

A Maturity-Structured Mathematical Model of Mutation Acquisition in the Absence of Homeostatic Regulation

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Abstract. Most mammalian tissues are organized into a hierarchical structure of stem, progenitor, and differentiated cells. Tumors exhibit similar hierarchy, even if it is abnormal in comparison with healthy tissue. In particular, it is believed that a small population of cancer stem cells drives tumorigenesis in certain malignancies. These cancer stem cells are derived from transformed stem cells or mutated progenitors that have acquired stem-cell qualities, specifically the ability to self-renew. Similar to their normal counterparts, cancer stem cells are long-lived, can self-renew and differentiate, albeit aberrantly, and are capable of generating tissue, resulting in tumor formation. Although identified and characterized in several forms of malignancy, the specific multi-step process that causes the formation of cancer stem cells is uncertain. Here, a maturity-structured mathematical model is developed to investigate the sequential order of mutations that causes the fastest emergence of cancer stem cells. Using model predictions, we discuss conditions for which genetic instability significantly speeds cancer onset and suggest that unbalanced stem-cell self-renewal and inhibition of progenitor differentiation contribute to aggressive forms of cancer. To our knowledge, this is the first continuous maturity-structured mathematical model used to investigate mutation acquisition within hierarchical tissue in order to address implications of cancer stem cells in tumorigenesis.

Key words: cancer stem cell hypothesis, maturity-structured mathematical model, mutation acquisition

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1. Introduction

Cancer is a multi-step process in which several mutagenic events occur to alter normal cellular characteristics. It has been estimated that three to ten genetic mutations are required to malignantly transform a cell [7, 18]. Ultimately, malignant growth requires a combination of mutations that gain cellular function in addition to those that remove tumor suppression. Specific types of mutations vary from one form of disease to another, but evasion of programmed cell death, genetic instability, and deregulated proliferation are commonly observed abnormalities [18].

To further the complexities involved in studying cancer, tumors themselves are heterogeneous in cellular composition. Specifically, all tumor cells do not have an equal capacity for initiating and promoting malignancy. Interestingly, it has been noted that the cells capable of initiating tumorigenesis share many characteristics of stem cells. Both malignant cells and normal stem cells are long-lived, evade apoptosis, have high proliferative potential, and are able to produce daughter cells of different phenotypes [2, 18, 21, 31]. Realizing these similar properties, tumor-initiating cells have been called cancer stem cells. The cancer stem cell hypothesis suggests that malignant growth is driven by a subpopulation of tumor cells capable of self-renewal and aberrant differentiation that contributes to heterogeneity. These cancer stem cells are believed to be mutated stem cells or progenitors that have acquired stem-cell characteristics [31]. It is theorized that cancer may only be fully eradicated by the elimination of these tumor-initiating cells, therefore understanding their role in tumorigenesis is crucial [31]. Since the cancer stem cell hypothesis originated, cancer stem cells have been identified in tumors of the breast, brain, colon, and blood, among others [2, 8, 16, 17, 21].

Due to the difficulty of isolating and studying stem cells experimentally, mathematical modeling provides further insight into the growth dynamics involved during tumorigenesis in hierarchical tissue. In order to simulate the cancer stem cell hypothesis mathematically, it is necessary to model cancer stem cells as a distinct subpopulation from other tumor cells. Furthermore, tissue hierarchy must be considered because stem, progenitor, and differentiated cells have very different properties. In this work, a continuous maturity-structured mathematical model is presented that investigates mutation acquisition in stem, progenitor, and differentiated cells. In particular, mutation pathways causing the fastest emergence of cancer stem cells are determined and investigated. In addition, tumor heterogeneity and composition are discussed.

2. A Maturity-Structured Mathematical Model of Hierarchical Tissue

It is well known that mammalian tissue is not a homogeneous collection of cells, but is instead a composition of different types of cells that each have specific roles. Healthy tissue is carefully organized in a hierarchical structure consisting of stem, progenitor, and differentiated cells. Rare naïve stem are long-lived and unique in that they both self-renew and differentiate [2, 27]. Progenitor cells are more committed in lineage, but too immature to carry out specific functions. As

they complete additional divisions, progenitors expand in number and become more specific, until they are fully differentiated [2]. Due to the varying properties of cells in hierarchical tissue, it is desirable to create a mathematical model that allows cellular kinetics to depend on cell maturity. Figure 1 provides a schematic of the maturity progression from stem to differentiated cell.

