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A 2D Mechanistic Model of Breast Ductal Carcinoma *in Situ* (DCIS) Morphology and Progression

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Abstract

Ductal carcinoma *in situ* (DCIS) of the breast is a non-invasive tumor in which cells proliferate abnormally, but remain confined within a duct. Although four distinguishable DCIS morphologies are recognized, the mechanisms that generate these different morphological classes remain unclear, and consequently the prognostic strength of DCIS classification is not strong. To improve the understanding of the relation between morphology and time course, we have developed a 2D *in silico* particle model of the growth of DCIS within a single breast duct. This model considers mechanical effects such as cellular adhesion and intra-ductal pressure, and biological features including proliferation, apoptosis, necrosis, and cell polarity. Using this model, we find that different regions of parameter space generate distinct morphological subtypes of DCIS, so elucidating the relation between morphology and time course. Furthermore, we find that tumors with similar architectures may in fact be produced through different mechanisms, and we propose future work to further disentangle the mechanisms involved in DCIS progression.

Keywords

tumor growth parameters; ductal morphology; computational model; breast cancer

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1. Introduction

Breast cancer is one of the leading causes of cancer-related deaths in women (Parkin et al., 2005). In this paper, we focus on a pre-invasive category of breast cancer that is comparatively straightforward to analyze computationally, Ductal Carcinoma *In Situ* (DCIS). DCIS has a number of recognizable morphological types: micropapillary, cribriform, solid and comedo. Our goal is to use computer simulations to uncover the relationship between these subtypes – for instance, whether the separate subtypes result from distinct growth processes or whether they evolve under the same growth conditions.

DCIS is a pre-invasive breast cancer that accounts for about 13% of all newly diagnosed cases of cancers worldwide (Quinn and Ostrowski, 1997). DCIS is characterized by excessive growth of abnormal epithelial cells within the duct. These cells grow and aggregate in an irregular fashion with noticeably abnormal histology and enlarged or multiple nuclei. At this stage, these abnormal cells remain within the duct, hence the term “*in situ*”. If left untreated, it is estimated that between 14% and 53% of DCIS will progress to invasive cancer after penetrating the basement membrane (Erbas et al., 2006). There is uncertainty over what circumstances cause the disease to progress to invasive cancer as well as great variability in the amount of time it takes for progression to invasive disease, ranging from 2 to 12 years (Collins et al., 2005; Polyak, 2001; Weinberg, 2007; Wiechmann and Kuerer, 2008). To improve the likelihood of a successful clinical outcome, it is desirable to understand the mechanisms that govern disease progression during early stages when the disease is relatively well established, but has not yet become invasive.

DCIS presents several distinct and identifiable morphologies that are used by pathologists to determine prognosis and future invasive potential of the cancer. These morphologies are classified into four subtypes, shown in Fig. 1: micropapillary, cribriform, solid and comedo (Jaffer and Bleiweiss, 2002; Silverstein, 2000). Micropapillary tumors are characterized by finger-like projections into the interior of the duct, as shown in Fig. 1a. Cribriform tumors have an interrupted morphology, where the interior of the duct is filled with tumor cells, but exhibits holes filled with interstitial fluid, as shown in Fig. 1b. Solid tumors are characterized by a duct filled with an uninterrupted mass of cells, shown in Fig. 1c, and finally comedo tumors consist of a filled duct with a central necrotic core, containing dead cells, shown in Fig. 1d.

Although these four morphologies are used by pathologists for diagnosis, we are limited in our knowledge of the progression of tumor morphology, on several accounts. First, with the exception of the comedo morphology which is typically associated with late-stage DCIS (Lagios, 1996), it is unclear how morphology relates to patient outcome (Cornfield et al., 2004; Wiechmann and Kuerer, 2008). Second, the correlation between DCIS grade and patient outcome is poor, depends on details of the classification method used and is complicated by the frequent presence of multiple morphologies within a single sample (Jaffer and Bleiweiss, 2002; Quinn and Ostrowski, 1997; Silverstein, 2000). Third, whereas morphology can be readily determined by a trained histologist, its utility as a benchmark of tumor progression is not well-established (Lagios, 1996; Wiechmann and Kuerer, 2008).

Finally, it is unclear whether there is a well-defined progression from one morphological subtype to another, and nor is it known under what conditions a given subtype may manifest itself. Thus it remains to be determined whether the different morphological structures are progressions of a single growth process under different growth conditions, or if each morphology represents a distinct growth process that develops over time. Likewise, it is not clear how much of a tumor’s history can be determined from histological analysis of ductal morphologies at a single time point.

In summary, it is fair to say that defining the correlation between stage, grade and progression remains problematic (Sharifi-Salamatian et al., 2000; Sontag and Axelrod, 2005). A model to improve the understanding of DCIS development would therefore be valuable. To address this need, we have developed a computational discrete particle model that permits the direct simulation of DCIS progression under various assumed scenarios. Our goal is to quantitatively analyze DCIS development and to consequently improve future capabilities of making predictive prognostic evaluations.

1.1 Review of pertinent literature

Modeling of Cancer in general—There is considerable literature on attempts to provide useful mathematical and computational treatments of cancer growth, dating perhaps to Gompertz (Applebaum, 2001; Gompertz, 1825). Recent studies have proposed that Gompertzian kinetics and fractal geometry may have an effect on the morphology of tumor growth (Norton et al., 2006; Sanga et al., 2007). In one example, the roughness of the tumor surface was shown to change periodically in a cellular automata model of breast cancer growth (Sedivy et al., 2002). This model was shown to have similar fractal dimensions to an experimental study using breast cancer cell lines (Sedivy et al., 2002). Similarly, in a model of avascular tumor growth, Ferreira et al. found that fractal dimension could characterize whether a tumor had a solid or papillary architecture (Ferreira et al., 2003). Other studies have also found that the fractal dimension of breast images were useful for predicting breast cancer risk (Rangayyan et al., 2007). Overall these models indicate that the morphology of the tumor, as determined by fractal dimension, is an important indicator of growth of a tumor.

Evidence from various studies have also indicated that cellular dynamics play an important role in tumor morphology (Alarcón et al., 2003; Anderson et al., 2006; Schmeichel and Bissell, 2003). These studies are well summarized in a recent review describing computational modeling of connections between tumor phenotype and tumor morphology (Sanga et al., 2007). Notably, one group has used techniques from fluid mechanics to evaluate changes in cell shape during cancerous growth (Rejniak and Anderson, 2008). These authors simulated the evolution of acini whose epithelial cells undergo growth, division, and death under different levels of polarity and found that distinct morphological changes appear as these parameters are varied. We are interested here in determining which features of cellular dynamics are especially influential in the development of DCIS morphology.

Many cancer models have focused on the study of tumor growth in exclusively solid morphologies (for a review see (Araujo and McElwain, 2004)), one advantage of solid models being that the results can be directly compared to 3D cell cultures, especially in tumor spheroids (Byrne and Preziosi, 2003; Debnath and Brugge, 2005; Gudjonsson et al., 2002; O'Brien et al., 2002; Schmeichel and Bissell, 2003; Wang et al., 1998). Notably, (Drasdo and Hohme, 2005) used a cell based model to study the growth of avascular tumor spheroids. The method used in that work is similar to ours in that it examines avascular tumor growth using an individual cell based model, theirs being a more general model focusing on necrotic cell death as compared to apoptotic (Drasdo and Hohme, 2005). Another study developed a PDE model of solid tumor growth in response to nutrient availability (Greenspan, 1976). The authors analyzed the stability of the tumor colonies under small perturbations in motion and under modifications in nutrient supply and pressure, and found that colonies forced to share the same supply of nutrients repel one another and separate, whereas colonies remain stable when their surface tension is not overcome by pressure (Greenspan, 1976).

