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Dynamics of tissue topology during cancer invasion and metastasis

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Abstract

During tumor progression, cancer cells mix with other cell populations including epithelial and endothelial cells. Although potentially important clinically as well as for our understanding of basic tumor biology, the process of mixing is largely a mystery. Furthermore, there is no rigorous, analytical measure available for quantifying the mixing of compartments within a tumor. I present here a mathematical model of tissue repair and tumor growth based on collective cell migration that simulates a wide range of observed tumor behaviors with correct tissue compartmentalization and connectivity. The resulting dynamics are analyzed in light of the Euler characteristic number (χ), which describes key topological features such as fragmentation, looping and cavities. The analysis predicts a number of regimes in which the cancer cells can encapsulate normal tissue, form a co-interdigitating mass, or become fragmented and encapsulated by endothelial or epithelial structures. Key processes that affect the topological changes are the production of provisional matrix in the tumor, and the migration of endothelial or epithelial cells on this matrix. Furthermore, the simulations predict that topological changes during tumor invasion into blood vessels may contribute to metastasis. The topological analysis outlined here could be useful for tumor diagnosis or monitoring response to therapy and would only require high resolution, 3D image data to resolve and track the various cell compartments.

Introduction

Topology theory

Topology is a field of mathematics that describes the connectedness of structures. It was pioneered by Euler and refined by Poincaré and Riemann among others [25]. Using the concepts of topology theory, it is possible to quantitatively describe the connectivity and compartmentalization of structures. To be specific, topological analyses can determine whether a structure is made of a single, connected mass or separated into multiple parts. It can also quantify the number of holes or cavities in a given object. The Euler number, designated χ , has a characteristic value for each topological configuration and is affected by the number of objects, holes and cavities as shown in figure 1 [3].

Tissue organization

These topological considerations are potentially useful for studies of tissue organization. Tissues are characterized by multicellular organization that results in well-defined compartments separated by physical and biochemical barriers. Cells within the compartments perform specific functions such as antibody production in lymph node B cell foci, milk production in breast acini and digestion of food in the gut. In general, the barriers that separate anatomical compartments are made of two-dimensional (2D) cellular sheets associated with laminar basement membranes that both stabilize the structure and provide geometrical cues for cell polarization and tissue assembly.

It is important that the various tissue compartments remain distinct. During development, injury and most pathologies, changes in topology are either well-orchestrated or quickly repaired, and there is no ‘mixing’ of the various compartments. Even when a wound disrupts epithelial and endothelial barriers to temporarily connect multiple compartments, the tissue eventually returns to its previous topology. The mechanisms by which topology is regulated are not completely understood. During development, it presumably is achieved by continuous growth of tissue ‘from within’, with in-plane extension of 2D barriers and growth of the contained compartments in the direction roughly perpendicular to the barriers. During gastrulation in the embryo, folding or involution of the cell sheets can thus produce the distinct anatomy of the various tissues while allowing the barrier structures to remain intact [30]. However, as tissue expands during development, compartments are temporarily interrupted and resealed to provide the correct spatial relationships between various compartments. A good example is in vascular development, where intussusceptive vessel splitting and sprouting angiogenesis change the overall Euler number of the vasculature by increasing the number of ‘holes’ or loops. But these changes are achieved in a deliberate way that does not allow mixing of intravascular cells into the extravascular space or vice versa [19].

As opposed to deliberate changes in topology during development, injury can disrupt the existing tissue topology indiscriminately, necessitating that the 2D barriers separating compartments be re-formed quickly and robustly. At the level of the microanatomy, this means that cells such as epithelial and endothelial cells at the newly-created edge of the barrier migrate in an appropriate way, preferably remaining connected with the trailing sheet structure, to find other ‘edge’ cells with which to re-establish junctions. This type of collective migration is currently an active area of research, but little is known about the environmental cues that guide the closure of the cell sheets. It has been shown that small defects in epithelium can be closed via a ‘purse-string’ mechanism in which cytoskeletal tension and junction remodeling can quickly cover denuded areas of epithelial monolayers [20]. In convoluted three-dimensional (3D) tissues, there must be strict rules that prevent sheet edges in different compartments from connecting (e.g. an epithelial sheet meeting an endothelial sheet). Presumably, this is automatically achieved if topology is always maintained locally.