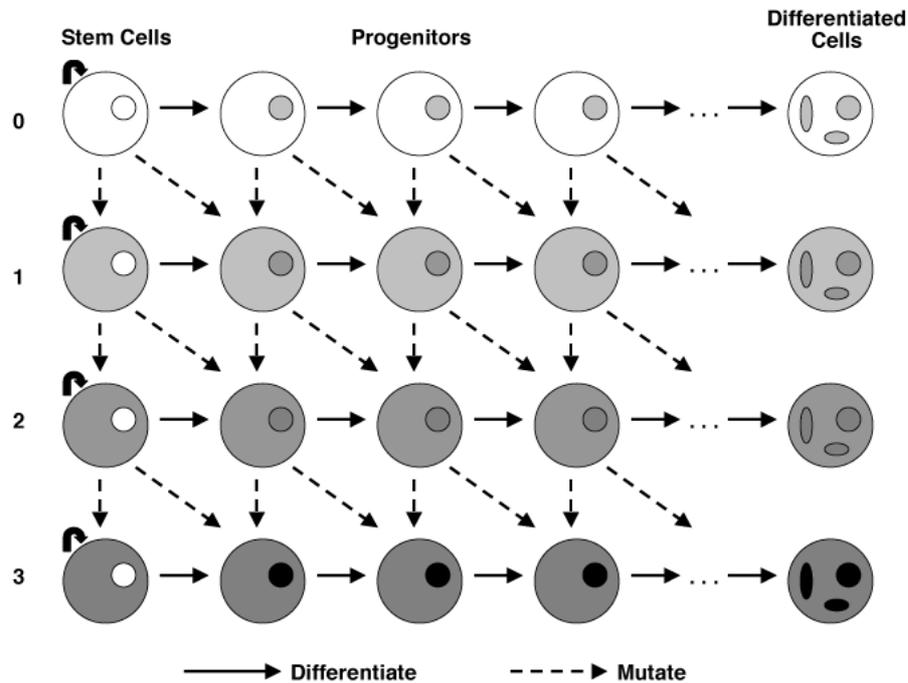


Figure 1: Schematic diagram of mutation acquisition in hierarchical tissues. Stem cells with zero, one, two, or three mutations may self-renew or differentiate to form progenitors, which in turn continue dividing and maturing. Each time cells divide, there is a small probability they will acquire a mutation. Cells can accumulate up to three mutations, at which point they are classified as cancer cells.

There are various ways of explicitly modeling each of the cell subpopulations in a tissue. Some mathematical models compartmentalize stem cells, progenitor cells, and differentiated cells [3, 9, 13, 24], while others distinguish cells based on maturity, be it through discrete cell divisions [5] or continuous cell maturity level [28]. Here, a differential equations model is presented in which both time and maturity are continuous. First, a model of healthy tissue is established, which is then used to investigate the process of mutation acquisition in hierarchical tissue. An ordinary differential equation is used to model the stem-cell population since it is assumed that stem cells remain immature. In contrast, a partial differential equation is used for non-stem cells that is dependent on both time and maturity. To our knowledge, this is the first mathematical model that addresses the emergence of cancer stem cells within a maturity-continuous structure.

2.1. Model Structure

It is known that the properties of stem, progenitor, and differentiated cells are markedly different from each other. Stem cells have the potential to remain in an undifferentiated state. Under homeostatic conditions, it is thought that the majority of stem cells are quiescent and divide infrequently [12]. In contrast, progenitors eventually reach full maturity through extensive proliferation that produces differentiated progeny. As progenitors mature, their proliferative and apoptotic behavior may also change. For instance, immature myeloid precursor cells, such as myeloblasts, divide faster than more differentiated myelocytes [30]. Consequently, it is desirable to mathematically model hierarchical tissue in such a way as to allow cell kinetics to depend on cell maturity.

In 2003, Ostby *et al.* presented a continuous maturity-structured model of granulopoiesis, which simulated cells from the myeloblast stage through terminally differentiated granulocytes in a normal, healthy system [28]. Stem cells were not modeled, but rather were assumed to be at a constant level in homeostasis that fed into the progenitor population. A one-dimensional hyperbolic partial differential equation dependent on time and cell maturity was employed for progenitor cells in the bone marrow in which rates of proliferation, mobilization from the bone marrow to blood, and apoptosis were maturity-dependent. Cell maturity was scaled such that the most immature cell had maturity level zero, the most mature cell had maturity level one, and the maturation rate determined how quickly cells progressed through each stage. This model assumed that cells in the blood were terminally differentiated, and thus were fully mature and did not proliferate, so that blood cells were modeled with an ordinary differential equation dependent on time only.