Of particular interest to many investigators is the development of quiescent and necrotic cellular zones within the tumor region (Nagy, 2005; Roose et al., 2007), which are relevant to our study since necrosis is often found in late-stage DCIS. Specifically, many studies of solid tumors focus on tumor cell diversity and nutrient competition leading to necrosis (for a review

see (Nagy, 2005)). One hypothesis arising from these models is that natural selection will favor cells with aggressive proliferation (Franks et al., 2003), efficient use of scarce nutrients (Ambrosi et al., 2002), and insensitivity to hypoxia within the tumor environment (Gatenby et al., 2003; Gatenby et al., 2004) (Nagy, 2005). The importance of acidosis and hypoxia on the growth of tumor has further been assessed in recent computational models of cancer invasion (Gatenby and Gawlinski, 2003; Gatenby and Gillies, 2008; Gatenby et al., 2007; Sanga et al., 2007).

A significant class of studies have also been performed in two related areas, namely angiogenesis and the development of invasive disease (for reviews see (Gatenby and Gillies, 2004; Mantzaris et al., 2004)). In a series of models of angiogenesis, Chaplain and colleagues (Chaplain et al., 2006; McDougall et al., 2006; Stéphanou et al., 2006), modeled tumor induced angiogenesis, focusing on blood flow and tumor angiogenic factors in relation to the development and remodeling of capillary networks. A goal of these models is to understand how growth of and transport through capillary networks affects the nutrient supply to the tumor, so as to improve drug delivery. Alarcon and coworkers have also developed models of cancerous and non-cancerous regions (Owen et al., 2009), focusing on the interplay between the generation of new vessels in under-perfused regions and vessel pruning under conditions of low wall shear stress. This multi-scale model concluded that long-term tumor size and vascular density are largely insensitive to initial vascular conditions angiogenesis and vasculature remodeling in (Owen et al., 2009). The study of angiogenesis has also been used to understand how cancer progresses during invasion; for example, recent computational modeling work has shown a connection between an invasive phenotype and a tumor's morphology (Sanga et al., 2007), and a previous study examined the effects of cellular and environment heterogeneity on tumor morphology (Anderson et al., 2006). In the latter work, a model examining the interactions between tumor cells and their environment showed that the distribution of extracellular matrix (ECM) and nutrients affected tumor morphology, with uniform ECM leading to a solid pattern, and grainy ECM or low nutrient availability leading to fingering patterns (Anderson and Quaranta, 2008). Although this area only peripherally relates to our topic at hand, we hope to incorporate these features into the model in later studies to investigate the development of invasive disease in DCIS.

Modeling of Ductal Carcinoma *in situ*—Modeling of tumor progression has shed light on the natural history of DCIS (for a review see (Byrne et al., 2006)). Notably, Franks et al. developed a continuum mathematical model to examine the effects of proteolytic enzymes on tumor growth and invasion, and the effects of hypoxia and pressure on enzyme production in comedo DCIS (Franks et al., 2005). Franks et al. report that intercellular pressure is more likely to affect enzyme production than hypoxia, and that invasion will most likely occur where duct deformation is the greatest, and hence where the membrane is the weakest, since this would be the point where the duct would exhibit the greatest strain. This approach is based on an earlier model that examined the shape of the tumor boundary and the extent of the cell's adhesion to the duct wall in early DCIS (Franks et al., 2003). By contrast, Bankhead and colleagues used a 3D cellular automata model to study the progression of hereditary and non-hereditary DCIS development (Bankhead et al., 2007). In another example, a 3D model was used to investigate the effects of stress in the transformation from DCIS to invasive ductal carcinoma (Paszek and Weaver, 2004). Lastly, Sontag and Axelrod used optimization of mathematical models to determine which transition pathway from DCIS to invasive cancer best fit the biological data (Sontag and Axelrod, 2005). These models have provided frameworks in which to study DCIS and have created hypotheses to be used for further testing.

Other computational models have also been used to study the development of architectural patterns of DCIS, most notably Rejniak and Dillon, whose model made predictions for DCIS morphologies by changing cellular reproduction and cell orientation variability (Rejniak and

Dillon, 2007). In that work, the authors used a model based on the immersed boundary method that treats cells as elastic bodies with the cytoplasm and extracellular matrix modeled as viscous incompressible fluids. That model includes cell growth, senescence, adhesion, and communication with other cells and the environment, and starts with a ring of cells surrounded by a basement membrane and six uniformly spread cells to initiate cancer growth. The authors found that differences in morphology result from changes in cell reproduction and orientation. Another notable paper is by Xu, who modified an existing model by Byrne et al. into a symmetric cylindrical geometry in which growth was nutrient limited, and found solutions that corresponded to cribriform, solid and papillary morphologies (Byrne et al., 2006; Xu, 2004). Although important, that work was continuous in nature; neglected necrosis, intra-ductal pressure and cellular interactions; and assumed cell density to be uniform. Our model, by contrast, includes forces as well as necrotic and apoptotic effects on each individual cell, but needs to be extended to 3D to understand the biomechanical influences that affect the architectural outcomes of DCIS.

1.2 Approach

In the present work, we simulate the progression of DCIS into distinct morphologies in an *in silico* model of cellular mechanics in a breast duct. Our goal is to understand how large-scale histological features in DCIS result from small-scale changes in cellular growth and death parameters. We use a discrete particle model where cellular interactions, such as cell-cell adhesion and polarity, can be directly simulated. Our model represents cells as discrete agents (spheres) that respond to compression and attract one another locally as damped springs (described in detail in the Appendix). Cells can migrate, reproduce, interact, die and are subjected to external, e.g., hydrostatic, forces (described in the Methods section), and for simplicity and speed of computation, we consider only spherical cells here. We start with a ring of cells, representing a duct of epithelia, surrounded by a tensile outer ring of myoepithelial cells, and we allow cells in the inner epithelium to reproduce stochastically. As will be described, we find that all of the known DCIS morphologies spontaneously self-assemble as intra-ductal pressure, proliferative rate, and apoptosis are parametrically varied.

In brief, we find from our simulations that proliferation causes new cells to buckle inward to form a fingering pattern, since the epithelial layer is constrained by a tensile myoepithelial layer. Intra-ductal pressure, by contrast, causes cells to be forced outward, stretching the duct and stabilizing buckling patterns that would otherwise appear. Finally, apoptosis stabilizes incipient patterns by eliminating high cell density regions. Apoptosis produces spaces between neighbors, and causes subsequent cellular rearrangements that lead to changes in the resulting ductal morphology. As we will show, each of these parameters plays an important role in breast duct morphogenesis, and as illustrated in Fig. 1, varying these parameters permits us to reproduce all identified DCIS morphologies.

The outline of this paper is as follows: in the Methods section, we describe the computational model that we use to simulate DCIS development. Then in the Results section, we define a classifier to distinguish between different morphologies, we construct a parameter space that determines the boundaries between subtypes, and we identify time progressions from one morphological subtype to another. Finally in the Discussion section, we analyze the changes in morphological subtype with variations in the parameters that most strongly affect biomechanical forces: intra-ductal pressure, cellular proliferation, and apoptosis.