Cancer is one notable pathology in which the robust mechanisms of topology repair apparently fail. Epithelial tumors (carcinomas) can remain in a single compartment (topologically connected), in which case they are considered benign and can be excised

completely by a surgeon. But when they cross basement membranes, they can mix with the surrounding tissues and are much more difficult to excise and control. By definition, epithelial cells that undergo an epithelial to mesenchymal transition (EMT) abandon the existing sheet anatomy to mix with the stromal tissue. But it is still not known how important this process is during invasion and metastasis, or whether collective migration of cells, which may at least partially maintain topology, may also play a role. Recently, much attention has focused on intratumor heterogeneity in cancer genotype and phenotype, with many studies concluding that there is incomplete mixing of cell populations within tumors [13, 27]. The biological and physical bases for mixing are not well-understood, but could have important implications for tumor progression and dissemination. For example, Yuan *et al* used Ripley's K function analysis of cell clustering in histological sections of breast cancer and concluded that moderate stromal cell dispersion was associated with poor survival [32]. A better understanding of the mechanisms involved could lead to more effective, targeted therapies to slow or stop tumor progression.

Because topological considerations play such a central role in tissue organization, an analysis of topological changes during tumor growth may uncover useful information about the cellular interactions involved. To date, the application of topology concepts to cancer have been limited to image analysis, where topology theory has been used to determine relationships between objects in 2D tissue slices as a method of segmentation [12, 16, 23]. Others have proposed that fractal dimension measurements at the tumor boundary can be used to characterize tumor growth [1, 11] or improve the selection of surgical margins [7, 16, 22]. In general, these studies use histological sections for analysis, which precludes determination of connectivity in 3D. Furthermore, no studies have analyzed the Euler characteristic of tumors or normal tissues during invasion and metastasis.

To establish a framework from which to approach this problem I present here a mathematical model of tumor invasion and metastasis as well as methodology for extracting relevant topological data from the resulting 3D structures. The model consists of a cellular automaton that explicitly considers endothelial cells, epithelial cells, laminar basement membrane (BM), stromal matrix (SM), and provisional matrix (PM) produced by tumors or wounds. It was formulated to reproduce the correct topology changes during tissue repair, and then used to analyze tumor invasion.

Methods/approach

Model formulation

The literature is rich with mathematical models of tumor growth and treatment [2, 4–6, 10, 14, 15, 17, 21, 23, 24, 26, 28, 29, 33]. In general, these models focus on the biological or mechanical determinants of growth and invasion using continuous or discrete methods, with the goal of predicting growth rates and/or responses to treatment. The model presented here simplifies the biology, instead focusing on the dynamics of the barrier structures and the associations between cells that are important for tissue compartmentalization.

The model is a 3D cellular automaton developed in Matlab. Connectivity is based on a neighborhood of 26, and each operation is associated with a defined probability (table 1).

Seven different binary arrays (one for each species) are updated at each time step. A key operation of the simulation is the binary dilation, which changes any '0' voxel to '1', if it is adjacent to an existing '1' voxel (in a neighborhood of 26). During each time step, the following operations occur (figure 2).

1. The cancer array is subjected to binary dilation; the cancer array is then updated by combining the dilated cancer array with the other species arrays using logical 'or' operations, with associated probabilities. The other (non-cancer) arrays are then updated to remove cells where new tumor has been produced using the logical 'not' operation with the cancer array. This step allows the cancer to extend into the surrounding spaces.
2. Cancer cells produce provisional matrix by replacing a fraction of the cancer cells with provisional matrix (according to the assigned probability). Both arrays are updated accordingly.
3. The epithelial and endothelial arrays are subjected to binary dilation. Where this array overlaps with the provisional matrix array, the provisional matrix is replaced by epithelium or endothelium, as appropriate, with defined probability. The endothelial, epithelial and PM arrays are updated accordingly. In this way, epithelial or endothelial cells digest and move into adjacent provisional matrix.
4. The lumen, BM, PM, epithelial and cancer arrays are dilated; lumen replaces epithelium where the dilated lumen array overlaps with the epithelium, except where it also overlaps with the dilated PM, BM or cancer arrays. This step is repeated for the endothelium, and causes any multi-layer endothelial or epithelial sheet to be 'thinned', with deleted cells replaced by lumen.
5. The epithelial, cancer and PM arrays are dilated. Where the dilated epithelium overlaps with the lumen array and either the dilated PM or cancer arrays, lumen is replaced with epithelium. This is repeated for the endothelium and causes any exposed edges of endothelium or epithelium to extend to cover adjacent provisional matrix or cancer cells; the extension is restricted to directions away from the edge of the existing basement membrane, into lumen space.
6. The epithelial array is dilated. Where this array overlaps with SM, SM is replaced with BM. The BM and SM arrays are updated. This is repeated for the endothelium and results in new BM being deposited adjacent to epithelium and endothelium at stromal matrix sites.
7. The endothelial and epithelial arrays are dilated. Where these arrays overlap with the original epithelial or endothelial array, BM replaces the existing epithelium or endothelium. This step effectively separates the endothelial and epithelial cells that occupy adjacent nodes.
8. The endothelial and epithelial arrays are dilated. Where these dilated arrays overlap, BM is inserted to replace existing PM or SM. This results in basement membrane formation between endothelial and epithelial cells that are separated by only one voxel of PM or SM.