In the present work, a mathematical model is introduced that imparts a similar continuous maturity structure, while making various significant alterations from the Ostby model. It is important to note that the Ostby model simulated granulopoiesis in homeostasis, which permitted the omission of explicitly modeling stem cells. In modeling tumorigenesis, and specifically the generation of cancer stem cells, it is essential to include an equation monitoring the dynamics of the stem-cell population. Therefore, the most significant difference between our model and the Ostby model is the inclusion of a stem-cell equation that fosters investigation of the effects that stem cells have on tissue dynamics.

Other differences between the two models are not as striking, but still noteworthy. For instance, the Ostby model was specifically tailored for granulopoiesis, and although the hematopoietic system will be simulated in this chapter as well, the mathematical model developed in the present work is general and can be applied to any hierarchical system. The models also slightly differ in how the measurement of maturity is handled. In the Ostby model, maturity was on a scale of zero to one, and a constant maturation rate was derived from the time needed for immature progenitors to develop into fully differentiated cells. In contrast, here maturity is not scaled, but rather progresses according to the time scale. A cell with a maturity level measured in weeks has completed a certain number of divisions depending on the proliferation rate, which determines its progress to terminal differentiation. Therefore, the models slightly differ in how maturity is defined, but in essence are comparable since both models rely on average proliferation rates to determine maturity.

Consider a time- and maturity- continuous model in which time is denoted by t and the maturity of cells is denoted a . Stem cells are the most naïve cells in the system. They do not mature,

and, therefore, the size of the stem cell population, denoted $S(t)$, is not dependent on a . Stem cells proliferate at rate k . Each stem cell encounters one of four fates during each division: symmetric self-renewal, asymmetric self-renewal, symmetric commitment differentiation, and apoptosis. Stem cells symmetrically self-renew with probability α_S , which increases the stem cell pool by one. Stem cells asymmetrically self-renew with probability α_A , which does not change the stem cell pool but increases the progenitor pool of maturity level zero by one. Stem cells symmetrically differentiate with probability α_D , which decreases the stem cell pool by one and increases the progenitor pool of maturity level zero by two. Finally, stem cells die with probability δ_S , and it follows that $\alpha_S + \alpha_A + \alpha_D + \delta_S = 1$. We denote the type of stem cell division that occurs by classifying the status of daughter cells after division is completed, and the model is not dependent on whether the various modes of division are dictated by chromosomal segregation. Due to the tight regulation of stem cells, it is assumed in this model that stem cells only die or differentiate when dividing, though the model equations could easily be slightly modified to allow for division-independent differentiation and apoptosis.

The maturity density of differentiated cells at time t is denoted $n(a, t)$, where the number of cells between maturity level a and $a + \Delta a$ is approximately $n(a, t)\Delta a$. The proliferation rate of differentiated cells, denoted by the function $\beta(a)$, allows immature to proliferate but tends to zero as maturity is reached. The death rate of differentiated cells, given by the function $\mu(a)$, increases after full maturity. If the initial stem cell population is S_0 , the initial maturity distribution of differentiating cells is given by $f(a)$, and the total number of non-stem cells in the tissue is $N(t)$, then the model equations follow:

$$\begin{aligned} \frac{dS}{dt} &= (\alpha_S - \alpha_D - \delta_S)kS & (2.1) \\ \frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} &= (\beta(a) - \mu(a))n \\ n(a, 0) &= f(a) \\ S(0) &= S_0 \\ n(0, t) &= (2\alpha_D + \alpha_A)kS \\ N(t) &= \int_0^\infty n(a, t)da. \end{aligned}$$

In this model, probabilities of stem-cell division are constant, but in future work, dynamic regulatory feedback mechanisms that influence the pattern of self-renewal and differentiation will be discussed.