2. Methods

Our model simulates the biomechanical evolution of simplified cells in the presence of cellular repulsion, attraction, proliferation, and death (apoptosis and necrosis) as sketched in Fig. 2, as well as cell polarity, intra-ductal pressure, microlumen formation and cell migration. The

approach is based on a computational model reported earlier (Caicedo-Carvajal and Shinbrot, 2008; Shinbrot, 2006) that has been used to simulate tissue and organ development, with the addition here of features specific to DCIS such as intra-ductal pressure, cell death and microlumen formation (described subsequently).

A synopsis of the simulation is as follows; algorithmic and other details are included in the Appendix. Cellular repulsion is simulated by using a Voigt model (Zhu et al., 2000) that causes cells to repel one another by applying linear damped spring forces where they overlap (see Figure 3). Likewise we use a Voigt model to simulate membrane-bound cellular adhesion, i.e. cells attract one another with a force proportional to the distance between them. Two types of cellular attraction are included: homotypic and heterotypic. Homotypic attraction is present between similar type cells, e.g. epithelial cells in a monolayer, and is applied only between a cell and its two neighbors. In this 2D model, we define a cell's neighbors to simply be the two cells on either side of it in the initial ductal configuration. When a cell divides, the new daughter cell of the same size as its parent is placed between two cells, the parent cell and its successive neighbor, and these two cells become the daughter cell's neighbors. Heterotypic attraction occurs between different type cells, i.e. between epithelial cells and surrounding myoepithelial cells. We take homotypic strengths to be larger than heterotypic ones, since the myoepithelial attraction is simply used to attach the epithelial cells to the ductal boundary. When a cell reproduces or dies (below), the list of adjacent neighbors is updated.

Proliferation (mitosis) occurs in the inner epithelial layer such that one-fourth of the cell population, chosen at random, reproduces once per cycle (a predefined number of computational time steps). Each selected progenitor cell reproduces to form two progenitors, which are each in turn eligible to proliferate. Cells reproduce asynchronously, with proliferation occurring in only a subset of the cells at a given instant in time, as has been reported in breast cancer cell lines (Kulp et al., 1996; Manni et al., 1992). We model cell death by assuming that cells die (again asynchronously) either by apoptosis, in which case the dead cell is removed from the simulation altogether, or by necrosis, in which case the cell remains as debris that can no longer adhere or reproduce (shown in gray in Fig. 1h).

Apoptosis is considered to be triggered by the mechanical stress of overcrowding, as has been previously proposed (Marceau et al., 2001; Shraiman, 2005). Apoptotic cells are removed from the simulation, in keeping with normal physiology in which apoptosis triggers a cascade of events leading to the exocytosis and digestion of a cell and its contents. Algorithmically, we remove a cell from the simulation with a fixed probability (typically between 0% and 2% in each time step), whenever there are more than 10 cells within a fixed distance (two and a half cell diameters) of the cell of interest. In this way, we model the effect of stress in a localized area. We find that by modeling apoptosis using these parameters, our model produces similar growth dynamics to DCIS growth reported in the literature (Mohsin and Allred, 2004). Necrosis occurs when a cell is 10 cell diameters away from the myoepithelial cell layer, since nutrients become limited at that distance without angiogenesis. The necrosis distance of 10 diameters was estimated by examining images of DCIS specimens in our laboratory, and is supported in the literature (Gatenby and Gillies, 2004; Nagy, 2005; Wolberg and Mangasarian, 1990).

Cells are initially placed in a circular monolayer that represents epithelial cells in a duct (shown in blue in Figs. 1 & 2), surrounded by a multiply-wrapped myoepithelium (green in the figures). *In vivo*, myoepithelial cells (encircled in Fig. 1a) surround normal ducts, forming a natural border that separates epithelial cells from the basement membrane and stroma. The myoepithelium also has a contractile function (Barsky and Karlin, 2005; Gudjonsson et al., 2002; Polyak and Hu, 2005). Similarly in the model, myoepithelial cells serve both as a barrier to invasion of the epithelial cells (as the epithelial cells have to break through multiple layers before they can breach the myoepithelial cell layer) and as a contractile layer that counteracts

intra-ductal pressure (since the cell-cell adhesion maintains the closed ductal structure and prevents the myoepithelial cells from unraveling). The number of myoepithelial cells remains constant during the simulation, although sufficient pressure on the myoepithelial cells can cause the myoepithelial layer to stretch such that gaps form between the myoepithelial cells.

We model cell polarity by constraining the epithelial cells to adhere laterally such that each cell adheres only to two epithelial cells at any one time, the cell's two neighbors (Shinbrot, 2006). The neighbors may change throughout the simulation as we have mentioned, but each epithelial cell adheres only to two neighboring epithelial cells. This is biologically relevant as normal epithelial cells grow in a monolayer and adhere to one another on their lateral sides (Paredes et al., 2002).

Since breast ducts contain interstitial fluid, and since cancerous tissue is notoriously leaky to blood and lymphatic fluid (Heldin et al., 2004), intra-ductal pressure caused by fluid buildup is included in the model. This is done by applying a normal, outward pressure to each epithelial cell, indicated in the right panel of Fig. 2. Intra-ductal pressure is modeled by exerting a force on epithelial cells in the direction perpendicular to the cell layer, determined by the locations of the two neighbors of a given cell (for more information see the Appendix). The direction of the intra-ductal pressure consequently varies with the position of the cells, and the amplitude of the pressure is fixed and determined at the beginning of the simulation.

An additional element included in the model is that new microlumens are permitted to form when cells come within a cell diameter of one another. A microlumen is a fluid-filled, non-cellular region within the cancerous DCIS tissue, commonly seen in the cribriform morphology. This occurs, for example, when the epithelial layer folds over (due to crowding of reproducing cells), and one projecting fold comes within close contact to another fold, as illustrated schematically in Fig. 4. When this occurs, cells at the point of nearest contact merge to form a new lumen. Algorithmically, they achieve this by moving closer to one another and being designated to be one another's neighbors. The cells within each microlumen are then relabeled and the neighbors of the "touching" cells are reassigned such that the cells form two separate lumens. Once a new microlumen is formed, intra-ductal pressure and mitosis are implemented separately within each microlumen but the cells in each microlumen can exert forces on one another if they overlap.

The cellular morphologies of interest are not simple amorphous aggregates, and to prevent such non-physiological aggregates from forming, we permit cells to form new microlumens only if they are separated along the epithelial monolayer by 10 or more cells. Without this requirement, papillae invariably degenerate into aggregated clumps. Microlumen formation by fusion of micropapillae has been seen *in vivo* (Jaffer and Bleiweiss, 2002); similarly in another well-studied system, the kidney, a pretubule aggregate develops and proliferation occurs to create a new lumen (Kroschewski, 2004). The smallest pretubules in that case are 4 to 6 cells, therefore after proliferation the resulting pretubule lumen would consist of around 10 cells (Kroschewski, 2004). Examples such as this from the literature indicate that a 10 cell limit on microlumen formation is physiologically justified as well as being computationally effective in producing microlumens without permitting large aggregates to form. Moreover, this procedure produces cribra similar in size and shape to those seen *in vivo* (cf. Fig. 1b, f).

Cell motion is calculated using velocity Verlet integration (Coté et al., 2001; Swope et al., 1982). The velocity Verlet approach is commonly used in molecular dynamics simulations and is more accurate than the simpler Euler integration scheme. Random cellular migration is modeled as an integrated random walk with viscosity (Shinbrot, 2006), in which velocities in x and y directions are incremented by noises taken from a uniform distribution up to a maximum

value. The acceleration is calculated using the forces from the cellular adhesion, cellular repulsion and intra-ductal pressure, see the Appendix for more information.

