

Using evolvable genetic cellular automata to model breast cancer

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Received: 15 November 2006 / Revised: 21 June 2007 / Published online: 4 October 2007
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Abstract Cancer is an evolutionary process. Mutated cells undergo selection for abnormal growth and survival creating a tumor. We model this process with cellular automata that use a simplified genetic regulatory network simulation to control cell behavior and predict cancer etiology. Our genetic model gives us the ability to relate genetic mutation to cancerous outcomes. The simulation uses known histological morphology, cell types, and stochastic behavior to specifically model ductal carcinoma in situ (DCIS), a common form of non-invasive breast cancer. Using this model we examine the effects of hereditary predisposition on DCIS incidence and aggressiveness. Results show that we are able to reproduce in vivo pathological features to hereditary forms of breast cancer: earlier incidence and increased aggressiveness. We also show that a contributing factor to the different pathology of hereditary breast cancer results from the ability of progenitor cells to pass cancerous mutations on to offspring.

Keywords Genetic cellular automata · DCIS · Progenitor hierarchy · Ductal simulation · Hereditary genetic predisposition · Hereditary breast cancer

1 Introduction

One in eight women will be diagnosed with breast cancer in their lifetime [21]. Carcinogenesis is an evolutionary phenomenon known to result from genetic mutations effecting cellular reproduction and survival [4, 12]. Breast cancer cells that are able to abnormally reproduce and survive, undergo a selection process that

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results in a tumorous growth. Here we use a computational simulation to model the evolution of a common form of breast cancer called ductal carcinoma in situ (DCIS). The simulation is based on a cellular automata (CA) design that uses mutable genes to model how somatic mutations lead to DCIS [7].

Using this simple genetic CA simulation, we model the evolution of DCIS and investigate the effects of hereditary genetic predisposition (HGP). Hereditary forms of breast cancer typically occur earlier in life and grow more aggressively than non-hereditary forms [10, 21, 22, 25]. Earlier cancer incidence due to genetic predisposition is well understood, but the aggressive growth is not. We hypothesize that hereditary forms of breast cancer are more aggressive because potent cells (able to reproduce) attain and propagate pathological mutations more effectively than non-hereditary forms of the disease. To investigate this hypothesis we run the model with and without HGP and examine the effects on the evolved DCIS.

A computer model is used for several reasons. Models are necessary because in vivo somatic mutations that contribute to DCIS are stochastic and difficult to quantify [4, 12, 14]. The natural history of human DCIS is poorly understood as it cannot be directly observed in vivo and is difficult to detect at its earliest stages [11]. Finally, computer models are useful in forming preliminary hypotheses about recently discovered biological paradigms that may take years for researchers to fully investigate [29].

CA models have been used to simulate various biological populations such as bacterial biofilms, plant growth, and tumor development [27, 28, 30]. These spatial models have provided insight to studying various cancers such as glioblastoma multiforme and esophageal adenocarcinoma [1, 17, 23, 31]. To examine the evolution of DCIS and effects of genetic predisposition, we add mutable *genes* to each cell. The resulting *genetic cellular automata* (GCA) provide an abstraction to illustrate cell heritage and somatic mutations.

Several mathematical models have been used to reproduce DCIS growth. Xu adapts a tumor growth model presented by Bryne and Chaplain to mimic nutrient and inhibitor dependent growth dynamics within a mammary duct [6, 34]. Recently, Franks et al. presented a model examining how mechanical stress and basal membrane degradation contribute to DCIS transition to invasive breast cancer [15]. Although these models are insightful and considered realistic, they do not focus on the underlying cause of DCIS: genetic dysfunction [4, 12, 14]. Instead of fitting our model to a tumor growth curve or chemical diffusion data, we use GCA to simulate the genetically evolved growth of DCIS. The simulation design is based on known histological morphology, cell types, and stochastic cell proliferation. Using a systems biology approach, we have created a mammary duct model that is ideal for examining features such as carcinogenesis, genetic selection, and population growth. To our knowledge, this is the first CA simulation used to examine the effects of genetic predisposition on the evolution of a tumor.

In the next section, the current understanding of DCIS and mammary progenitors will be reviewed. Breast cancer resulting from HGP is then discussed. In the ‘Methods’ section the model design and implementation will be described in detail. Finally, data from the simulation are presented and discussed.

