifies the number of tumor cells per unit volume.

The conservation equation for tumor cell density n is:

$$\frac{\partial n}{\partial t} + \nabla \cdot J = S_n, \quad (1)$$

where J is the flux of tumor cells and S_n is a quantity relevant to both the proliferation and death of tumor cells. We mainly focused on tumor proliferation and diffusion in the surrounding tissues. The flux J is expressed in the form:

$$J = -D\nabla n, \quad (2)$$

where D is the diffusive coefficient of tumor cells [61, 62]. The term S_n is related to the proliferation and death of tumor cells and is formulated by the logistic growth function [60, 62–65]:

$$S_n = \lambda n \left(1 - \frac{n}{K} \right), \quad (3)$$

where λ is the growth rate of the tumor cell and K is the carrying capacity of the environment [62, 66].

2.2 Model with nondimensionalization and parameterization

We performed nondimensionalization on these Eqs. (1, 2, and 3) by rescaling the length with an appropriate scale L (the maximum invasion distance of cancer cells), time with τ (the average time of cell mitosis) and the tumor cell density with n_{ref} (the maximum number of cells per unit area). We reduced the quantities in the partial differential equation (Eq. 1) with the relevant reference variables and obtained new dimensionless parameters:

$$\tilde{n} = \frac{n}{n_{ref}}, \quad \tilde{x}' = \frac{x'}{L}, \quad \tilde{y}' = \frac{y'}{L}, \quad \tilde{t} = \frac{t}{\tau}, \quad (4)$$

$$\text{and } d = \frac{\tau \cdot D}{L^2}, \quad \tilde{\lambda} = \lambda, \quad \tilde{K} = \frac{K}{n_{ref}}.$$

Dropping the tildes for notational convenience, we obtained the dimensionless differential equation:

$$\frac{\partial n}{\partial t} = d \nabla^2 n + \lambda n \left(1 - \frac{n}{K} \right). \quad (5)$$

We defined the vertical direction on the left as the starting line of a tumor invasion. The periodic boundary conditions were used along the vertical direction, whereas open boundaries were used for the horizontal direction, which represents the direction of tumor invasion. Our approach is similar to the scenario when Bindsschadler and McGrath [67] and Khain et al. [68] used parallel strips surrounded by cell-cultured strips made with microfabricated stencils for observation of collective cell migration. For the sake of simplification, we assumed that an initial tumor seed is located at

$x' = 0$ and $y' = 0.5$ and spreads isotropically. A Gaussian distribution was used to depict the initial tumor seed in the host tissue:

$$\begin{cases} n(x', y', t)|_{t=0} = \exp\left(-\frac{(y'-0.5)^2}{\varepsilon}\right), & x' = 0, \quad y' \in [0, 1], \\ n(x', y', t)|_{t=0} = 0, & x' \in (0, 1], \quad y' \in [0, 1]. \end{cases} \quad (6)$$

where ε is the standard deviation of n .

2.3 Percolation clusters

We used the Monte Carlo method and the percolation theory to produce a discrete two-dimensional percolation cluster given by a square lattice of sides $X \times Y$ to represent the inhomogeneous tissue microenvironment [55, 57]. Figure 1a is a typical tissue biopsy of tumor diffusion and invasion in a surrounding tissue and it was used to illustrate how to construct a percolation counterpart imitating tumor cells movement in surrounding tissue, as shown in Fig. 1b. To describe the percolation cluster conveniently, we define each site of the lattice with coordinates (x, y) that could be empty or occupied by cells. The relationships of the coordinate (x, y) and the realistic location (x', y') of a site are:

$$x = x' \cdot X, \quad y = y' \cdot Y, \quad (7)$$

The value of a grid point in the percolation cluster $A(x, y)$ was set randomly as either *zero* or *one* and it impacts the tumor's growth and diffusion. The value *zero* indicates that the grid point is a blocked site or an obstacle and that tumor cells cannot occupy or pass through it. The