All of the parameters in the model are taken to be dimensionless, using values (see Appendix) that produce biologically realistic and computationally stable results (Caicedo-Carvajal and Shinbrot, 2008; Shinbrot, 2006). Cellular responses to compression or interaction are calculated from *strains* (dimensionless deformations) imposed between cells, and not from *stresses* (dimensional forces per unit area). This approach has two benefits: first, it is consistent with prior approaches used in the literature (e.g. Reynwar et al., 2007), and second, it permits comparisons to be made between our simulations and *in vivo* or *in vitro* snapshots, without measuring or calculating actual forces between cells. Although the parameters are dimensionless, we used average DCIS apoptosis and proliferation rates (Mohsin and Allred, 2004) as well as an average doubling time (Kopans et al., 2003) from the literature to estimate that one iteration in our model is approximately 17 hours in real time.

The parameters in this model are governed by multiple factors, such as the interplay of hypoxia, growth factors, cell signaling, etc. that we represent using a phenomenological lumped parameter model. For example, numerous factors influencing necrosis create a length scale that regulates when cells die, so rather than constructing a complex model using multiple (largely unknown) dimensional parameters involved in necrosis, we use the observation that cells die when they exceed an average phenomenological distance of 10 cell diameters from a vascularized outer membrane. Similarly, there are many ways to model apoptosis and we use a phenomenological approach using overcrowding as the triggering effect which takes into account many factors such as mechanical stress, lack of nutrients, etc. This approach seems to generate a veridical model for cell death.

In summary, the model developed here for the time evolution of DCIS incorporates several additional features over prior models (Shinbrot, 2006), namely myoepithelial cells, cell death, intra-ductal pressure, and microlumen formation. We allow for two forms of cellular death: necrosis and apoptosis, so the cellular diversity at a given instant in time includes cancerous epithelial cells, surrounding myoepithelial cells, and necrotic cells.

3. Results

3.1 Generation of Morphological Types

We find that all four architectural subtypes can be reproduced by variations of the parameters that we have described, as shown in Fig. 1. The simulations in this figure used at least 700 time steps with different parameter values for apoptosis, intra-ductal pressure, and mitosis rate (see figure caption), starting from 60 initial progenitor cells surrounded by 270 myoepithelial cells. Although the coincidence between *in silico* and *in vivo* morphologies by itself does not imply that the model is correct, the availability of a model permits us to predict the effects of parameter variations – subject to the model’s simplifications – on morphological development. Our approach is to use this simplified model to make hypotheses about DCIS morphogenesis as a function of these parameters.

3.2 Decision Tree Classifier

In order to objectively determine when each morphology appears, we created a “decision tree classifier” (Hastie et al., 2001) that separates samples into one of the four histological subtypes (See Fig. 6). The first branch of the decision tree classifier discriminates between cribriform and other states. The reason for using this as a first branch is that the cribriform subtype is the most variable of the subtypes, insofar as it may or may not fill the entire ductal space and may or may not display necrosis, but is easily recognized by having fluid filled circular spaces, or

“holes,” between the cancer cells. By identifying these holes, we can thereby distinguish the cribriform architecture and then move on to distinguishing the other architectures from the non-cribriform remaining structures.

We identify spaces from our images as follows. First, we locate the centers of each cell by eye, which is a straightforward and unambiguous process both *in vivo* and *in silico*. Then cell locations were converted to black and white, with white being cellular and black being “empty” or noncellular. If the sample is a histological section, this was done by estimating the size of each cell to be the average distance from the closest four cells. If the sample is obtained by simulation, we use the model cell size for each cell location recorded for each cell in the simulation. Each cell location is represented as a disc with the estimated cell size, and these cell sizes are enlarged slightly to fill any small gaps for the purpose of producing unambiguous classifications by creating a smoothed, continuous representation of the cancerous region. The internal empty regions are then identified and holes that are larger than the largest cell size are counted. If there are more than two holes of this size in the interior of the DCIS mass, the specimen is classified as cribriform.

Once cribriform and non-cribriform structures are distinguished, the second branch discriminates between comedo and non-comedo specimens. This is done simply by determining whether or not necrotic cells are present. For *in vivo* specimens, this information was provided by a co-author (NB), who is a trained and practicing cancer pathologist, while for *in silico* snapshots, the state of each cell is known *a priori*.

Of the remaining morphologies that have been identified as containing no necrotic cells, we note that DCIS specimens can be separated into either uniform or annular cell distributions of average cell density; solid and cribriform (filled) patterns are both examples of uniform distributions, while micropapillary and comedo states are distinctly annular. Accordingly, if the cross-section of the remaining morphologies yields a bimodal (large cell density at diametric extremes) distribution, the structure must be micropapillary; otherwise it must be solid. To obtain the cross-sectional modality, we azimuthally averaged the density distributions of the duct image as described in Fig. 6.

In detail, starting with images of histological DCIS specimens, our approach first used the cell locations to calculate the centroid of the image. Then 100 radially oriented boxes (depicted in Fig. 7) were oriented randomly through the centroid, and cell density was assessed by averaging across density vs. radius plots obtained from these 100 boxes. The widths of the boxes used were chosen arbitrarily to be 1 standard deviation of the distribution of the distances from the cell population to a line through the centroid. The boxes were then divided into 20 evenly spaced bins, from which a series of histogram profiles were generated. These were ensemble averaged to produce a mean density profile that was used to distinguish uni-modal from bi-modal distributions as described above. We determine the modality of the distribution by comparing the maximum density in a bin to the value of the median bin. If the difference between the maximum and the median value is greater than 2 standard deviations, then the distribution is defined to be bimodal; otherwise it is uni-modal. If the distribution is found to be uni-modal, it is classified as solid, otherwise it is classified as micropapillary.

This method was tested on a set of DCIS histology specimens and a set of DCIS model figures. It takes about 0.3 seconds to create and analyze each figure. On the training set of 68 *in silico* images that could be easily classified by eye, including cribriform patterns with and without necrosis, the classifier correctly classified the data 88% of the time. This training set consisted of 15 micropapillary, 20 cribriform, 20 solid and 13 comedo subtypes. On the training set of histology images, it correctly classified DCIS subtypes with 92% accuracy for a set of 12 *in vivo* images from histological cross-sections taken from human breast biopsy samples,

as determined by consultation with NB. These 12 images consisted of 3 micropapillary, 4 cribriform, 3 solid and 2 comedo subtypes. To validate that this approach correctly classifies model images, the same method was used to classify a test set of *in silico* images and a test set of *in vivo* images. Using a test set of a randomly generated model images that were between 400 and 700 iterations, the test set was 94% accurate correctly identifying 65 out of 69 images. This test set generated 53 micropapillary, 7 cribriform, 4 solid and 5 comedo subtypes that were easily classifiable. Simply looking at the 16 cribriform, solid, and comedo subtypes, the classifier was accurate 81% of the time. On a test set of 5 *in vivo* images, the classifier was 100% accurate. We deemed that this was a suitable classifier that could be used to objectively identify both *in vivo* and *in silico* images.

3.3 Parameter Space

In order to identify the parameter values that lead to the various DCIS subtypes, we varied intra-ductal pressure, the mitosis rate and the apoptotic rate in independent simulations, each lasting for 600 iterations. We ran each parameter set 20 times and used the decision tree classifier described previously to produce the phase diagrams shown in Fig.8. Each parameter region was determined to be of a particular subtype if 80% or more of the runs were classified as one specific subtype, otherwise, the region was identified as mixed. Apoptosis rates were varied from 0% to 2%, intra-ductal pressure from 0 to 0.18, and proliferation from 6 mitosis events to 20. At parameters outside of these ranges, the morphology either changed little or became non-physiological. For example, if the apoptotic rate is much above 2%, all cells die; similarly if the intra-ductal pressure is set at too high, the duct expands substantially and cells break through the myoepithelial layer.