1.1 DCIS and progenitor hierarchy

A human mammary duct is a cylindrical tube that transports milk during lactation. This bi-layered tube consists of luminal epithelial and myoepithelial cells surrounded by a basal membrane; Fig. 1A shows a stained cross-section [16]. The inner luminal epithelial cells undergo frequent reproduction via estrogen and progesterone hormonal control throughout a female's lifetime. Upon reproduction, somatic genetic mutations result from the genome copying process. These somatic mutations may accumulate in areas of the genome controlling survival and reproduction; cancerous cells are mutated to abnormally survive and reproduce. Ductal carcinoma typically originates from luminal epithelial cells and is considered in situ until the cancerous growth expands/breaks out of the duct to the surrounding stromal tissue [11, 19].

Recently mammary *stem cells* have been identified as progenitors of ductal epithelial cells [8, 32]. These cells reproduce rarely throughout an individual's life time to sustain the mammary duct cell population. Other progenitor cell types have been identified and are included in the *progenitor hierarchy* illustrated in Fig. 2. The bi-potent progenitor cells produce luminal epithelial or myoepithelial populations. Luminal epithelial progenitor cell types produce luminal epithelial cells incapable of reproduction. Myoepithelial progenitors produce myoepithelial cells also unable to reproduce [5, 33].

1.2 Hereditary genetic predisposition

Women that are genetically predisposed to breast cancer have inherited genetic mutations that make them more susceptible to breast cancer. It is estimated that 5–10% of all breast cancer cases are due to hereditary genetic predisposition (HGP) [21]. Although not all hereditary gene mutations have been identified, mutations in

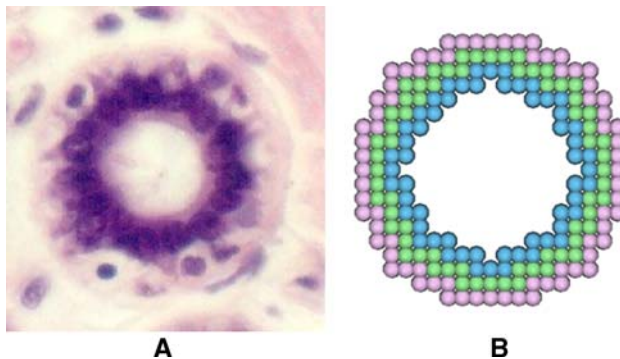
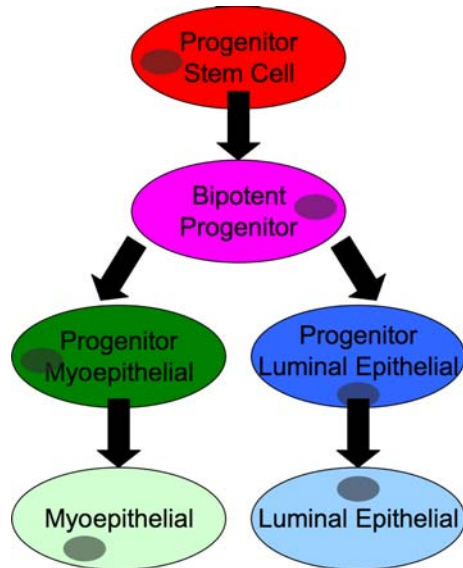


Fig. 1 (A) Human mammary duct cross-section with bi-layered epithelium consisting of an inner luminal epithelial, myoepithelial layer surrounded by a basal membrane. (B) Genetic cellular automata simulation. luminal epithelial = blue, myoepithelial = green, basal membrane = pink. Histological slide copyright 1998 by Gwen V. Childs, Ph.D. Reprinted with permission. Color version available online only

Fig. 2 Recent literature describes a progenitor hierarchy originating from a single stem cell type that produces differentiated myoepithelial and luminal epithelial cells. A mammary duct consists primarily of myoepithelial and luminal epithelial cells that are incapable of reproduction [5, 33]



genes *BRCA1*, *BRCA2*, *PTEN*, and *TP53* are known to increase the likelihood of breast cancer [10].

It has been shown that breast cancer resulting from HGP often has a different pathology than non-hereditary breast cancer. Women with HGP are more likely to be diagnosed at a younger age; Marcus et al. demonstrated this difference to be approximately 10 years younger [10, 21, 22, 23]. Earlier cancer incidence due to HGP is an established phenomenon often explained using Knudson's multiple hit hypothesis. Knudson studied HGP in the 1970's and linked earlier cancer diagnosis with the increased likelihood of somatic mutations further damaging cancer-related genes [18].