The code is completely vectorized in Matlab and operates on 2D or 3D domains. Using a Macintosh (2.7 GHz Intel Core i7), each time step takes approximately 5 s (wall clock time) for a domain size of $100 \times 100 \times 100$.

Assessment of tissue topology

At each time step, the topologies of various tissue compartments are analyzed using the imEuler3D code developed by David Legland [18]. The code extracts the 3D Euler number, χ_i , defined as:

$$\chi_i = N_i - G_i + H_i$$

where N_i , G_i and H_i are the numbers of objects, holes and cavities for species i , respectively.

For contiguous, uninterrupted endothelial/epithelial structures or homogeneous spheroids of cancer cells, χ has a value of 1. Each hole that penetrates and passes through a structure decreases χ , while cavities within structures increase χ . Similarly, breaking the connectivity of a structure will increase χ .

From this perspective, a tumor that grows as a 'ball' will maintain its topology at $\chi = 1$ as will a tumor that extends finger-like projections that remain connected to the tumor mass. On the other hand, tumors that break up into multiple parts during invasion will have increased χ , and those that remain connected but become porous will have decreased χ (figure 1).

For the purpose of this analysis, the provisional matrix is considered to be part of the tumor. Also, to avoid ambiguity, lumen spaces within the epithelial cavities are not counted; i.e., the lumen spaces are merged with the epithelium or endothelium before calculation of the Euler number.

Results

Tissue repair (case a)

Before examining tumor growth, the model was validated using simulations of wound healing (figure 3(A)). Wounds introduce perturbations in topology that are robustly corrected by the intrinsic mechanisms. In the following simulation, a puncture wound is introduced that extends through the skin epithelium, the stromal matrix, a blood vessel and penetrates an underlying gland lined with epithelium (figure 3(B)). The wound is assumed to be made of provisional matrix components (e.g. fibrin, fibronectin) but would also contain aggregated erythrocytes, platelets and other structural components, as well as released growth factors. Because we are only interested in the topology of repair, we can simplify the biology and assume a single component. As in the actual biological process, the endothelial and epithelial cells migrate on and digest the provisional matrix (figure 3(A)). Subsequent thinning of the epithelial and endothelial structures, combined with the rules that prevent their mixing (rules 7 and 8, figure 2), results in dissolution of the wound and re-establishment of the cell compartments. Analysis of the Euler numbers during this simulations show initial decreases, as epithelium and endothelium form small looping

structures into the provisional matrix, followed by increases, as particles of provisional matrix are encapsulated by the epithelium and endothelium (figure 3(C)). Upon complete dissolution of the wound, the Euler numbers return to baseline values, indicating that the tissue topology has been restored. For simplicity, the model does not include the mechanisms that would help the tissue structures remodel to form more accurate planar, cylindrical or spherical geometries. Nevertheless, the resulting compartmentalization is correct.