There are many potential functions that would be suitable choices for $\beta(a)$ and $\mu(a)$. Generally, $\beta(a)$, should be greater than $\mu(a)$ for immature cells that have not yet completed terminal differentiation in order to allow for progenitor expansion. Furthermore, as $a \rightarrow \infty$, $\beta(a) \rightarrow 0$ and $\mu(a)$ increases well beyond $\beta(a)$. By not explicitly defining functions for the proliferation and death rates of differentiating cells, the model provides a general enough framework to be used modeling various types of hierarchical tissue. In Section 2.3., specific examples are given in order to generate numerical simulations of the hematopoietic system.

2.2. Model Analysis

The solution of the stem-cell equation is $S(t) = S_0 e^{(\alpha_S - \alpha_D - \delta_S)kt}$. The method of characteristics may be used to solve the differentiated cell equation [26]. Assuming cell maturity is determined by time passed since a differentiated cell was formed gives $\frac{da}{dt} = 1$, and the differentiated cell population equation is simplified to $\frac{dn}{dt} = \beta n - \mu n$. Note that $\frac{da}{dt} = 1$ implies that $a = t + C$, for some constant C. For $a > t$, $C = a_0$ and for $a < t$, $C = -t_0$.

When $a > t$, the following is true:

$$\begin{aligned}\frac{dn}{dt} &= \beta(a)n - \mu(a)n \\ \frac{dn}{dt} &= (\beta(t + a_0) - \mu(t + a_0))n \\ \int \frac{dn}{n} &= \int (\beta(t + a_0) - \mu(t + a_0))dt \\ \int \frac{dn}{n} &= \int_0^t (\beta(\tau + a_0) - \mu(\tau + a_0))d\tau \\ \int \frac{dn}{n} &= \int_{u=a_0}^{t+a_0} (\beta(u) - \mu(u))du \\ n &= K \exp \left[\int_{a_0}^{t+a_0} (\beta(u) - \mu(u))du \right] \\ n &= K \exp \left[\int_{a_0}^a (\beta(u) - \mu(u))du \right].\end{aligned}$$

At time $t = 0$, $a = a_0$, and so $K = n(a_0, 0)$. If the initial age distribution is given by $n(a, 0) = f(a)$, then since $a_0 = a - t$,

$$\begin{aligned}n(a, t) &= n(a_0, 0) \exp \left[\int_{a_0}^a (\beta(u) - \mu(u))du \right] \\ n(a, t) &= n(a - t, 0) \exp \left[\int_{a_0}^a (\beta(u) - \mu(u))du \right] \\ n(a, t) &= f(a - t) \exp \left[\int_{a_0}^a (\beta(u) - \mu(u))du \right].\end{aligned}$$

Note that if $f(a) = 0$, then $n(a, t) = 0$ for $a > t$. That is, if there are no differentiating cells in the tissue at time $t = 0$, then it is impossible for the maturity level of a cell to be greater than the time that has elapsed. For instance, if $n(a, 0) = 0$, then at two weeks all differentiating cells must have maturity that is less than or equal to two weeks.

When $a < t$, then the following holds:

$$\begin{aligned}
\frac{dn}{dt} &= \beta(a)n - \mu(a)n \\
\frac{dn}{dt} &= (\beta(t - t_0) - \mu(t - t_0))n \\
\int \frac{dn}{n} &= \int (\beta(t - t_0) - \mu(t - t_0))dt \\
\int \frac{dn}{n} &= \int_{t_0}^t (\beta(\tau - t_0) - \mu(\tau - t_0))d\tau \\
\int \frac{dn}{n} &= \int_{u=0}^{t-t_0} (\beta(u) - \mu(u))du \\
\int \frac{dn}{n} &= \int_0^a (\beta(u) - \mu(u))du \\
n &= K \exp \left[\int_0^a (\beta(u) - \mu(u))du \right].
\end{aligned}$$

When $a = 0, t = t_0$, so that $K = n(0, t_0) = n(0, t)$, which in this model gives $K = (2\alpha_D + \alpha_A) S$. Therefore, for $a < t$,

$$n(a, t) = (2\alpha_D + \alpha_A) S(t) \exp \left[\int_0^a (\beta(u) - \mu(u))du \right]. \quad (2.2)$$

The differentiated-cell population will go to steady state as long as the stem-cell population is in steady state, as seen in Equation 2.2. The stem-cell population is in steady state if $\alpha_S - \alpha_D - \delta_S = 0$. Otherwise, it either grows or decays exponentially, which causes the differentiated-cell population to behave accordingly. Thus stem-cell population dynamics dictate differentiated-cell population dynamics, which correlates with previous modeling results from Hardy and Stark that predicted stem-cell equilibrium determines tissue homeostasis [19].