Fig. 8 summarizes cross-sections of the states produced along planes of fixed apoptotic rate. Between parameter regimes separated by recognizable morphologies such as in Fig. 1, we found a range of intermediate morphologies that were not easily classifiable into any unique subtype. These intermediate morphologies often contained regions that could be characterized by 2 or 3 different subtypes. For instance, for apoptosis of 1%, at lower levels of pressure and at a very high mitosis rate (before the subtype becomes cribriform), we find a mixture of cribriform, solid and micropapillary subtypes. At apoptosis of 0%, we see that at high mitosis rates (about 12 times over the simulation) and moderate pressure, the mixed regions consist mostly of solid and comedo subtypes, and at higher pressures mostly comedo and micropapillary result. This raises the possibility that these are genuine intermediates between morphological subtypes, which would suggest that one morphology might progress to another over time: a possibility that we discuss shortly.

We ran one set of simulations that covered the parameter space for 1000 iterations to see if we saw any long standing trends over the iterations. We found that most of the outcomes that covered the parameter space appeared to approach a steady state where they stayed micropapillary at low mitosis and high apoptosis, cribriform at high pressures or reproduction rates with apoptosis, or comedo at very low apoptosis. We also found evidence of a cribriform subtype that is transient between the regions of steady state micropapillary and cribriform, indicating that some subtypes switch either between cribriform and micropapillary or between cribriform and solid during their progression to a steady state, although many of the images found in the micropapillary to cribriform switches had characteristics of both subtypes, which may have led to this classification. The transient states occurred principally at 1% apoptosis rate, but there were some instances at 2% apoptosis rates at moderate pressure and reproduction rates. For both of these levels of apoptosis, moderate to high pressure and reproduction mostly led to a steady cribriform pattern, suggesting that there may be two different cribriform patterns: one that is transient and one that is steady.

In general, we find that high apoptotic rates produce mostly micropapillary tumors, changing to cribriform subtypes when the mitosis rate and pressure is high. We only see other subtypes when mitosis occurs more than once every 50 iterations. This supports the hypothesis that in order to progress to a higher grade subtype (solid or comedo), cells must develop a resistance to apoptosis and/or an increased mitosis rate.

On the other hand, very low or nonexistent apoptotic rates produce comedo tumors for a high rate of mitosis, solid tumors at low pressure and high mitosis (rates between 8 and 10), and micropapillary tumors with high pressure and moderate mitosis rate or with a low mitosis rate. Intriguingly, the level of intra-ductal pressure affects whether the subtype will be solid or comedo: a result in accord with some data correlating pressure with cancer development in more advanced Invasive Ductal Carcinoma (IDC) (Boucher and Jain, 1992; Nathanson and Nelson, 1994). Based on our results, it seems that a low apoptosis rate is necessary to form a comedo morphology. At 1% apoptosis, we observe that very high mitosis rates and low intra-ductal pressure produces a mixed subtype that transforms to a cribriform structure.

3.4 Progressions Between Morphological Types

Apparently, each parameter affects the morphology in ways that are not intuitive, and morphological subtypes seem to progress from one to another over time. To more fully explore these transitions, we recorded the model outcomes as a function of time. A total of 700 time steps were simulated with different parameter values for apoptosis, intraductal pressure, and mitosis rate, within the same parameter ranges and using initial cell conditions previously discussed. Images of the simulated cell morphologies were recorded every 100 timesteps, resulting in 7 consecutive images in time for each set of parameter values. These images were then examined for evidence of progressions from one morphological subtype to another.

This examination suggested three distinct types of progressions that occurred frequently within the parameter space. In the first of these, exemplified in Fig. 9, a simple progression from micropapillary to cribriform appeared, with the cribriform pattern sometimes exhibiting necrosis when it grew far from the myoepithelial layer. In this case, sparse foliations of papillae joined to form cribra, which ultimately progress until the center of the tumor develops necrosis. This occurs at a moderately low rate of apoptosis, a moderately high pressure, and a very high rate of mitosis. In this parameter range, the tumor displays local regions in which cells are absent throughout the entire observed time span, and necrosis is seen only at very late stages of development. Interestingly, evidence of cribriform patterns with a central necrotic core similar to those shown in Fig. 9 have also been reported in human DCIS cross-sections (Jensen, 2000). These necrotic cells occur because the cells have grown farther away than 10 cells from the myoepithelial cell layer (and thus the vascular system) and die.

A second type of progression, shown in Fig. 10, was seen when a non-filled solid subtype, defined as a uniform growth of cells that does not completely fill the duct, progressed to a solid filled subtype, which then progressed further to a comedo subtype. This occurred when there was no apoptosis, a low rate of intra-ductal pressure and a high rate of mitosis. In this regime, the tumor grows uniformly and eventually fills the entire ductal region, leading to necrosis.

A third type of progression, shown in Fig. 11, was seen when there is moderately high intra-ductal pressure, in which a micropapillary subtype grows quickly but fairly uniformly under pressure such that a hole is left in the center of the duct that eventually fills with necrotic cells. This third type of progression is similar to the second type, with the difference that the duct does not completely fill with cells before necrosis occurs, so that a solid subtype is never formed. This occurs because the large intra-ductal pressure forces the duct to expand before it is completely filled. Thus cells mitose and reach a critical distance at which they necrose before

they fill the duct, and so necrosis occurs before a solid subtype emerges. This progression appears when there is a high mitosis rate and no apoptosis.

In Table 1 we summarize the progressions that lead up to the final morphologies shown in Fig. 8. We note that the progressions seen occur in different locations in parameter space, such that there are transitions from one type of progression to another as the parameters are varied. For instance at very high mitosis rates and no apoptosis, we see at lower pressures the progression goes from solid to comedo but as the pressure increases past 0.08, the progression goes directly to comedo from the micropapillary subtype. In this case, it seems that intra-ductal pressure prevents the cells from completely filling the central lumen before they necrose. We also find that the solid subtypes that appear at no apoptosis, moderate reproduction and low pressure also arise from a micropapillary subtype. Each type of progression occurs in different parameter regimes, which provides evidence that given morphologies may not be solely dependent on attributes of the tumor cells, but may also depend on the history of growth of the DCIS. This also suggests that additional metrics may need to be developed to adequately characterize a specific subtype.

4. Discussion

Our model readily reproduces each of the four morphological subtypes of DCIS: micropapillary, cribriform, solid and comedo. Thus, despite the evident simplifications, we suggest that the present model is sufficient to reproduce DCIS behaviors, and consequently permits us to probe the anticipated effects of changes in these parameters on tumor architecture in particular, and on ductal carcinoma development in general. Our model indicates that adhesion, intra-ductal pressure, proliferation, apoptosis, necrosis, and cell polarity affect the development of breast cancer in ways that are complex and inter-dependent. We examined timescales between one to two years, which is within the range for how long it takes for normal ducts to progress to DCIS (Collins et al., 2005; Weinberg, 2007). Specifically, to generate Fig. 8, we ran our simulation for 600 iterations for the parameter space, which corresponds approximately to 14 months real time. For our studies of transitions between states, we ran simulations up to over 1000 iterations, which is roughly equivalent to 23 months real time. We limited the model evolution to this upper bound because larger values tended to produce results in which the epithelial cells burst out of the myoepithelial layer under the influence of proliferative pressure, and would therefore not be considered DCIS.