Another feature associated with hereditary forms of breast cancer is increased aggressiveness. It is not understood why the breast cancers of individuals with a family history often have higher proliferation rates than non-HGP cases [10, 21, 22, 25].

Using our GCA simulation we impose genetic predisposition on all initial stem cells of the duct. Cancer simulations with HGP will be labeled *HGP*. Simulations of the onset of cancer where HGP is not used are labeled *Normal*. We validate our model by showing *HGP* simulations elicit earlier DCIS incidence and more aggressive growth than *Normal* simulations. The resulting *Normal* and *HGP* simulations are examined to propose an explanation for increased aggressiveness.

2 Methods

We model the ductal structure using a CA design. We will refer to each CA lattice point as a *cell*; each epithelial cell is represented as a CA *cell*. Cell populations exist on a 3D fixed lattice and each cell may assume a finite number of *states*. A cell's

state changes according to the cell's *rules* which react to the cell's current state and local *neighborhood*. The entire lattice is updated in discrete generations.

Obvious parallels emerge between CA cell *state* and *in vivo* cell genetics. We previously published a *genetic* CA model in which rules are controlled by the fidelity of binary bit strings called *genes* [2]. A cell contains a *pseudo-genome* consisting of four genes listed in Table 1. Upon reproduction, cells inherit a parent's *pseudo-genome*; 'somatic' mutations are applied to parent and child *pseudo-genomes* [13]. As the pseudo-genome becomes mutated, the cell's rules (and behavior) dysfunction. In our previous publication we showed that a genetic CA can evolve realistic tumorous behavior.

2.1 Model design and implementation

The 3D lattice consists of $20 \times 20 \times 200$ points. The lattice is asynchronously updated by randomly selecting a point and, if a cell is present, updating the cell's state. 80,000 lattice point selections constitute a generation.

The duct is initialized by creating a cylindrical basal membrane and placing a small number of progenitor stem cells within. Progenitor cells reproduce and form a bi-layered duct analogous to a human mammary duct. The 20×20 lattice cross-section results in a simulated duct cross-section containing ~ 50 luminal epithelial cells (Fig. 1B); this is a realistic size for studying ductal carcinoma [24]. Duct ends are connected so a torus is formed to prevent unrealistic boundary conditions that are not present in *in vivo* mammary ducts. The majority of the duct consists of two cell types: (1) Myoepithelial cells that line the basal membrane and (2) inner luminal epithelial cells allowed adjacent to myoepithelial cells. All cells remain inside the fixed basal membrane.

Figure 3 illustrates lattice initialization. The following algorithm is used:

1. Draw the basal membrane by designating outer boundary points in a cylindrical orientation
2. Place stem cells within the basal membrane

Table 1 Each cell's *pseudo-genome* consists of four *genes*. Damaged genes, cause the cell to behave dysfunctionally [2]

Gene	Normal function	Effect when damaged
Housekeeping	Allows cell to sustain itself	Cell dies
Proto-oncogene	Allows normal reproduction	Cancerous reproduction
Tumor suppressor	1. Inhibits cancerous reproduction 2. Activates apoptosis when damaged proto-oncogene	1. Cancerous reproduction 2. No apoptosis when damaged proto-oncogene
Apoptosis	1. Cell suicide when damaged proto-oncogene 2. Allows hormonally induced cell suicide 3. Cell dies when not in bi-layer structure	Cell's survive when they shouldn't

3. If *HGP*, mutate all genes in all stem cells by 10%
4. Allow progenitor cells to reproduce into vacant neighboring lattice points without cells according to progenitor hierarchy (Fig. 2)
5. Allow non-stem cells to migrate one point to a vacant neighboring lattice point consistent with the bi-layer ductal structure
6. Repeat Steps 4 and 5 until there is no room for potent cells to reproduce

Current literature estimates that stem cells make up less than 5% of the ductal population [8, 33]—here we place ~ 200 stem cells to make up $\sim 1\%$ of the initialized ductal population, $\sim 24,000$ cells.