2.3. Parameter Estimation

Due to the difficulty of isolating and studying stem cells *in vivo*, there are limited data for stem-cell kinetics. The hematopoietic system is perhaps the best quantified system, and as a result, parameter values for model simulations are derived from hematopoietic stem cells and progenitor and differentiated cells of the granulocytic lineage. The parameters used in numerical simulations are presented in Table 2.3..

Although hematopoietic stem cells are better understood than stem cells in other tissues, there is still much uncertainty concerning *in vivo* measurements. Part of the discrepancy comes from the process of isolating stem cells. There are several markers that isolate immature cells from those that are more differentiated, but it can be difficult to separate stem cells from early progenitor cells. Therefore, it is not uncommon for a population of “stem” cells to also include early progenitor cells, which can taint the true measurements of stem versus early progenitor cells. As a result,

Parameters Used			
Parameter	Biological Meaning	Value Range	Value Used
S_0	Initial number of stem cells	11,000 - 22,000 cells [1]	18,000 (cells)
α_S	Probability SSR	(derived from [33])	0.20
α_A	Probability of ASR	(derived from [33])	0.60
α_D	Probability of SD	(derived from [33])	0.15
δ_S	Probability of stem-cell death	0.05 [24]	0.05
k	Proliferation rate of stem cells	0.08-0.40 $weeks^{-1}$ [12]	0.2471 $weeks^{-1}$
b	Max. prol. rate of progenitors	9.0 - 10.6 $weeks^{-1}$ [30]	9.7 $weeks^{-1}$
ρ_β	Steepness of proliferation switch	No information	2
ω_β	Maturity at proliferation switch	No information	1.85 weeks
d	Max. death rate of diff. cells	15 - 18 $weeks^{-1}$ [6, 9]	16.8 $weeks^{-1}$
ρ_μ	Steepness of death switch	No information	10
ω_μ	Maturity at death switch	No information	3.20 weeks

the current literature includes a wide range of values regarding hematopoietic stem-cell kinetics. Because this mathematical model separates stem cells from all other cells, the parameters used here attempt to reflect the most purified stem-cell population

As cells differentiate, they lose their ability to proliferate. Stem and early progenitor cells have high proliferative potential, whereas terminally differentiated cells are unable to complete further divisions. Although some terminally differentiated cells are long-lived, such as lymphoid cells, this model assumes that fully mature cells have a short half-life and, consequently, a significant rate of apoptosis [6, 9, 23]. In order to capture the dependence of proliferation and apoptosis on cell maturity, functions that smoothly transition between two different baseline rates are used. There are many possibilities for such functions, but for simplicity, this model assumes that the proliferation rate is approximately constant until terminal differentiation, after which it approaches zero. In contrast, the death rate is assumed to be approximately zero until terminal differentiation, after

which it is approximately constant with a short half-life. Instead of incorporating step functions that introduce discontinuity, the functions used to model proliferation and death rates for non-stem cells are as follows:

$$\begin{aligned}\beta(a) &= \frac{-b}{2} \tanh(\rho_\beta(a - \omega_\beta)) + \frac{b}{2} \\ \mu(a) &= \frac{d}{2} \tanh(\rho_\mu(a - \omega_\mu)) + \frac{d}{2} + \delta_S k.\end{aligned}$$

The maximum rate of proliferation is given by b and the maximum rate of death is given by d . The maturity at which non-stem cells proliferate at half the maximum rate is ω_β , and ρ_β is the steepness of the decreasing switch. Similarly, the maturity at which non-stem cells die at half the maximum rate is ω_μ and the steepness of the increase in the switch is ρ_μ . It is also assumed that progenitors die at a rate at least as great as stem cells. The proliferation and death rate functions defined above in 2.3 are plotted in Figure 2.

2.4. Numerical Simulations and Results

In Section 3., mutation pathways generating cancer in hierarchical tissue will be explored, but before this can be investigated, the steady state of healthy tissue must be first determined. In order to conduct numerical simulations, model equations were discretized with the upwind method and simulations were conducted in MATLAB. Step sizes for Δt and Δa satisfy the CFL condition, with $\Delta t = \Delta a$ to correlate with the assumption that $\frac{da}{dt} = 1$.