Fig. 1 Schematic diagram of tumor proliferation and diffusion into surrounding tissue. Physiological diffusion and invasion in a tumor (*left-hand side*) and in a percolation cluster (*right-hand side*). The tumor cells diffusion is affected by the inhomogeneity of surrounding tissue and shows an anisotropic distribution. To investigate the influence of the inhomogeneity of the surrounding tissue, the Monte Carlo method and the percolation theory were used to produce a discrete two-dimensional lattice given by a square lattice with sides $X \times Y$, which represented the inhomogeneous surrounding tissue. The tumor cell could pass through the open bonds (*bold lines*) and occupy these open sites (*solid points*) but was hindered by the blocked bonds and sites. More details are given in the text

value *one* indicates that the grid points are open sites available occupied by tumor cells and that the grid points can communicate with each other. In this difference-equation algorithm, tumor proliferation and diffusion are closely linked to or, more precisely, depend upon not only the theoretical model PDE (Eq. 5) but also the site occupation probability of the percolation lattice. In our finite difference implementation, we stipulate that tumor cells, due to the diffusion term, cannot survive at the block site due to $A(x, y) = 0$; more detailed descriptions are given in the supplemental material (File S1). A bond (connectivity) comes into being between two open sites but breaks between blocked sites. The proportion of overall open sites in the lattice was defined as the site occupation probability of the percolation cluster P . The different occupation probabilities P of the percolation cluster signify the different homogeneity degrees of the surrounding matrix distribution in this simulation. The cluster is very homogeneous if P is close to 1.0. The cluster is completely blocked if P approaches 0. Biologically, P can reflect the compressive stress of the surrounding matrix to the tumor; P may also relate to the surrounding matrix stiffness or the adhesion between tumor cells and the surrounding matrix [33]. A percolation cluster with different site occupation probabilities emulates a sort of inhomogeneous environment of the tissues surrounding a tumor, similar to the ‘seed and soil’ concept described by Paget [22].

The finite difference method was employed to quantify the proliferation and diffusion behavior of a tumor in inhomogeneous surrounding tissue. The selection of parameters satisfied the stability condition and the accuracy was set as $O(\tau + h^2)$. The parameters selected for reduction and used in the subsequent simulations are specified in Table 1. The peak of the tumor cell density is the maximum value of tumor cell density (n); the peak location of tumor cell density is the spatial location of the maximum tumor cell density on a percolation cluster. The time evolution of the peak location of tumor cell density was computed to decipher the velocity of tumor proliferation and invasion based on the general diffusion theory [62, 69]. The root-mean squared displacement of Brownian motion particles was used to illustrate the random motion of tumor cells over time. Tumor cell density (n) varies spatiotemporally with time (t) and spatial length (x, y) and the peak location of the n curve, signifying a sort of diffusion front similar to the spatial root-mean squared displacement, was recorded to quantify the diffusion process. The peak location of tumor cell density was calculated as $r_m = (x_m^2 + y_m^2)^{1/2}$, where x_m and y_m are the corresponding spatial length (x, y) at the peak location. The algorithm for the discretized theoretical model (Eq. 5) and its implementation using the Monte Carlo method and the percolation theory to simulate the inhomogeneous microenvironment are described in the supplemental material (File S1).

Table 1 Reference variables and parameters used in this study

Description		Dimensional value	Refs
L	Length	1 cm	[62, 74, 75]
τ	Time	8 to 24 h (16 h)	[62, 74, 75]
n_{ref}	Reference tumor cell density	6.7×10^7 cells.cm ⁻³	[58, 62]
D	Diffusion coefficient	1×10^{-11} cm ² /s	[62, 76]
λ	Tumor growth rate	$10/\tau$	[62, 63]
K	Carrying capacity of the environment	$10.n_{ref}$	[62, 63]
ε	Positive parameter	0.00025	[62, 77]

The pathological and clinical images of tumors were acquired from ordinary medical examinations of patients at the Sun Yat-sen University Cancer Center. These examinations were carried out for therapy only and no additional drugs or measures were administrated. Every effort was made to maximize the protection of the patients' privacy; the patient records and information were anonymized and de-identified prior to analysis.