Tumor interactions with the epithelium

Tumor within the epithelium with low production of provisional matrix (case b)

To simulate tumor growth, a single tumor cell is seeded within the domain and allowed to expand following the assigned probabilities. We first analyze a tumor initiated in the planar epithelium that produces little provisional matrix and cannot cross the basement membrane (see table 2 for a summary of the parameter values for the tumor simulations). The tumor expands and mixes with the epithelium, but remains isolated from the stroma by the basement membrane, thus simulating a benign skin tumor (figure 4(A)). The tumor exhibits exponential growth, and the majority of cancer cells remain in a single, connected mass (as indicated by the number of objects, figure 4(C)). The epithelial cells, however, are occasionally engulfed by the growing tumor mass, thus breaking the epithelium into multiple pieces (figure 4(C)). The increase in the number of cavities within the cancer mass (which are either filled with epithelial objects or empty) results in an increase in χ_c . Fragmentation of the epithelium should cause χ_{ep} to increase, but this is overwhelmed by the large number of holes (loops) in the epithelial structure, as indicated by the decreasing χ_{ep} values (figure 4(D)).

It is important to note that there is not a quantitative correspondence between the number of cavities in the cancer space and the number of individual epithelial objects (compare the yellow line in figure 4(C) and the black line in (D)). This is due to the connectivity of 26 used in the calculations for the number of objects and the Euler numbers. With this connectivity, yellow voxels can be contained within a black cavity, but still connected diagonally to another yellow voxel. In other words, connectivity is enhanced, skewing the numbers toward more cavities and less fragmentation. If the calculations are performed using a neighborhood of 6, then the opposite occurs—the results tend toward more fragmentation and fewer cavities.

Tumor seeded within the epithelium with production of provisional matrix (case c)

In this simulation, I change only the parameter that allows the cancer cells to produce provisional matrix (see table 2). A tumor that produces such matrix can resemble a wound, triggering repair responses in the surrounding stromal endothelial and epithelial cells [9]. With this change to the simulation, there is a reversal in the topological dynamics. Because of the provisional matrix, which provides pathways for epithelial migration, there is more infiltration of the tumor by epithelial cells (figure 5(A)). The epithelium remains as a cohesive mass, but the cancer cells break up into multiple objects (figure 5(C)). However, χ_c and χ_{ep} both become increasingly negative, indicating that the system is dominated by

structures with interconnected loops (figure 5(D)). Thus, the tumor is growing as two interdigitating, porous structures (epithelium and cancer cells; figure 6).

Tumor seeded within the epithelium with production of provisional matrix and increased epithelial migration (case d)

Another parameter that dramatically affects the Euler numbers is the affinity of epithelium for the provisional matrix [P(E, PM)]. In this simulation, I increase this parameter, keeping everything else the same as in case c. This leads to dramatic mixing and fragmentation of the tumor (figure 7). Again, overall growth is slowed due to loss of cancer cells at the expense of endothelial migration (figure 7(B)). As small pieces of tumor are encapsulated within cavities in the epithelium, χ_{ep} increases (figures 7(C) and (D)). At the same time, the cancer cells form loops into the epithelium, decreasing χ_c .

Figure 8 summarizes the effects of P(E, PM) and provisional matrix production by the tumor on fragmentation and Euler numbers. Increasing the migration of epithelium into PM regions inhibits fragmentation of the epithelium, but increases that of the tumor (figure 8(A)). Similarly, increasing the amount of PM in the tumor allows the epithelium to stay together (figure 8(B)). This also fragments the cancer cells initially, but at high levels of PM production, the increase in cancer fragmentation is countered by loss of tumor fragments that have been invaded by epithelium, stopping tumor growth. The Euler number for epithelium initially decreases from 1 with increasing P(E, PM), but then becomes increasingly positive (figure 8(C)). This shows the transition from a topology dominated by a looping, porous structure to a single mass with multiple embedded cavities. The cancer Euler number is positive at low P(E, PM), goes through a minimum and then shows a slight increase with increasing P(E, PM). This reflects encapsulation of epithelium by the cancer cells, looping topology and fragmentation, respectively. For increasing PM production (figure 8(D)), χ_{ep} starts negative, goes through a (positive) maximum, and then returns toward 1. Thus, with low PM production, the epithelium is porous; in the mid-range, it encapsulates tumor fragments, while at higher levels, it becomes a single mass of epithelium at the expense of the tumor. χ_c follows the inverse behavior, dominated by cavities at low PM production (positive χ_c), a minimum (negative) value and then returning toward one when PM production is high.