To determine the steady state maturity-distribution of cells, it is necessary to generate the progeny resulting from a steady state of stem cells. Since hematopoietic stem cells are arguably the most observed, the hematopoietic system is used to illustrate the dynamics of hierarchical tissue. It is estimated that there are between 11,000 - 22,000 hematopoietic stem cells in an adult human [1]. We begin by assuming that the system is infused with 18,000 stem cells and zero progenitor and differentiated cells. Stem cells divide with probabilities that maintain equilibrium within the stem-cell compartment so it possible to determine the steady state maturity distribution of progenitor and differentiated cells resulting from this number of stem cells. Progenitors are formed through asymmetric and symmetric commitment divisions of the existing stem cells. Progenitors proliferate and expand in number to generate fully-differentiated cells, and eventually a steady-state distribution of progenitor and differentiated cells is reached.

Figure 2 plots tissue dynamics as stem cells generate progenitor and differentiated cells. The maintained homeostasis of stem cells and the generation of the resulting progeny of non-stem cells is displayed in a log plot in Figure 2. Initially, there is a low number of non-stem cells, which in fact are all progenitors formed from the stem-cell population. As time progresses, progenitors proliferate and expand in number as the stem-cell source continues to form early progenitors. As equilibrium is reached, the total number of non-stem cells includes all progenitors and fully-differentiated cells. Using the parameters in Table 1, the total number of non-stem cells, N , reaches 5.6×10^{11} cells, which is within the range of $5 - 15 \times 11$ cells of the granulocytic lineage estimated in a human adult [20, 28, 30]. It takes approximately 3.3 weeks for N to reach 90% of its

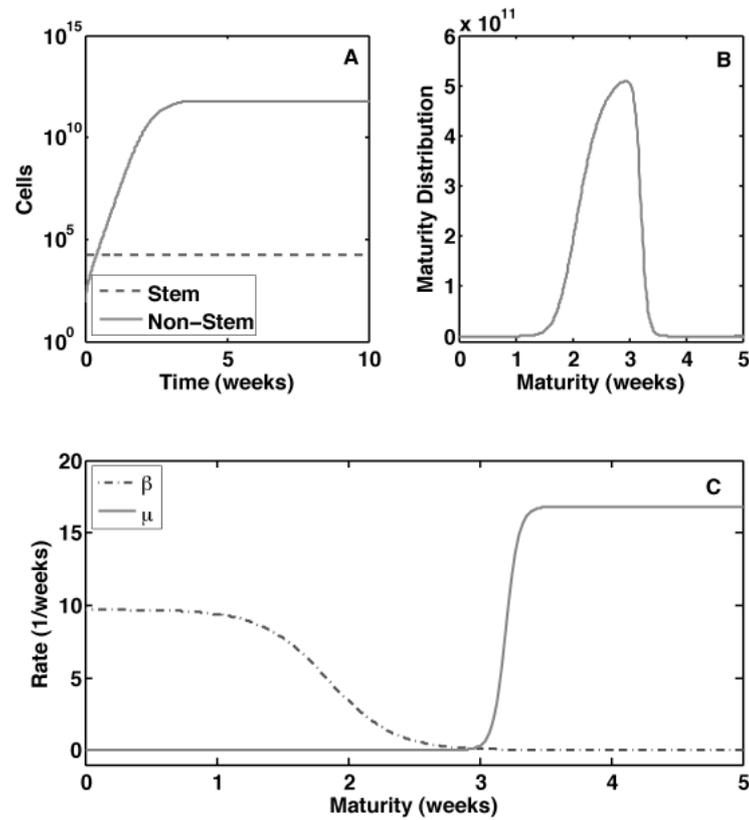


Figure 2: The generation of hierarchical tissue from stem cells. (A) A log plot of stem (blue) and non-stem (red) cells in the tissue versus time. Starting at stem-cell homeostasis, the stem-cell population remains constant over time, while the non-stem cell population expands until it reaches homeostasis. (B) The maturity distribution of non-stem cells at steady state demonstrates the majority of cells are fully mature. (C) Proliferation and death rates of differentiating cells depend on cell maturity. The functional forms presented in Equations 4.6 are plotted versus cell maturity.

equilibrium value, though the time to constitution can be altered by the rate of proliferation in both stem and progenitor cells. In Figure 2, the maturity distribution of non-stem cells at steady state demonstrates that the majority of stem cells are fully mature. At homeostasis, it is estimated that 1.35×10^{11} cells are fully differentiated, which correlates with the estimated 1.2×10^{11} granulocytes that are released from the bone marrow to the blood daily.