Our phase diagrams indicate that there are distinct parameter regions in which we each architectural subtype predominates. Thus, comedo subtypes are found in regions with very low apoptosis rates and high mitosis rates, and solid subtypes are found at very low apoptotic rates with low pressure and moderate to high mitosis rates. We find that provided that there is limited apoptosis and mitosis occurs at least at a moderate rate, morphologies invariably become comedo. As shown in Table 2, it seems that if a model duct has a solid or comedo DCIS, then their cancer cells must have developed a resistance to apoptosis, as well as some self-sufficiency in growth signals, or a resistance to anti-growth signals.

Cribriform subtypes, on the other hand, are found in our simulations either as a transient DCIS presentation or as an advanced presentation with apoptosis, inter-ductal pressure greater than 0.08 and high mitosis rates. This indicates that a duct with longstanding cribriform DCIS will have little resistance to apoptosis, and this also implies that the cancer cells will tend to have self-sufficiency in growth signals or a resistance to anti-growth signals. Finally, micropapillary subtypes are found for lower mitotic rates and more frequently with larger apoptosis rates, indicating that a duct with micropapillary DCIS should have cancer cells with little self-sufficiency in growth signals and little resistance to anti-growth signals - essentially where apoptosis is high enough to counteract proliferation.

Some of our results find partial support in the literature; for example, among the findings that are in accord with *in vivo* studies are the following:

1. Our simulations indicate that patterning in ductal carcinoma is tightly connected with the intra-ductal pressure present – thus for the same proliferative and apoptotic rates, at low pressure we find that solid patterns emerge, while at high pressure, comedo, micropapillary or cribriform patterns predominate. The mechanism for this morphological change is simply that increased pressure tends to inflate the duct, producing more luminal volume, and in further studies (not shown here), we have found that high intra-ductal pressure invariably compromises the myoepithelial sheath of the duct, causing escape of epithelial cells. By contrast, studies comparing normal breast tissue, benign breast tumors, DCIS, and Invasive Ductal Carcinoma (IDC) have concluded that IDC had significantly higher levels of intra-ductal pressure than normal samples (Boucher and Jain, 1992; Nathanson and Nelson, 1994). The higher levels of intra-ductal pressure can occur for numerous reasons, including leaky blood and lymphatic vessels, and compression due to the expanding mass of tumor cells (Butcher et al., 2009). Thus our results suggest that some attributes of the advancement of DCIS to IDC may be associated with biomechanical stresses, rather than being purely due to biochemical changes as is commonly assumed (Wiechmann and Kuerer, 2008).
2. The observation in a computational model that different subtypes may progress from one to another with mixed specimens forming in intermediate regions of the parameter space, combined with the clinical observation that multiple subtypes can coexist in the same specimen or duct, i.e. invasive cancer can coexist with DCIS in the same specimen and/or multiple morphologies can be seen in the same duct or in nearby ducts, suggests that new metrics may be needed to fully distinguish one state from another. Thus our results indicate that a cribriform pattern may either be an advanced, steady state for a rapidly dividing cancer subject to active apoptosis, or it may be a transient state associated with the merging of growing papillae in the absence of apoptosis. Determining the treatment and prognosis of a particular presentation thus requires more information than is currently obtainable from snapshots taken from biopsy specimens obtained at an instant in time.

We have also seen that three distinct time progressions may be present: one that progresses from micropapillary to cribriform, one that progresses from solid to comedo, and one that progresses from micropapillary to comedo. Consistently we see that in each progression, a central necrotic core eventually develops after a sufficient time has elapsed. Therefore necrosis, either in the comedo or in the cribriform subtype, seems to be associated with late stage DCIS development, and indeed this is in agreement with some clinical data, identifying necrosis as being associated with worsened patient prognosis (Bodis et al., 1996; Cornfield et al., 2004; Jaffer and Bleiweiss, 2002; Lagios, 1996).

3. Evidence of progressions between subtypes has also been reported in clinical studies. For example, one study found evidence for an atypical hyperplasia to a comedo progression and evidence of a high grade solid DCIS which might also be associated with this path (Mariuzzi et al., 2002), and an association between tubular carcinoma and cribriform and micropapillary DCIS has also been reported (Fernandez-Aguilar et al., 2005). Moreover, a study of karyometric features from H&E stained slides reported distinct low grade and high grade progressions (Mariuzzi et al., 2002). That work reported two trends of evolution: 1) hyperplasia to atypical hyperplasia and comedo DCIS, and 2) hyperplasia to cribriform to solid DCIS.

Furthermore, our results show that solid and comedo morphologies can be produced in substantially different parameter regimes than are micropapillary and cribriform structures. Consequently, these subtypes may be characterized by distinct genetic or phenotypic profiles and may as a result have different potentials for invasion. Our simulations also indicate that the comedo subtype can progress from a solid subtype without any changes in our parameters, and so the comedo state may be an “end point” in DCIS progression.

4. In a related finding, our model sometimes generates regions in the parameter space that can be unambiguously identified as one of the known subtypes, but also produces co-existing subtypes in certain regions of the parameter space, such that a single duct has similar qualities to two different morphological subtypes, and instances in which the model produces different classification results in different computational trials under the same parametric conditions. This ambiguity may itself indicate the presence of time dependent progressions from one morphological subtype to another, such that the coexisting subtypes occur because the duct is in an intermediate stage between two subtypes. This is consistent with recent reports of differences in histological subtypes within distinct regions of a single DCIS or Invasive Ductal Carcinoma (IDC) duct (Allred et al., 2008).

In conclusion, we have elucidated relationships between morphological subtypes of ductal carcinoma of the breast by computer simulation. Our model suggests that each morphological subtype tends to result from a well-defined sub-range of parameter values and that certain morphologies will progress to others over time. These findings present several open questions. Firstly, it remains to be demonstrated whether the predicted changes in patterns (Fig. 8) as a function of pressure and proliferative and apoptotic rates are borne out *in vivo*. Secondly, our simulations indicate that cribra can be produced in one of two ways – either through merging of papilla in a less aggressive cancer, or through apoptosis of a solid tumor in a more aggressive/advanced tumor. It would be valuable to find additional markers that can discriminate between these different presentations of cribra. Thirdly and closely related, our model has generated distinct progressions, suggesting that the morphology observed at an instant in time is not a complete characterization of the state of a patient’s DCIS. Again, this points to the importance of identifying markers that can provide a complete representation of the history, as well as the current state, of a tumor.

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Appendix

Appendix:

1. Setup

Cells are modeled as spherical particles that interact through linear spring forces such that they repel one another when overlapping. The cells are initially placed in a circular monolayer of 60 cells, which is a realistic size (Bankhead et al., 2007; Bussolati et al., 2000) that represents epithelial cells in the duct (shown in blue in examples of Fig. 1), surrounded by a multi-wrapped myoepithelium (green in Fig. 1). The initial cell population consists of two cell types, myoepithelial cells that maintain the structure of the duct and epithelial cells whose growth pattern determines the architecture of the tumor. The epithelial cells are larger than the

myoepithelial cells such that the epithelial cell radius r_e is .5 while the myoepithelial cell radius is r_m is .25. In the model, the myoepithelial cells serve both as a barrier to invasion of the epithelial cells and as a contractile layer that counteracts intra-ductal pressure. The myoepithelial cells prevents escape of the epithelial cells outwards because they are wrapped multiple times around the epithelial cells and so an epithelial cell would have to break past multiple myoepithelial cells before it can breach the layer. The myoepithelial cells counteract intra-ductal pressure by pushing back an epithelial cell under pressure. Since each cell adheres to its neighbor a myoepithelial cell being pushed outwards by a epithelial cell will feel an adhesive force from its two neighbors pulling it back towards its neighbors.