Misell et al. [26] measured a cell turnover rate of .69% per day for epithelial cells of the duct. To simulate a minority of cells undergoing hormonal reproductive/apoptotic cell turnover, progenitor luminal epithelial cells are prompted to reproduce and non-progenitor luminal epithelial cells undergo apoptosis at a constant rate of 1% per generation.

Upon reproduction, the child cell inherits a copy of the parent's pseudo-genome and both pseudo-genomes are mutated at a rate of 0.05% per bit. Because in vivo mutation rates vary with cell type, genetics, and environment, we assume a fixed arbitrary mutation rate. As genes controlling reproduction and apoptosis are mutated to dysfunction, cells become cancerous (Fig. 4).

The rules of each cell are controlled by a pseudo-genome containing four genes listed in Table 1. Each gene consists of 32 binary bits. Intact bits are represented as a 1 and mutated bits are represented as a 0. Each time a cell reproduces there is a .05% chance a bit will be permanently mutated. Equation 1 describes the probability of a bit being mutated where p is the mutation rate (.0005) and M is the number of reproductions.

$$q = 1 - (1 - p)^M \quad (1)$$

The number of DNA mutations required to make in vivo genes dysfunction varies greatly between specific genes and from individual to individual [13]. Furthermore,

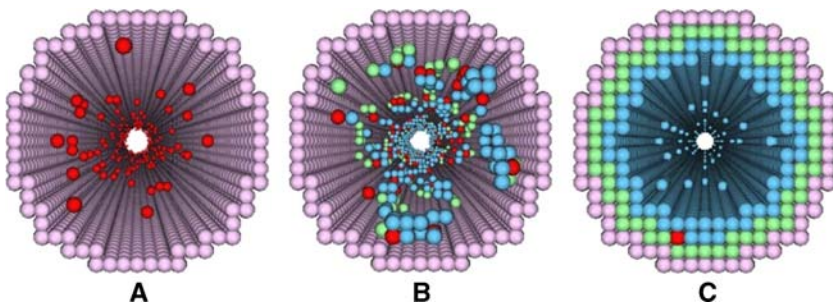


Fig. 3 Lattice initialization begins with a basal membrane and placement of mammary stem cells (A). Stem cells and other progenitors reproduce (B) until a complete cell bi-layer covers the inside of basal membrane (C). basal membrane = pink, mammary stem cells = red, myoepithelial = green, luminal epithelial = blue. Color version available online only

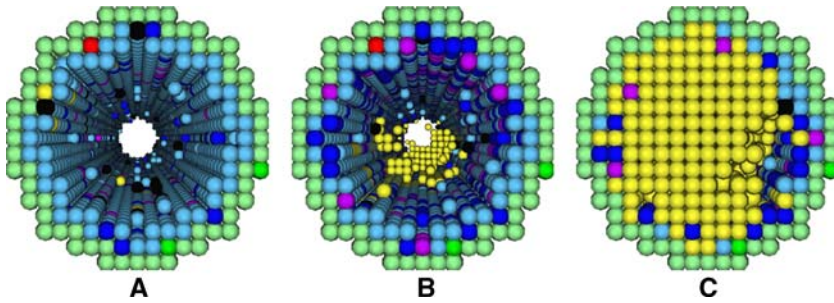


Fig. 4 Cells with at least one dysfunctional gene are colored yellow. (A) Initially, the majority of cells have intact genomes. (B) Genetically divergent cell populations arise with mutations that favor reproduction and survivability. (C) DCIS has completely blocked the duct lumen. bi-potent progenitors = purple, luminal epithelial = light blue, luminal epithelial progenitors = dark blue, myoepithelial = green, apoptotic cells = black, cells with genetic dysfunction = yellow. Color version available online only

mutations in one or two in vivo alleles may cause drastically different susceptibilities to cancer. For these reasons, we represent both alleles as a single in silico gene and assume that *some* arbitrary mutational threshold must be acquired before that gene dysfunctions. Genes may have a functional or dysfunctional state; genes have a functional state until 50% of their bits are mutated. Functional and dysfunctional behavior is listed in Table 1. A mutation rate of 0% allows all genes to remain in a functional state and the ductal structure remains intact despite cell turnover.