3 Results

3.1 Simulation results

3.1.1 Influence of tissue inhomogeneity on tumor proliferation and diffusion

We analyzed the influence of tissue inhomogeneity on both tumor proliferation, quantified by the population density of tumor cells n and diffusion, demonstrated by an actively expanding contour of tumor cell density, through adjusting site-occupation probability P . Some typical results are shown in Figs. 2a–d. As P decreased, tumor evolution was inhibited and the active contour of the tumor cells shrunk. In this study, the tumor cells were able to survive and proliferate from a small tumor into a widespread one when P was greater than the threshold of

Fig. 2 The inhomogeneity of the percolation cluster affects tumor proliferation and diffusion. Figs a–d show the tumor proliferation and diffusion at different site-occupation probabilities P . (a) $p = 1.0$. (b) $p = 0.75$. (c) $p = 0.54$. (d) $p < 0.54$. As P decreased, tumor evolution was inhibited and the active contour of the tumor cells shrunk. In this study, the tumor cells were able to survive and proliferate from a small tumor into a widespread one when P was greater than the threshold of 0.54. When the occupation probability P was lower than the threshold, the tumor cells in the region died. Simulation parameters: $X*Y = 50*50$, $t = 100$. The remaining parameters were fixed

0.54. When the occupation probability P was lower than the threshold, the tumor cells in the region died. The DTCs fail to proliferate and diffuse with this kind of tumor homogeneity, as shown in Figs. 2a–d. The characteristic proliferation and diffusion of a tumor on the percolation cluster were analogous to what Nguyen et al. [8] and Paget [22] claimed in that both the components and microenvironment of each organ have specific homogeneity for the infiltration of tumor cells. Tumor metastasis to different organs becomes difficult because of the inhomogeneity of the surrounding tissues. Therefore, we speculated that a percolation cluster with a different occupation probability should be suitable for describing the proliferation and spread of a tumor in an inhomogeneous surrounding matrix, which itself should be compressive to tumor proliferation and invasion. The results are basically in accordance with previous studies that showed that the surrounding matrix possesses a specific external pressure on a solid tumor [42–44, 70].

3.1.2 Comparison of several lattice constructors

To confirm that a threshold for the proliferation and spread of tumor cells on a percolation cluster really exists and to compare the thresholds for different kinds of lattices, we constructed three kinds of lattices with 4, 6 and 8 linked near neighbors (LNN). The variable α was defined as the probability that tumor cells could pass through the percolation cluster, which was defined as $\alpha = M/N \times 100\%$, where N is the total number of percolation clusters for each occupation probability P , which was set to 10 000 in this study and M is the statistical number of tumor cells that could pass through and reach the right edge of the lattices after 10 000 repetitions. We assumed that if the tumor cells could reach the right edge, the cells could escape from one tissue and then invade another.

The relationship between α and P depicts the influence of tissue inhomogeneity on tumor proliferation and diffusion. An obvious critical transition relating to tumor survival and diffusion is observed in Figs. 3 and 4. A turning point appeared in the curve of α versus P with a change in the lattice site-occupation probability P , namely tissue homogeneity. Few tumor cells could pass through and reach the right edge of the lattice when P was less than 0.54. This finding can be interpreted to mean that tumor cells are hampered by the sparse distribution of the surrounding matrix on an inhomogeneous percolation cluster and their further diffusion and migration are scarce when the occupation probability P is sufficiently low. The results are in accordance with research by Cheng et al. [43] and Desmaison et al. [44], showing that inhomogeneity in the mechanical properties of a confining environment could affect tumor cells distribution and morphology by inducing apoptosis.

The turning point of the curve of α versus P was independent of the passage of time when the duration was sufficient. The turning point was stabilized at a p value of 0.54 as the evolution time t exceeded 100, as shown in the inset of Fig. 3. The turning point at a p value of 0.54 corresponds to the threshold of tumor cells penetrating the percolating cluster. Theoretically, as for a simple square lattice with four nearest neighbors ($LNN=4$), its threshold for percolation is a stable value (0.5927) when a particle can move at random completely within the lattice [49]. For a complex system in relation to proliferation and diffusion, however, the percolation threshold depends definitely upon the lattice size/shape as well as specific partial differential equations that describe the growth and spreading of tumor cells with an effective interaction between the cells.