The intra-ductal pressure, apoptosis rate and cell mitosis rate are set at the beginning of each run of the simulation and remain constant during each simulation. We assume that all cells in the duct have a determined rate of proliferation, apoptosis, and have a certain “leakiness” that would create a fixed intra-ductal pressure rate. All other parameters are shown in the table below:

Table 3

List of parameter values

Parameter	Explanation	Value
r_e	Epithelial cell radius	0.5
r_m	Myoepithelial cell radius	0.25
D	Maximum intensity	0.1
$1-\kappa$	Viscosity of cells	0.8
k	Spring constant	2
β	Damping coefficient	0.05
ω_1	Homotypic attraction	-10
ω_2	Heterotypic attraction	-1
Δt	time step	0.5

2. Microlumen formation

Initially the breast duct consists of one lumen, with a layer of epithelial cells surrounded by a multilayer of epithelial cells and a fluid filled space within, but local microlumens are known to form in DCIS, therefore we have included this aspect in our model. A microlumen is formed when a cell and its neighbor are closer than 1 epithelial cell radius apart, r_e , from two other neighboring cells and the cell is greater than 10 cells away on the monolayer from the cell it is forming the loop with. When the microlumen is formed, one cell from each microlumen is moved halfway between its initial position and its new neighbor to close the loop and the cell neighbors are reassigned to two separate lists, one for each microlumen. These microlumens are considered separate for intra-ductal pressure, proliferation, and polarity (maintenance of monolayer).

3. Mitosis rate

Mitosis occurs every set number of iterations as determined at the start of the simulation. When mitosis occurs, one fourth of the cells are randomly chosen to reproduce and make a daughter cell. This is done by placing a new daughter cell between the reproducing cell and one of its neighbors.

4. Cell death

Apoptosis occurs where there are more than 10 cells within two and a half cell diameters from the cell of interest. This cell will die a certain predetermined proportion of the time (the apoptosis rate defined at the beginning of the simulation) whenever this condition is met. When the cell dies from apoptosis, it is removed completely from the system and its two neighbors become neighbors in the subsequent time step. We also assume in this system that cells do not automatically die when they become detached from the basement membrane because otherwise the lumen would always be empty, as shown in other work (Debnath et al., 2002; Rejniak and Anderson, 2008)

Necrosis occurs when a cell is determined to be greater than 10 cell diameters from the multi-layer of myoepithelial cells. When this occurs, the cell is labeled as dead but continues to take up space. It no longer takes part in proliferation, adhesion, intra-ductal pressure, microlumen formation, etc. but it does repel cells that overlap it.

5. Intra-ductal Pressure

The intra-ductal pressure, $P_{i,n}$, of cell i at time step n , is calculated by applying pressure perpendicular to the epithelial cell layer, and $P_{i,n}$ is calculated separately within each microlumen. The program uses the order of the cells (since all cells must remain on a monolayer) to exert a pressure on the cell perpendicular to the average of the two vectors connecting it to its two neighbors. In short, the intra-ductal pressure is calculated by taking a normal unit vector $\mathbf{x}_p$ multiplied by a pressure constant p .

$$P_{i,n} = p \mathbf{x}_p \quad \text{E A1}$$

6. Cellular Interactions

Cellular interactions include cellular repulsion, which occurs when cells overlap, and cellular attraction, which represents cell-cell adhesion and cell-substrate adhesion. If two cells overlap one another, they repel as if connected by springs; neighboring cells are attracted by a force proportional to the distance between the two cells. Each epithelial or myoepithelial cell has two neighbors, one to the left and one to the right, to which it adheres. This represents apical-basal polarity, in which a cell attaches to its neighboring cells and forms tight junctions laterally, with its basal side facing the basement membrane (or myoepithelial cells in the case of the model) and its apical side facing the lumen. The cells are catalogued such that cell $i-1$ is the right neighbor of cell i and cell $i+1$ is the left neighbor of cell i .

For each cell we follow the same procedure:

a. Overlapping Cells

First, we find any cells that are overlapping with cell i . For these cells, we find the force that each overlapping cell exerts on cell i , first, by finding the cell's displacement due to the overlap, $s_{i,n}$. We find $s_{i,n}$ by letting d_o be the overlapping distance, d_c be the distance between cell i and cell o (the overlapping cell), $\mathbf{x}_o$ be the overlapping cell coordinates, and $\mathbf{x}_{i,n}$ be the coordinates of cell i at time step n :

$$\mathbf{s}_{i,n} = \frac{d_o (\mathbf{x}_o - \mathbf{x}_{i,n})}{d_c} \quad \text{E A2}$$

We take the unit vector of $s_{i,n}$ and multiply this by the overlapping cell velocity, v_o to get the normal component of the overlapping cell velocity, v_o , which is determined in the previous timestep using equation E A5. We then use a spring-dashpot model to calculate acceleration due to overlapping cells:

$$\alpha_{i,n} = k s_{i,n} - \beta v_o \quad \text{E A3}$$

where k is the spring constant and β is the damping coefficient.

b. Cellular Attraction

There are two types of cellular attraction in this model: homotypic cellular attraction, i.e. myoepithelial-myoepithelial cell attraction or epithelial-epithelial cell attraction, and heterotypic cellular attraction, i.e. myoepithelial-epithelial cell attraction. Homotypic attraction occurs only between a cell and its two neighboring cells in order, in this way they are forced to remain on a monolayer. Both are modeled such that attraction is proportional to the distance between the two cells, with homotypic attraction being larger than heterotypic. The acceleration for cell i for time step $n+1$ is then calculated by adding all the α_o s of the overlapping cells to the sum of the homotypic and heterotypic attractions and the intra-ductal pressure $P_{i,n}$:

$$\begin{aligned} a_{i,n+1} = & \sum_{i=1}^l \alpha_{i,n} \\ & + \sum_{j=1}^2 \omega_1 s_{j,n} \\ & + \sum_{k=1}^q \omega_2 s_{k,n} + P_{i,n} \end{aligned} \quad \text{E A4}$$

where l is the number of overlapping cells, 2 represents the two homotypic neighbors, q is the number of heterotypic cells close by (within 2.5 cell radii), ω_1 is the homotypic attraction strength, ω_2 is the heterotypic attraction strength and $P_{i,n}$ is the intra-ductal pressure on cell i at time n (See E A1).

7. Cellular Movement

Cellular movement is calculated using the velocity Verlet integration method. The velocity Verlet algorithm updates the position $r_{i,n}$, velocity $v_{i,n}$, acceleration $a_{i,n}$ of a cell i from time step n to time step $n+1$ as follows:

$$\begin{aligned} r_{i,n+1} &= r_{i,n} + \Delta t v_{i,n} + (\Delta t)^2 a_{i,n} / 2 \\ v_{i,n+1} &= \kappa (v_{i,n} + \Delta t [a_{i,n} + a_{i,n+1}] / 2) + D x_r \end{aligned} \quad \text{E A5}$$

where κ is a viscous term, and $D x_r$ is the i -th cell's random motion. To calculate a cell's random motion, we take a random direction through a unit vector x_r and multiply it by a maximum intensity within range $[0, D]$. Acceleration is calculated the same as in cellular interactions.

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Figure 1.