Intravasation of a tumor into a blood vessel

Tumor invades with low production of provisional matrix (case e)

Because the rules for vascular endothelium are similar to those of the epithelium (indeed, endothelial cells are just a special class of epithelium), the behavior of the tumor as it encounters blood vessels is similar to that described above. Again, three distinct topological mechanisms are observed as the tumor expands into the blood vessel lumen.

The first is analogous to case b, where provisional matrix production is low and the cancer cells dominate the system. Endothelial cells are unable to keep up with the cancer growth, and fragments of endothelium become encapsulated in the tumor (figure 9(A)). Tumor growth is rapid (figure 9(B)), and it remains cohesive and integral with the vessel wall. Multiple endothelial objects appear (figure 9(C)), but there are also many endothelial loops

through the tumor, leading to a decrease in χ_{en} (figure 9(D)). The single tumor mass contains cavities with endothelium resulting in increased χ_c .

Endothelial–cancer mixing in the vessel (case f)

Increasing the production of tumor provisional matrix (table 2) results in more mixing of the two compartments (figure 10(A)), slower tumor growth (figure 10(B)) and moderate fragmentation of both the endothelium and cancer spaces (figure 10(C)). The increasingly negative Euler numbers confirm that the system is dominated by interconnected porous structures (figure 10(D)).

Encapsulation of cancer cells by endothelium (case g)

If we increase the affinity of the endothelium to the provisional matrix spaces, then the endothelium more effectively encapsulates the cancer cells (figure 11(A)). The tumor growth is slower due to replacement of cancer/PM space by endothelium (figure 11(B)), and the cancer connectivity is lost to a larger extent (figure 11(C)). The increasingly positive χ_{en} values indicate a large number of cavities filled with cancer cells, although many loops also exist in the cancer space, resulting in negative χ_c values (figure 11(D)).

In general, the effects of PM production and $P(E, PM)$ are similar for endothelium (figure 12) and epithelium (figure 8). Increasing the affinity of endothelium to PM (figure 12(A)) or cancer production of PM (figure 12(B)) induces fragmentation of the tumor, and at high levels, inhibits tumor expansion.

Discussion

The concept of a tumor as a non-resolving wound is intriguing and rich with unexplored implications. One implication is that normal cells in the vicinity of a tumor may attempt to repair the wound, and in the process, contribute to tumor growth or invasion. It has long been assumed that mixing of cancer cells with normal tissue is a pseudo-random process without well-defined rules. However, if at least some of the cells are following the normal program of tissue repair, then the problem becomes more approachable.

Using a simple set of rules designed to reproduce tissue repair, the model presented here is able to simulate a wide range of tumor behaviors similar to that seen *in vivo*. Focusing on collective migration rather than diffusive invasion, it cannot be used to compare tumor growth rates (due to the lack of an accurate mass balance on the various populations). Instead, it was formulated to reproduce the topological dynamics that cells perform in disrupted tissue and thus makes reliable and interesting predictions of population mixing.

One of the key processes appears to be the interaction of normal cells with provisional matrix produced by the tumor. The production of PM within the tumor—and the subsequent infiltration of endothelium or epithelium—has a profound effect on compartment mixing. As the tumor produces more PM or the tendency of the epithelium to migrate on the PM increases, the epithelium first extends fingers and loops into the tumor and can eventually encapsulate cancer fragments, which are disconnected from each other. Similarly, endothelium can interact with cancer cells as they invade blood vessels through porous

mixing or encapsulation. These processes could have implications not only for tumor invasion, but also angiogenesis. As the endothelium performs collective migration to loop into the cancer compartment, it is in effect forming new blood vessels in a manner topologically analogous to sprouting angiogenesis.

It remains to be seen how these topological considerations relate to tumor progression. Logically, tumors with negative Euler numbers should be able to infiltrate easily, forming a highly interdigitating mixture with the surrounding tissue. Such a tumor would also be difficult to resect completely by a surgeon. On the other hand, a tumor that is largely fragmented and contained in cavities that present barriers for further expansion might become dormant. This would depend on the ability of the epithelium (or endothelium) to keep pace with the cancer cells and contain their growth as well as the ability of the cancer to continuously disrupt these barriers.

A potentially important prediction of the simulations is that during intravasation into a blood vessel, tumors with high PM expression can become fragmented and encapsulated within endothelium. If this occurs *in vivo*, the endothelial-coated clumps of cancer cells could easily enter the circulation and contribute to distant metastasis. The protective coat of endothelium, as well as the inclusion of stroma and other cells within the capsule, could enhance the survival of the cancer cells in the blood stream and at the secondary site [8].