The critical transition relating to tumor survival and diffusion was also observed in the different lattices with 4-neighbor, 6-neighbor and 8-neighbor lattice percolations ($LNN=4, 6$

Fig. 3 The dependence of probability α on site-occupation probability P . The inset is the relationship of P and α over time. α is the probability that tumor cells can pass through the cluster and is defined as $\alpha = M/N \cdot 100\%$, where N is the total number of percolation clusters for each occupation probability P , N was set to 10 000 and M is the statistical number of tumor cells that can reach the right edge of the cluster for 10 000 repetitions. For each value of α , the statistical errors were estimated from 10 independent simulation results (error bars, SD). Simulation parameters: $X \cdot Y = 50 \cdot 50$. The remaining parameters were fixed

and 8). The curves of α versus P for various LNN values are very similar in trend but their turning points differ in that a larger LNN corresponds to a lower threshold value, as shown in Fig. 4. A lattice with a larger LNN generally has better symmetry and directionality available

Fig. 4 The relationship between P and α in three kinds of lattice constructors. Turning points were observed in the 4-neighbor, 6-neighbor and 8-neighbor lattice percolations ($LNN=4, 6$, and 8) and are marked with arrows. The curves of α versus P are similar in trend but the locations of the turning points, i.e., the thresholds, shift from 0.54 to 0.44 and 0.37, respectively, with increasing LNN . Simulation parameters: $X \cdot Y = 50 \cdot 50$, $t = 100$. The remaining parameters were fixed

for cell mobility. In this simulation, this situation was equivalent to better tissue homogeneity surrounding a tumor. Therefore, a site with a greater number of near-linked neighbors implies that tumor cells have more opportunities to reach another site with a smaller threshold.

3.1.3 Characteristic diffusion pattern in tumor-leaping metastatic diffusion

The continuous proliferation of tumor cells increases the pressure inside a tumor. Tumor cells proliferate and invade toward the direction of low pressure [37]. There are two possible modes of a tumor infiltrating host tissue. In the first mode, the center of a tumor is fixed and tumor cells detach from the maternal tumor and diffuse randomly into the surrounding tissues. In the second mode, the tumor's center moves as the tumor cells detach from the maternal tumor and diffuse into the surrounding tissues.

To investigate tumor invasion behavior on percolation clusters, the peak location of the tumor density contour, as displayed in Fig. 5a, was recorded to identify the diffusion process. Figure 5b shows the evolution of the tumor peak location with an emerging and leaping characteristic over time t . Take the peak p_9 for example. It appears at $t=71$ and remains unchanged until $t=210$, which we considered to be a quasi-steady state for the peak p_9 . Another new peak p_{10} emerges at $t=211$ as evolved from the previous peak p_9 . The peak location then keeps unchanged until $t=824$, indicating that the tumor has entered a new quasi-steady-state period from $t=211$ to $t=823$. The original peaks disappear gradually while a new peak emerges over time at a new site on the percolation cluster. Double peaks clearly develop during a tumor spreading. The phenomenon of double peaks was observed with certain spatial tissue homogeneities independent of the random distribution of spatial open sites, as shown in Fig. 6a–d. More detailed results are given in the supplemental material (File S2). A weak link or tunnel connecting the new peak to its original counterpart was found on the percolation cluster, as demonstrated in Fig. 7b. The simulated tumor metastasis can be ascribed to tumor cells simply passing along the link between the two peaks. The tumor cells inside the original area were driven to the new area by the mechanical stress of the surrounding matrix. An

Fig. 5 The peak location of tumor diffusion at different times. **a** The schematic peak location. **b** The specific location of the tumor cell peak emerging on the percolation cluster at different times at $p=0.90$. The time to monitor the peak is listed in brackets, i.e., from $t=71$ to $t=210$ in the form $p_9, 71 \leq t \leq 210$. The dashed line represents a link between two peaks. A new peak appears at $t=71$ and remains unchanged until $t=210$, which we consider to be the quasi-steady state for the peak. Another new peak p_{10} emerges at $t=211$ as an increase from the previous peak p_9 . The peak location then remains unchanged until $t=824$, indicating that the tumor entered a new quasi-steady-state period from $t=211$ to $t=823$. Simulation parameters: $X \times Y = 50 \times 50$. The remaining parameters were fixed

Fig. 6 Snapshots of the tumor cell density contour over time on the percolation cluster. The peak location of the tumor density contour exhibits a leaping metastatic diffusion characteristic with the evolution of time. The evolution of tumor diffusion at time (a) $t = 1$, (b) $t = 100$, (c) $t = 211$, (d) $t = 500$, (e) $t = 750$ and (f) $t = 1000$. A new peak appears and moves away from the original peak area at $t = 211$ and remains unchanged between $t = 211$ and $t = 750$. Another new peak emerges at $t = 750$ and increases over time, such as $t = 1000$. The original peak disappears as the new peak appears over time. Double peaks clearly develop. Simulation parameters: $X \times Y = 50 \times 50$, $p = 0.90$. The remaining parameters were fixed

obvious directionality was observed during the migration of tumor cells. The tumor cells constantly moved away from the initial center. The new peak location r_m of the tumor cell density always varied in a stepwise manner, as shown in Fig. 7a–b.