Histologies of human ductal carcinoma *in situ* biopsy specimens (left), alongside corresponding structures from our *in silico* simulations (right). Panels (a) & (e) show “micropapillary” structures. In the simulations, the duct is surrounded by myoepithelial cells (green) that constrain the growth of the epithelial cells (blue). The myoepithelium is identified in (a) as the circled region. Panels (b) & (f) show the “cribriform” morphology; exemplar cribrum identified in (b) by arrow. Panels (c) & (g) show “solid” tumors, where the entire ductal lumen is filled with proliferating cells, and panels (d) & (h) show the “comedo” morphology, in which a central region, deprived of nutrients, undergoes necrosis. These necrotic cells are identified by the arrow in (d) and are shown in gray in (h). The *in vivo* cross-sections were classified by subtype by pathologist Dr. N. Barnard, after being acquired by biopsy, stained with Hematoxylin & Eosin (H&E), embedded into paraffin, sliced, and imaged. In the simulation, the “micropapillary” structure was formed at iteration 500 with an apoptotic rate of 1%, intra-ductal pressure of 0.14, mitosis every 50 iterations (see text for details). The “cribriform” structure was formed at iteration 700 with an apoptotic rate of 1%, intra-ductal pressure of 0.02, mitosis every 30 iterations. The “solid” tumor formed at iteration 700 with no apoptosis, no pressure and mitosis occurring every 88 iterations. Lastly, the “comedo” structure formed at iteration 700, with no apoptosis, intra-ductal pressure of 0.06 and mitosis occurring every 50 iterations.

Figure 2.

Model Elements. In the left panel, we show representations of repulsion, attraction, proliferation, apoptosis and necrosis used in the model. The solid arrows on the cells indicate forces, and the Xs denote cell death. In apoptotic cell death, the cell is completely removed from the simulation whereas in necrotic cell death, debris is left behind that occupies space. The larger open arrows represent transitions over time. In the right panel, we show the counteracting influences of outward intra-ductal pressure (black arrows) and inward myoepithelial contraction (red arrows) on the epithelial layer.

Repulsion

Adhesion

Figure Key:

r_c = Cell a radius

r_o = Cell b radius

d_c = distance
between cell
centers

$\vec{s}$ = vector
between cell
surfaces

Figure 3.

Cellular Repulsion and Adhesion. We calculate the forces on cell **a** due to repulsion using a spring-dashpot model, such that the net force equals $k\vec{s} - \beta\vec{v}_o$, where k is the spring constant, $\vec{s}$ is defined as above, β is the damping coefficient and $\vec{v}_o$ is the cell velocity. The acceleration on cell **a** due to adhesion is calculated simply the attraction strength (homotypic or heterotypic attraction constants) multiplied by $\vec{s}$, the attractive force (see Appendix E A2).

Figure 4.

Microlumen Formation. When two papillae, separated along a monolayer by 10 or more cells, come within 1 cell diameter from one other, the cells are attached by reordering cell positions so that they form new adhesions with the nearby papillae, thus forming two separate lumen regions (or “microlumens”), in which mitosis and pressure are calculated separately.

Figure 5.

Overview of the simulation program executed at each time step. Cell motion is implemented first using random additions to cell velocities, and interactions calculated from the cell positions from the previous time step. The program then checks whether microlumens should be formed and, if so, assigns the cells into separate microlumens in which pressure and mitosis are implemented separately. Next, mitosis is implemented, followed by apoptosis, and finally, necrosis. Cellular interactions (attractive and repulsive forces and intra-ductal pressure) are then calculated for the next iteration, and the cells chosen for apoptosis are identified. The cell locations are then logged for plotting, and the cycle repeats.

Figure 6.

Overview of decision tree classifier used to identify morphological subtype. First the number of cribra are determined (1), if there are more than 2 cribra the specimen is classified as cribriform. Afterwards, the presence of necrotic cells is established (2). If there are necrotic cells, the specimen is classified as comedo. Lastly, the modality of the distribution of binned cells across 100 random line segments is determined (3). Modality is illustrated in Fig. 6. If the distribution is uni-modal, the figure is classified as solid. If the distribution is bi-modal, it is classified as micropapillary. (See text for details)

Figure 7.

Classification of DCIS subtypes by cell distribution. Top: cell centers are identified, the centroid is calculated, and boxes are placed radially through the centroid at random orientations. Middle: cell densities are calculated in such 100 boxes. Bottom: these 100 densities are ensemble averaged to yield either uni- or bi-modal distributions. Note that micropapillary (far left) and comedo (far right) patterns produce bi-modal distributions, while filled cribriform (center left) and solid (center right) patterns produce uni-modal distributions.

Figure 8.

Characterizations of simulated DCIS subtypes as functions of apoptosis rate, proliferation rate, and intra-ductal pressure. Note that an ordinate of 20 means that mitosis occurs 20 times over the 600 computational iterations over which the simulation was run. White regions indicate multiple co-existing patterns. The numbers shown indicate the locations of the different progressions that were found, see Table 1 for further explanation. a) State space with apoptosis of 0%. Notice that, as expected, without apoptosis and with moderately high mitosis rates and low pressure (upper left), solid tumors predominate, becoming comedo at very high mitosis rates. At lower mitosis rates and higher intra-ductal pressures, the duct is enlarged by the applied pressure, causing the solid morphology to become micropapillary. b) State space with apoptosis of 1%. In this case, for sufficiently rapid mitosis, cribriform tumors are able to grow when the pressure or mitosis events are moderately high. c) State space with apoptosis of 2%, at which micropapillary tumors predominate. Mixed tumors arise at very high mitosis rates, and cribriform patterns arise at very high pressure and mitosis rates. At higher apoptosis and lower proliferation rates, only micropapillary morphologies survive, as cells within other structures are literally killed off.

Figure 9.

A time progression from micropapillary to cribriform with necrosis. At iteration 200 through 400 the state is micropapillary, at iteration 500-600 it is cribriform and at iteration 700 it becomes cribriform with central necrosis. In this simulation, the apoptosis rate is 1%, the pressure is 0.16 computational units, and mitosis occurs every 30 iterations.

Figure 10.

A time progression from solid to comedo. At iteration 400 the state is micropapillary, at iteration 500 it becomes a non-filled solid, at iteration 600 it is solid and by iteration 700 the state is comedo. In this simulation, the apoptosis rate was 0%, the pressure was 0.04 computational units, and mitosis occurred every 50 iterations.

Figure 11.

A time progression from micropapillary to comedo. At iteration 200, the state is micropapillary, at iteration 300 and 400 there is continued growth, and by iteration 500, the state is comedo. In this simulation, the apoptosis rate is 0%, the pressure is 0.12 computational units, and mitosis occurs every 50 iterations.

Table 1

Analysis of time progression transitions seen in Figure 8. For mitosis, 15 means that a mitosis event occurs 15 times over the 600 iterations. For apoptosis of 0% there is a transition at mitosis rate of 15, with low pressure resulting in transitions from solid to comedo and with high pressure resulting in transitions from micropapillary to comedo. At no apoptosis all the solid subtypes start out as micropapillary. At apoptosis of 1%, in the parameter space, all cribriform subtypes resulted from micropapillary subtypes originally.

	Progression	Apoptosis	Mitosis	Pressure
1	Solid to Comedo	0%	15	<0.1
2	μpap. to Comedo	0%	15	> 0.14
3	μpap. to Solid	0%	12 & 9	0 - 0.04
4	μpap. to Crib.	1%	24	> 0.1
		1%	20	> 0.16

Table 2

Biological events that may be correlated with the seen architectural subtypes.

DCIS subtype	Resistance to apoptosis	Self-sufficiency in growth signals or resistance to anti-growth signals
Comedo	yes	yes
Solid	yes	yes
Cribriform	no	yes
Micropapillary	no	no