As presented, the model has several limitations. Future refinements should include other cells such as fibroblasts, which can contribute to PM production and remodeling. Rather than the random production modeled here, fibroblasts may pattern the PM in specific ways to influence the topological changes. Macrophages may also contribute to matrix remodeling, and have been shown to organize collective migration of cancer cells [31]. Once more is known about these processes, they can be easily incorporated into the model. In addition, the simulations presented here were limited to growth within the epithelial or endothelial/lumen compartments to demonstrate the practical application of topological characterization. Future models should extend this analysis to tumor invasion through the stroma, which will potentially involve mixing of three or more compartments. Fortunately, because the Euler number for a given compartment is independent of the number of compartments with which it mixes, the concepts outlined here will still hold. These modifications should be guided by topological comparisons with actual tumor tissue in which the cancer, epithelial, endothelial and stromal cell populations can be visualized in 3D.

From a practical standpoint, analyses of tissue topology could have clinical relevance. There is a long-standing need to predict how a given tumor will progress or how a tumor is responding to treatment. For the most part, pathologists still rely on semi-quantitative analysis of two-dimensional tissue slices to make these determinations. However new imaging technologies such as multi-photon microscopy are able to extract multi-color, 3D image data from relatively thick tissues. In principle, it should be possible to apply the concepts here to such data sets to make pathological analyses more objective and quantitative.

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Figure 1.

Topological characteristics of 3D solids. In this illustration, the Euler numbers for the orange and black objects are designated χ_E and χ_C , respectively. The Euler number for a solid object with no holes or cavities is 1, regardless of surface features (A). Each penetrating hole decreases χ by one unit (B). χ for an ensemble of objects is the sum of the individual χ values (C). When two solids interact, each has a characteristic Euler number that is determined by its own topology (D). Cavities increase χ when completely contained within the solid (E).

Figure 2. Operations of the cellular automaton model. Single 2D slices of the domain are shown, but all operations extend to the third dimension.

Figure 3.

Simulated wound healing. Shown are cross-sections of the 3D domain through the center of the wound at selected time steps (A). The domain contains the surface of the skin (planar structure at top, yellow epithelium, blue basement membrane), a cylindrical blood vessel (middle, green endothelium with blue basement membrane), and a spherical gland (bottom, yellow epithelium, blue basement membrane). At $t = 0$ a puncture wound is simulated, leaving behind a cuboid of provisional matrix that connects these structures (B). Following the rules outlined in figure 2, the endothelium, epithelia and basement membranes close the wound and return to the correct anatomy. During this process, the Euler numbers transiently decrease due to loop formation, then increase as provisional matrix is encapsulated by the migrating endo- and epithelium. By the end of the simulation, the Euler numbers return to the baseline value of 1 ((C); green: χ_{endo} , yellow: χ_{epi}).

Figure 4.

Growth of a tumor initiated by a cancer cell (black) in a plane of epithelial cells (yellow, (A)). A single y-z plane of the 3D domain is shown. A portion of a cylindrical blood vessel appears at bottom. Over 30 time steps, the tumor expands and mixes with the epithelium, but is not allowed to produce provisional matrix or cross the basement membrane. Exponential tumor growth (B) is associated with an increase in the number of epithelial objects (fragmentation; (C)), along with increases in the cancer Euler number, χ_c , and decreases in the epithelium Euler number, χ_{ep} (D). Plots in (B)–(D) depict the mean $\pm$ sd from 16 independent simulations.

Figure 5.

Growth of a tumor initiated by a cancer cell (black) in a plane of epithelial cells (yellow, (A)). The tumor expands and produces provisional matrix (red), which provides pathways for epithelial migration. Exponential tumor growth (B) is slower than in case b because the epithelial cells are not included in the calculation of tumor volume, and their migration replaces cancer cells. The epithelium remains contiguous, but its migration partially fragments the tumor (C). Overall, this process results in Euler numbers that become increasingly negative (D), indicating that the epithelium and cancer cells interdigitate, forming two interconnected porous structures. Plots in (B)–(D) depict the mean $\pm$ sd from 16 independent simulations.

Figure 6.
Illustration of two interdigitating spaces, both with negative Euler numbers.