The relationship between the peak location r_m and time t was employed to quantify the double peak during a tumor invasion. The peak location is unchanged during the invasion and then alters stepwise with time, as shown in Fig. 7a. Note that the new tumor peak is never a result of the continuous movement of the original peak. Instead, the new peak emerges from a specific site on the percolation cluster and a link is created that connects the new peak with its

Fig. 7 The time evolution of the peak location with a link between the new and original peaks. **a** The stepwise evolution of the peak location at $p = 0.90$. **b** The tumor distribution was determined with a meticulous division at $t = 750$, revealing a link between the new and old peaks of the tumor density contour. The link was similar to the dashed line connecting the two peaks in Fig. 5b. Simulation parameters: $X*Y = 50*50$. The remaining parameters were fixed

original counterpart and provides a tunnel or pathway for tumor cells invasion. During the process of a tumor cell detaching from the maternal tumor and diffusing into the surrounding tissues, the locations of the tumor center exhibit leaping metastatic diffusion movement. A similar phenomenon was also observed in the clinical results of some types of tumor metastases (Fig. 9a–c and File S3). More detailed descriptions are given in the following clinical results section.

3.1.4 Inhomogeneity of the tissues surrounding a tumor affects the velocity of tumor diffusion and invasion

The time evolution of the tumor peak location was computed to investigate the influence of surrounding tissue inhomogeneity on the velocity of tumor diffusion and invasion by changing the occupation probability P . To quantify the velocity of tumor diffusion simply and directly, the slope k of the linear-fitting function between r_m and t was calculated using the least-squares method. A typical graph is shown in Fig. 8a. A larger slope k corresponds to a faster tumor diffusion process. In this simulation, the velocity of tumor diffusion and invasion was positively correlated with the occupation probability P of the percolation cluster, as shown in Fig. 8b. Tumor diffusion and invasion slow down with the reduction of the occupation probability P , indicating that the inhomogeneous surrounding matrix can inhibit tumor diffusion and invasion. Similar results were obtained in the clinical data analysis and they are presented in the Clinical Results section. The simulation results concur with those by Jiang et al. [62] and Friedman and Kim [71] in the studies for one-dimensional inhomogeneous matrix distribution, which make clear that a more homogenous matrix distribution can accelerate tumor proliferation and invasion.

3.2 Clinical results

In silico images of tumor proliferation and invasion on the percolation cluster at different times provided a heuristic approach for assessing physiological tumor invasion that could be quantified through image analysis. The preliminary clinical imaging results of some cancer

Fig. 8 Slope k is positively correlated to the site-occupation probability P . **a** The peak location r_m versus evolution time t at the occupation probability $p = 0.85$. The simulated data points were linearly fitted using the least squares method. **b** The measured value of slope k for different occupation probabilities P . The process of tumor diffusion and invasion slows down with a reduction in the occupation probability P , indicating that inhomogeneous surrounding tissue can inhibit tumor diffusion and invasion. For each value of slope k , statistical errors were estimated from seven independent simulation results (error bars, SD). Simulation parameters: $X^*Y = 50 \times 50$, $t = 100$. The remaining parameters were fixed

patients were employed to verify the results of the simulation. The maximal optical density of the clinical images, analogous to the tumor cell density *in silico*, was used to characterize the peak of the tumor cell density. Figure 9a–d displays a typical growing liver metastasis with a characteristic of leaping metastatic diffusion. The tumor cells diffuse and infiltrate from the original location to a new location. Three new peak areas of a tumor appear and grow over time. Their optical densities are even greater than that of the original. The location of the maximum optical density of the tumor was observed to move from the original location at $t = 1$ day to the third peak area at $t = 45$ day and then to the fourth peak area at $t = 72$ day. The emergence of the peak location of a tumor over time is in accord with the simulation, as shown in Figs. 5b and 6e–f. Tumor cells are also distributed among the peak areas via an obvious link to both the second and third peak areas, as indicated by the double arrow in Fig. 9b–c. Like the simulation, the link connects the new and old peaks of the tumor density contour, as shown in Fig. 7b. Similar clinical results have been observed in some other types of tumor metastases. Included in the supplemental material (File S3) are the clinical images data of three other typical cancer patients who had been clinically diagnosed with brain metastases of lung adenocarcinoma (patient A), the liver metastases of stomach cancer (patient B) or the liver metastases of liver cancer (patient C); all of these processes have the characteristics of leaping metastatic diffusion.