The proliferation and death rate functions, β and μ , respectively, are those displayed in Figure 2. Cells are considered to be fully mature when $\mu > \beta$, which occurs at approximately 3.0 weeks in this simulation. The cycle times of immature myeloblasts, myelocytes, and promyelocytes are estimated to be 11, 27, and 39 hours, respectively [30]. Therefore, the proliferation rate is greater for lower maturity levels, with a maximum rate of 9.7 divisions per week to correlate with the doubling time of myeloblasts. Neutrophils reside in the bone marrow for approximately 4 days after their final division before being released into the blood [15]. Once in the blood, the half-life of neutrophils is estimated at seven hours, thus the maximum of the death rate is achieved soon after cells have reached full maturity.

We have presented a maturity-structured model of hierarchical tissue that is continuous both in time and cell maturity. Through mathematical analysis, we determined the solution for both stem and non-stem cell populations and concluded that the number of progenitor and differentiated cells is directly dependent on the number of stem cells present in the system. Through numerical simulations in which cells of the granulocytic lineage were produced from an initial population of hematopoietic stem cells, it was possible to determine the steady state of non-stem cells as well as the maturity distribution of progenitor and differentiated cells at homeostasis. These findings will be used as the baseline values of healthy hierarchical tissue in exploring the process of mutation acquisition and tumorigenesis in the next section.

3. Mutation Acquisition in Stem, Progenitor, and Differentiated Cells

It is believed that tumorigenesis does not result from a single mutation, but rather is a multistep process [11, 18]. Although it is known several events are needed to cause the malignant transformation of a normal cell, the order in which these mutations are acquired can affect tumor dynamics. Deregulated proliferation, evasion of apoptosis, and genetic instability are likely involved in the early stages of cancer, whereas mutations causing angiogenesis and metastasis are probably acquired in later stages, after a tumor has grown beyond a certain threshold size [18]. We will apply our continuous maturity-structured model to investigate mutation acquisition in hematopoietic cells; consequently, angiogenesis and metastasis may be disregarded.

The hierarchical organization of most mammalian tissues may offer protection against cancer. The vast majority of tissues consist of differentiated cells that have a high rate of turnover and generally don't live long enough to accumulate enough mutations to become malignant [29, 31]. In addition, most differentiated cells do not self-renew, and when they reach full maturity, mutations are not passed on to any progeny. Generally, stem cells, or progenitors that have gained self-

renewal capability, are the only tissue cells that live long enough to acquire a sufficient number of mutations and possess a sufficient proliferative potential that allows the propagation of mutations to their progeny [14, 31].

Now the pathways leading to tumorigenesis in hierarchical tissue are explored. This mathematical model is one of the first that permits the investigation of sequential mutation acquisition within hierarchically structured tissue [5]. Because mutation order is monitored, it is possible to quantify the increased advantage gained through each transformation. Furthermore, tissue composition can be determined that is based on the percentages of cells with a certain number of mutations. An additional feature of this model is the explicit inclusion of all three models of stem-cell division: symmetric self-renewal, asymmetric self-renewal, and symmetric commitment differentiation. This differs from other mathematical models of cancer in which asymmetric divisions are often ignored. To our knowledge, this is the first model to incorporate all of these novel features within a maturity-continuous framework.

3.1. Model Structure for Mutation Acquisition

To study the process of oncogenesis in hierarchical tissue, the model presented in Equations 2.1 is expanded to incorporate mutation acquisition in both stem and differentiated cells. Normal stem cells, S_0 , acquire their first mutation at rate m_0 , at which time they are labeled as S_1 . Likewise, S_1 cells acquire the second mutation at rate m_1 to become S_2 cells, and S_2 cells acquire the third mutation at rate m_2 to become S_3 cells. Stem cells with i mutations, S_i , form progenitor cells with i mutations, n_i , when they differentiate. Committed cells may also mutate as they continue to divide, and M_i is used to denote the mutation rates from n_i to n_{i+1} . It is assumed that cells may only acquire one mutation at a time. Cells with i mutations may alter any of the model parameters, depending on which mutation is acquired, thus each parameter is denoted with an i -subscript to allow these values to differ from the baseline value. A schematic diagram is displayed in Figure 1 and the model equations for mutation acquisition are presented below.