After the duct is initialized, the following algorithm is used to update a selected cell:

1. If housekeeping or apoptosis genes are dysfunctional (>50% mutated), mark the cell for death
2. If apoptosis genes are functional and cell is not adjacent to the basal membrane or a myoepithelial cell, mark the cell for death
3. If dead, remove from lattice
4. Progenitor cells may reproduce into neighboring positions according to progenitor hierarchy for three reasons:
 - *normal reproduction*—if an adjacent neighboring point is vacant and does not break the bi-layer ductal structure (Fig. 3C), the cell reproduces
 - *hormonal reproduction*—if cell is stochastically chosen (1% chance), cell pushes a adjacent neighboring cell to a vacant lattice point and reproduces
 - *cancerous reproduction*—if the cell has a dysfunctional (>50%) proto-oncogene and tumor suppressor gene, cell reproduces into any vacant neighboring lattice point or by pushing a neighboring cell to a vacant lattice point and reproducing
5. Allow non-stem cells to migrate one point to a vacant neighboring lattice point consistent with the bi-layer ductal structure (Fig. 3C)

Normal reproduction allows progenitors to reproduce into legal neighboring points that are consistent with the bi-layer ductal structure (Fig. 3C). *Cancerous*

reproduction results in DCIS growth (Fig. 4C) as cells are able to reproduce into vacant points that are inside the normal duct and disrupt the bi-layer structure.

The CA model was implemented in C++ and run on a 155 node Beowulf cluster for 15,000 generations. Data shown results from 300 *Normal* simulation runs and 300 *HGP* simulation runs. All genes in all stem cells were mutated 10% in *HGP* simulations. Because all gene mutations leading to breast cancer are not known, we assumed a worst case scenario and mutate the entire *pseudo genome* [10].

In summary, our model incorporates the following *biological* features from scientific literature:

- <5% mammary epithelial stem cells [8, 33]
- ~50 cells per cross-section [24]
- stochastic luminal epithelial cell turnover [26]
- mammary duct epithelial bilayer morphology [5, 32]
- 6 cell progenitor hierarchy [5, 33]
- epithelial cell genetic heterogeneity [4, 19]

We have taken a reductionist approach to biological modeling. For example, instead of trying to accurately simulate a 4-base DNA genome consisting of millions of bases, we use a simple 2-base binary bit *pseudo-genome* consisting of 128 bits. Modeling abstractions are necessary because biological systems are too complex to accurately replicate. Furthermore, model abstractions provide a coarse-grained framework to understanding complex behavior by breaking the complexity up into essential pieces.

3 Results

3.1 DCIS incidence

DCIS incidence measures the amount of time it takes for a simulation to evolve a cancerous cell type. Specifically, cells with a dysfunctional apoptosis gene and proto-oncogene are labeled cancerous. Figure 5 shows the proportion of cancerous simulations over time. There were initially no cancerous simulations and all simulations attain a cancerous state by 15,000 generations.

With 10% of the genome damaged initially, earlier carcinogenesis is expected because less additional mutations are necessary to cause a cancerous state. This is expected, but important for the validation of the GCA model because it is consistent with Knudson's multiple hit hypothesis established in the 1970's [18]. We use a stochastic mutation process analogous to in vivo somatic mutations to allow a cancerous state to evolve. Just as there is variance in in vivo breast cancer onset, there is variance in our in silico results. This variance was significantly different between *Normal* and *HGP* runs. *HGP* simulations evolved cancers at an average of 4615 generations (s.d. = 839) whereas *Normal* simulations evolved cancers after an average of 6804 generations (s.d. = 699).

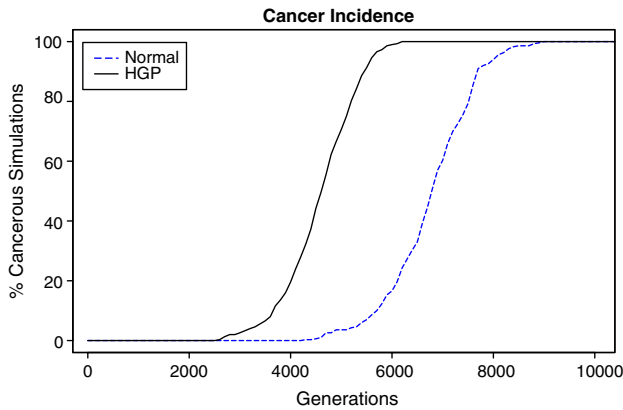


Fig. 5 Cancer incidence for 300 simulations without genetic predisposition (*Normal*) and an initial 10% genome damage (*HGP*). As expected, genetic predisposition causes DCIS to occur significantly sooner

3.2 Aggressiveness

Pathologists use cell reproduction rates as a prognostic feature for breast cancer aggressiveness. Greater reproductive activity indicates a faster growing tumor and a poorer patient prognosis [20, 9]. Here we observe the exact cancerous growth by counting the number of simulated cells after DCIS incidence. Figure 6 shows the total number of cells immediately after DCIS incidence.