To investigate the effects of the inhomogeneity of surrounding host tissues on tumor diffusion and invasion, we also measured, in addition to the location of the tumor mentioned above, the size of an identical tumor metastasizing in different organs by recording the time of the clinical observation from the clinical medical images. Two typical cancer patients (Patients D and E) were selected for evaluation and each of them who had been diagnosed suffered with bone, lung, liver and brain metastases simultaneously. Patient D received a clinical diagnosis of breast cancer and patient E of lung adenocarcinoma. Their clinical image data are shown in the supplemental material (File S4). All of these medical images were processed using the commercial image analytical software Image Pro Plus (IPP) for biology and medicine to extract precisely the outlines of the tumors and to segment surrounding tissues from the tumor and then the inhomogeneity value of the surrounding tissues could be obtained by using IPP

Fig. 9 Diffusion of growing liver metastasis with the characteristic of leaping metastatic diffusion. **a** 1st day. **b** 45th day. **c** 72nd day. The red cross is the anchor point for image measurement and the white arrow in **a** indicates the original location of the tumor. The insets within **(a) ~ (c)** display the measured optical density of the tumor peaks versus the peak location r_m . **(d)** The location of the maximum optical density in the liver at different clinical observation times. The statistical errors of these values were estimated from three independent measurements (error bars, SD). The maximum optical density was used to characterize the peak of the tumor cell density. The tumor diffused and infiltrated from the original location to a new location. Three new peak areas appeared and grew over time. Their optical densities were even greater than that of the original. The maximum optical density moved from the original location to the third peak area at $t = 45$ th day and then to the fourth peak area at $t = 72$ nd day. Tumor cells were also distributed among these peak areas via an obvious link to both the second and third peak areas, as indicated by the double arrow in **(b)** and **(c)**. As in our simulation, the link connected the new and old peaks of the tumor density contour, as shown in Fig. 7b

through working out the image grayscale distribution of the surrounding tissues and then calculating the fraction of the pixels that deviate more than a certain range (10% default) from the average intensity. A detailed introduction of the method that was used to quantify the inhomogeneity of the surrounding tissues in the clinical images can be found within the guidelines of Image Pro Plus (<http://www.mediacy.com/imageproplus>). The clinical data of the two patients were evaluated and presented in Figs. 10 and 11. The average radiuses of the metastases over time are plotted in Figs. 10a and 11a, respectively. The average radius was fitted linearly with the observation time and the slope k was used to appraise the diffusivity of

Fig. 10 Differential diffusivity of metastasized breast cancer in bone, lung, liver and brain. **a** The radius of the tumor versus the time of clinical observation (t). **b** The inhomogeneity of the surrounding tissues versus the time of clinical observation (t). The dotted lines in Fig (b) mark the averaged inhomogeneity and the initial inhomogeneity of the surrounding tissues are also enumerated above the first histogram, respectively. The statistical errors of these values were estimated from three independent measurements (error bars, SD). The patient received a clinical diagnosis of breast cancer and suffered with bone, lung, liver and brain metastases. The metastases of the breast tumor diffused faster in bone and lung than in liver and brain. The initial and average inhomogeneity of the surrounding tissue microenvironments are in good agreement with the order of diffusion velocities in breast metastases of bone, lung, liver and brain

tumor cells. A larger slope means that the proliferation and invasion of a tumor proceed more easily. The inhomogeneity of the different surrounding tissues over time is also given in

Fig. 11 Differential diffusivity of metastasized lung adenocarcinoma in bone, left lung, liver and brain. **a** The radius of the tumor versus the time of clinical observation (t). **b** The inhomogeneity of the surrounding tissue versus the time of clinical observation (t). The dotted lines in Fig (b) mark the averaged inhomogeneity and the initial inhomogeneity of the surrounding tissues are also enumerated above the first histogram, respectively. The statistical errors of these values were estimated from three independent measurements (error bars, SD). The patient received a clinical diagnosis of lung adenocarcinoma and suffered with bone, left lung, liver and brain metastases. The metastases of the lung adenocarcinoma diffused faster in bone and lung than in liver and brain. The initial and average inhomogeneity of the surrounding tissue microenvironments are in good agreement with the order of diffusion velocities in lung adenocarcinoma metastases of bone, lung, liver and brain

Figs. 10b and 11b. The statistical errors of these values were estimated from three independent measurements (error bars, standard deviation (SD)).