Figure 7.

Growth of a tumor with increased epithelial affinity to provisional matrix. Again, tumor growth (B) is slowed due to epithelial migration. In this case, the epithelium remains contiguous, and there is more fragmentation of the tumor (C). The positive χ_{ep} indicates many cancer-containing cavities within the epithelium (D); however, the decreasing, negative χ_c shows that the cancer space also contains a looping, porous structure. Plots in (B)–(D) depict the mean $\pm$ sd from 16 independent simulations.

Figure 8. Topological consequences of changing the affinity of epithelium to provisional matrix or its production rate. Parameter sets are listed in table 2; number of objects and Euler numbers are from time step = 40.

Figure 9.

Intravasation of a tumor (black) into a blood vessel. The tumor is initially seeded outside the basement membrane (blue, (A)), It crosses the basement membrane and interacts with the endothelium (green) over 30 time steps. As the tumor grows (B), it encapsulates endothelial cells, causing fragmentation (C). In the process, χ_c increases, while χ_{en} decreases (D). Plots in (B)–(D) depict the mean $\pm$ sd from 16 independent simulations.

Figure 10.

Intravasation of a tumor (black) into a blood vessel with increased production of provisional matrix (red). The cancer cells and endothelium mix, but there is minimal fragmentation of both spaces (C). Negative χ_c and χ_{en} values indicate interconnected looping structures (D). Plots in (B)–(D) depict the mean $\pm$ sd from 16 independent simulations.

Figure 11.

Intravasation of a tumor (black) into a blood vessel with increased endothelial migration into the provisional matrix (red). The endothelial cells dominate the system, breaking up the cancer space (C). Negative χ_c indicates interconnected looping structures, although many cancer cells are encapsulated by the endothelium (positive χ_{en} , (D)). Plots in (B)–(D) depict the mean $\pm$ sd from 16 independent simulations.

Figure 12.

Topological consequences of changing the affinity of endothelium to provisional matrix or its production rate. Parameter sets are listed in table 2; number of objects and Euler numbers are from time step = 40.

Table 1

Model parameters.

Parameter	Definition—probability of:	Biological rationale
P(C, L)	Cancer invading adjacent lumen site	Growing tumors easily expand into empty lumen space
P(C, PM)	Cancer invading adjacent provisional matrix site	Cancer cells can digest and migrate through provisional matrices
P(C, SM)	Cancer invading adjacent stromal matrix site	Cancer cells can digest and move through stromal matrices such as collagen I
P(C, Ep)	Cancer invading adjacent epithelial site	During uncontrolled growth, tumors may displace epithelial cells
P(C, En)	Cancer invading adjacent endothelial site	During uncontrolled growth, tumors may displace endothelial cells
P(C, BM)	Cancer invading adjacent basement membrane site	Tumors may produce matrix metalloproteinases that digest basement membrane (e.g. collagen IV)
P(PM, C)	Cancer producing provisional matrix	Many cancers produce provisional matrices (fibronectin, laminin, etc) or recruit stromal cells that do
P(E, PM)	Epithelium or endothelium extending into adjacent provisional matrix site	Endothelial and epithelial cells can digest and migrate through provisional matrices
P(E)	Overall epithelial or endothelial speed	The speed of these cells depends on activation state

Table 2

Summary of parameter values for the simulations.

Parameter (probability of:)	Case a-d				Case e-g					
	a	b	c	d	Figure 8	e	f	g	Figure 12	
Cancer producing provisional matrix: P(PM, C)	na	0.01	0.3	0.3	0-0.9	0.3	0	0.3	0-0.9	0.3
Epi- or endothelium extending into adjacent PM : P(E, PM)	0.2	0.2	0.2	0.6	0.4	0-0.8	0.2	0.2	0.6	0-0.8
Cancer invading adjacent basement membrane site: P(C, BM)	na	0	0	0	0	0	0.5	0.5	0.5	0.5
Cancer invading adjacent lumen site: P(C, L)	na	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Cancer invading adjacent provisional matrix site: P(C, PM)	na	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
cancer invading adjacent stromal matrix site: P(C, SM)	na	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Cancer invading adjacent epithelial site: P(C, Ep)	na	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cancer invading adjacent endothelial site: P(C, En)	na	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Overall epithelial or endothelial speed: P(E)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5