Results show that we were able to reproduce the increased aggressiveness of *HGP* breast cancer examined in biological literature [10, 21, 22, 25]. Both *Normal* and *HGP* runs have similar starting populations at the point of carcinogenesis. *HGP* ductal cell populations grew faster than the *Normal* simulations. Eventually the total number cells for each type of simulation converge as the duct fills and there is no room for cancerous cells to reproduce.

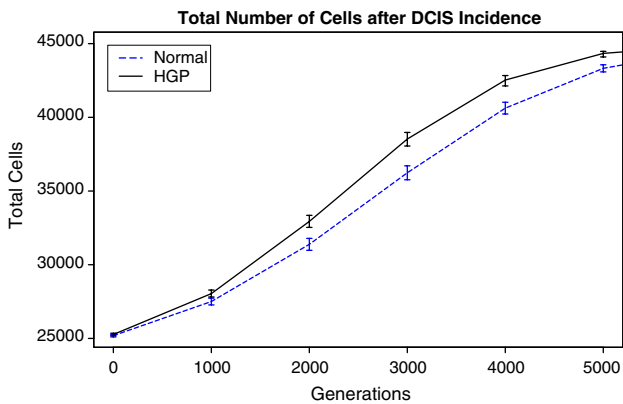


Fig. 6 *HGP* simulations had significantly faster growth after DCIS incidence. Both *Normal* and *HGP* population sizes converge as the duct is filled with cancerous cells. Error bars indicate a 95% confidence interval

To validate that the cause of the aggressiveness was due to an evolving cancerous genotype, the cumulative number of *cancerous reproductions* (Sect. 2.1) is shown in Fig. 7. Note the corresponding change in the total number of cells (Fig. 6) and cancerous reproductions (Fig. 7). Each cell has the same likelihood of being updated and having the opportunity to reproduce. Because there are more *cancerous reproductions*, it follows that there are more cancerous cells reproducing to cause the increased aggressiveness.

3.3 Mutational burden after carcinogenesis

Cancer is known to be an evolutionary process resulting from the accumulation of stochastic mutations [4, 12]. The only difference between the *Normal* and *HGP* simulations is the initial 10% mutation applied to the genome. Here we examine how that initial genetic predisposition has propagated and effected the evolution of the cancerous population. Figure 8 shows the average number of mutations for each cell type 3000 generations after cancer incidence. Simulations were examined at 3000 generations after cancer onset because this time point shows the greatest growth difference between *Normal* and *HGP* simulations.

As expected, there are a greater number of mutations in *HGP* cells than *Normal* cells. The stem cells have the least number of mutations because they reproduce less often. At each level of the hierarchy (Fig. 2) mutations accumulate and cells at the bottom accumulate the greatest number of mutations.

Figure 7 shows that the difference in aggressiveness comes from the number of *cancerous reproductions*. The *HGP* is being passed from progenitor cells making it easier for cells to attain a cancerous genotype and abnormally reproduce. More cells are attaining a cancerous state because the probability of genetic dysfunction increases since the number of initial mutations is greater. The number of mutations

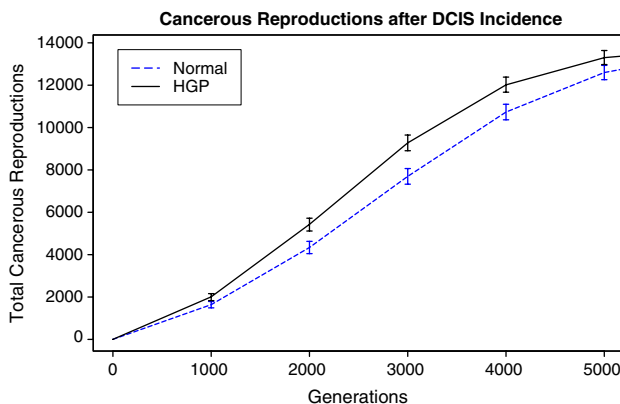


Fig. 7 The cumulative number of *cancerous reproductions* immediately after carcinogenesis are reproductions produced by a cancerous cell type. There were more *cancerous reproductions* for *HGP* simulations versus *Normal* in correlation with the growth curve shown in Fig. 6. Error bars indicate a 95% confidence interval