Judged by their slopes, the metastases of a breast tumor occur with more difficulty in an ascending sequence; bone, lung, liver and brain, respectively (Fig. 10a). Noticeably, the sequence fits well with the order of the initial and average inhomogeneity of the surrounding tissues; the metastases of the breast tumor diffused faster in bone and lung than in liver and brain because liver and brain have higher inhomogeneity than bone and lung (Fig. 10b), indicating that a more homogenous matrix distribution can promote tumor proliferation and invasion. The metastases of the lung adenocarcinoma also proceed faster in bone and lung than in liver and brain (Fig. 11a), which basically coincide with the order of the initial and average inhomogeneity of the host tissues microenvironment. Generally, the bone and lung metastases of lung adenocarcinoma have low initial and average inhomogeneity and the liver and brain metastases of lung adenocarcinoma have a higher initial and average inhomogeneity (Fig. 11b). This topic is addressed in detail in the following discussion.

4 Discussion and conclusions

We combined mathematical modeling and clinical validation approaches to reveal the influence of the inhomogeneity of surrounding tissue on tumor invasion and attempted to understand why some kinds of metastasized tumors often invade certain tissues or organs from the viewpoint of diffusivity on a percolation cluster.

In this study, the inhomogeneity of a tissue has an obvious effect on tumor proliferation and diffusion. A critical transition relating to tumor survival and diffusion occurs when the homogeneity of the host tissue is near to a threshold; tumor cells stop proliferating and diffusing in this type of host tissue (Figs. 2a–d, 3 and 4). Not all DTCs can grow eventually as macrometastases and a very inhomogeneous tissue environment will hinder the proliferation and diffusion of tumor cells. During the process of tumor metastasis, DTCs either lack the ability to colonize or are prevented from forming new metastases by the inhomogeneous host tissue environment. Tumor cells tend to invade and colonize those organ sites more evenly. Bone, lung, liver and brain tissue are dense and very rich in the surrounding matrix, which contributes to the proliferation and development of DTCs into macrometastases. Some organs have relatively poor homogeneity and almost no epithelial cells; DTCs do not settle easily in these organs and tumor cells fail to proliferate and grow as macrometastases.

Biologically, the diversity of tumor cell proliferation and migration into different tissues is significant, as is the selectivity of the specific tissues suitable for their growth and dissemination. The leaping metastatic diffusion movement of the tumor peak was observed in the simulation (Figs. 5b and 7) and tumor peaks always emerged selectively at *specific sites* on the percolation cluster. These results explain the above-mentioned selectivity of a tumor for a specific area that is suitable for its proliferation and invasion into the surrounding host tissues.

It is worthwhile to note the double peak and the link between the new and original peak areas observed on the percolation clusters (Fig. 7b and File S2). The results are basically consistent with the experimental results by Riching et al. in that highly aligned regions or tracks in the surrounding tissue can provide tumor cells that have a highly migrating capability with the most reliable and robust escape route [34]. Similar clinical results were observed (Fig. 9a–c and File S3). This double peak can be explained by the inherent characteristic of percolation [72]. The flowing pressure distribution varies considerably depending on the local

spatial distribution of the percolation sites. The fluid micelles always flow in a direction with significant pressure changes; fluid micelles flow slowly in regions with low-pressure differences. The percolation cluster includes a tunnel in which the pressure difference for flowing micelles is very significant and there is little diffusion resistance. Tumor cells diffuse easily via the tunnel and form a new tumor peak at a suitable *specific site*. Depending on the occupation probability P and the local spatial distribution of percolation sites, only sites that can develop a link with the original tumor peak site and then a tunnel between the two peaks may be suitable for tumor diffusion on the percolation cluster. When the occupation probability P is sufficiently high, e.g., close to 1.0, tumor cell micelles have more paths to flow and tumor cells diffuse randomly and steadily without the feature of a double peak.