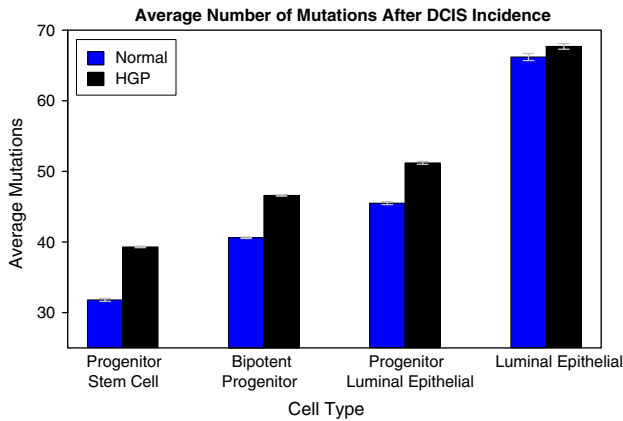


Fig. 8 Average number of mutations for each cell type 3000 generations after carcinogenesis. Refer to Fig. 2 for hierarchal structure. Note the drastic difference in the number of progenitor mutations that is not reflected in the non-reproducing luminal epithelial cells. Error bars indicate 95% confidence interval

per cell type can be explained by progenitor hereditary relationships shown in Fig. 2. HGP causes more mutations to occur higher in the hierarchy and produce more offspring with a greater number of mutations. It is easier for all cells in the hierarchy to attain a cancerous state and ultimately produce offspring that are cancerous.

4 Conclusion

Because cancer is an evolutionary process it is critical to use a modeling technique that is able to incorporate evolutionary paradigms such as mutation, heretability and selection. We have presented a genetic cellular automata (GCA) simulation of breast cancer that allows cancerous behavior to evolve as a result of random mutations that are propagated by a progenitor hierarchy. This model is used to understand why hereditary forms of breast cancer evolve more aggressive growth. Our model implements genetic rules to study the transition of a reproductively controlled cell population to a highly proliferative cancer population. Although a simple abstraction of the *in vivo* mammary duct, our GCA reproduced pathological features specific to hereditary forms of breast cancer.

Applying hereditary genetic predisposition to the starting stem cells causes the simulations to show markedly different behavior. Carcinogenesis occurred significantly earlier in hereditary genetic predisposition (HGP) simulations as expected (Fig. 5). Cancerous populations grew faster in the HGP simulations even after starting at a similar population size of $\sim 25,000$ cells. The increased growth rate was attributed to cancerous reproduction (Fig. 7); there were more cells that had evolved a cancerous state.

An advantage to using simple computer models is that we can thoroughly analyze *in silico* populations. It is not realistic for a biologist to observe an *in vivo* duct with

and without HGP to breast cancer. If this were possible, the biologist would still not be able to count the exact number of mutations in each cell type and compare them as done in Fig. 8. This figure shows that cancerous mutations are being propagated from each progenitor cell type as proposed by our hypothesis. It is easier for a cell with HGP to attain a cancerous state and pass on this state to its offspring.

It is possible there may be other selective mechanisms causing increased aggressiveness. For example, there may be a specific proto-oncogene that is activated to cause high proliferation in ductal carcinoma in situ (DCIS). However, from our results we believe that the progenitor hierarchy structure plays a major role in early DCIS incidence and increased aggressiveness of HGP breast cancers.

This work has been extended to examine the effects of progenitor hierarchy structure on genetic heterogeneity, DCIS initiation and aggressiveness [3].

We plan to continue this research in several ways. Treatment simulations could be used to examine the cancer population's evolving response to gene-specific or cell-specific treatments. Chemical diffusion could be included to simulate nutrient deficiencies and barriers of treatment. As cell type data becomes available, the model could be trained to reproduce cell type proportions of the ductal population. Finally, the same GCA framework could be used to model other forms of cancer and assist in understanding their growth.

Acknowledgments This work is supported by NIH COBRE Grant P20 RR15587, NIH INBRE Grant P20 RR16448-01, NIH R01 CA104470 and NSF Grant EPS80935.

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