The simulation indicates that the diffusion velocity of tumor cells in tissue is positively correlated to the homogeneity of the surrounding tissue environment. Analysis of the clinical data shows that metastases of breast tumors diffused faster in bone and lung than in liver or brain (Fig. 10a). The initial and average inhomogeneity of the surrounding tissue fit with this order of diffusion velocities well in bone, lung, liver and brain metastases of breast cancer (Fig. 10b). Both the simulation and the clinical results were consistent with each other. These results were also confirmed and proved by previous studies that showed that the inhomogeneous environment could influence tumor proliferation and invasion through compressive stress [42–44, 70]. The most common metastasis site for breast cancer is bone (48%), followed by lung (26%), liver (30%) and brain (7%) [6–8]. Bone and lung tissues are considered to have a low inhomogeneity, which corresponds to the homogeneous tissues in our mathematical model. Liver and brain tissues possess greater inhomogeneity than bone and lung tissue (Fig. 10b). The metastases of a lung adenocarcinoma diffused faster in bone and lung than in liver and brain (Fig. 11a). The initial and average inhomogeneity of these host tissues was also basically in agreement with this order of diffusion velocities in bone, lung, liver and brain metastases of lung adenocarcinoma (Fig. 11b). According to the clinical literature, lung adenocarcinoma often leads to either bone (39%) or lung metastases (22%) [73]. Tumor cells diffuse faster and invade more easily in a more homogeneous tissue microenvironment. A less-homogeneous tissue can limit tumor proliferation and diffusion, such as the brain metastasis in patient D and the liver metastasis in patient E (Figs. 10 and 11).

The clinical and simulation results presented above indicate that the inhomogeneity of the surrounding host tissue can have an obvious effect on tumor proliferation and diffusion and that an inhomogeneous tissue environment can limit the formation of new tumor metastases. Clinically, bone, lung, liver and brain are prone to metastasis because of the greater homogeneity of their tissues. Despite their mobility, DTCs have difficulty diffusing and are unable to form macrometastases in surrounding tissues with poor homogeneity. Note that the inhomogeneity of some surrounding host tissue changed over time (Fig. 10b and 11b). This could be an important reason for the infiltration of cancer into many other organs that have no metastases during the early stage of tumor invasion.

In conclusion, tumor diffusion and migration are complicated processes that strongly depend on the surrounding tissue microenvironment. Our study made use of simulations and clinical medical imaging to reveal that tumor proliferation and diffusion depend on the homogeneity of the surrounding tissue. Here we summarize the main findings of this study. (1) The proliferation and diffusion of tumor cells inside host tissues with different inhomogeneity can be described by mathematical modeling on a percolation cluster. The thresholds for tumor proliferation and diffusion in three kinds of lattices with 4, 6, and 8 connecting near

neighbors were calculated to be 0.54, 0.44, and 0.37, respectively. Two key features were observed in the percolation cluster; the existence of a critical transition relating to tumor survival and diffusion versus site occupation probability or in silico tissue inhomogeneity; and the existence of *specific sites* that qualify for tumor invasion and the link that forms between the *specific site* and the original tumor center. (2) When hampered by inhomogeneous surrounding host tissue, few tumor cells can diffuse to a further location. (3) During tumor diffusion and invasion, tumor cells detach from the maternal tumor and diffuse to the surrounding host tissue. The evolution of the tumor center takes on a leaping metastatic diffusion movement. Tumor cells accumulate and form double peak areas due to the surrounding tissue inhomogeneity. (4) Tumor diffusion in different tissues correlates positively with the homogeneity of the surrounding tissues. The inhomogeneity of the host tissue microenvironment can change over time, which may be an important cause of systemic metastases.

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Compliance with ethical standards

Ethical approval This study did not involve any human experiments or treatment processes. The physiological and clinical images of tumors were acquired from ordinary medical examinations of patients at the Sun Yat-sen University Cancer Center. These examinations were carried out for therapy only and no additional drugs or measures were used. This study was approved by the ethics committee of the Sun Yat-sen University Cancer Center and every effort was made to maximize the protection of the patients' privacy (e.g., anonymous analysis of data). The research materials and results were used for scientific purposes without any conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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