Stem Cells:

$$\begin{aligned}
 \frac{dS_0}{dt} &= [(1 - 2m_0)\alpha_{S_0} - m_0\alpha_{A_0} - \alpha_{D_0} - \delta_{S_0}] k_0 S_0 \\
 \frac{dS_1}{dt} &= [(1 - 2m_1)\alpha_{S_1} - m_1\alpha_{A_1} - \alpha_{D_1} - \delta_{S_1}] k_1 S_1 \\
 &\quad + [2m_0\alpha_{S_0} + m_0\alpha_{A_0}] k_0 S_0 \\
 \frac{dS_2}{dt} &= [(1 - 2m_2)\alpha_{S_2} - m_2\alpha_{A_2} - \alpha_{D_2} - \delta_{S_2}] k_2 S_2 \\
 &\quad + [2m_1\alpha_{S_1} + m_1\alpha_{A_1}] k_1 S_1 \\
 \frac{dS_3}{dt} &= [\alpha_{S_3} - \alpha_{D_3} - \delta_{S_3}] k_3 S_3 \\
 &\quad + [2m_2\alpha_{S_2} + m_2\alpha_{A_2}] k_2 S_2
 \end{aligned}$$

Differentiating Cells:

$$\begin{aligned}\frac{\partial n_0}{\partial t} + \frac{\partial n_0}{\partial a} &= [(1 - 2M_0)\beta_0(a) - \mu_0(a)] n_0 \\ \frac{\partial n_1}{\partial t} + \frac{\partial n_1}{\partial a} &= [(1 - 2M_1)\beta_1(a) - \mu_1(a)] n_1 + 2M_0\beta_0(a)n_0 \\ \frac{\partial n_2}{\partial t} + \frac{\partial n_2}{\partial a} &= [(1 - 2M_2)\beta_2(a) - \mu_2(a)] n_2 + 2M_1\beta_1(a)n_1 \\ \frac{\partial n_3}{\partial t} + \frac{\partial n_3}{\partial a} &= [\beta_3(a) - \mu_3(a)] n_3 + 2M_2\beta_2(a)n_2\end{aligned}$$

Birth and Death Functions for Differentiating Cells:

$$\begin{aligned}\beta_i(a) &= \frac{-b_i}{2} \tanh(\rho_{\beta_i}(a - \omega_{\beta_i})) + \frac{b_i}{2} \\ \mu_i(a) &= \frac{d_i}{2} \tanh(\rho_{\mu_i}(a - \omega_{\mu_i})) + \frac{d_i}{2}\end{aligned}$$

for $i = 0, 1, 2, 3$.

Initial Conditions:

$$\begin{aligned}n_0(a, 0) &= f(a) \\ n_{1,2,3}(a, 0) &= 0 \\ S_0(0) &= S_0 \\ S_{1,2,3}(0) &= 0\end{aligned}$$

Boundary Conditions:

$$\begin{aligned}n_0(0, t) &= [2(1 - m_0)\alpha_{D0} + (1 - m_0)\alpha_{A0}] k_0 S_0 \\ n_1(0, t) &= [2(1 - m_1)\alpha_{D1} + (1 - m_1)\alpha_{A1}] k_1 S_1 \\ &\quad + [2m_0\alpha_{D0} + m_0\alpha_{A0}] k_0 S_0 \\ n_2(0, t) &= [2(1 - m_2)\alpha_{D2} + (1 - m_2)\alpha_{A2}] k_2 S_2 \\ &\quad + [2m_1\alpha_{D1} + m_1\alpha_{A1}] k_1 S_1 \\ n_3(0, t) &= [2\alpha_{D3} + \alpha_{A3}] k_3 S_3 \\ &\quad + [2m_2\alpha_{D2} + m_2\alpha_{A2}] k_2 S_2.\end{aligned}$$

3.2. Exploring the Pathways to Tumorigenesis

Several types of genetic transformations have been implicated in oncogenesis, but in this investigation, focus is directed towards somatic mutations that occur during DNA replication. In their

review and classification of cancer cells, Hanahan and Weinberg identified commonalities in malignant cells: independence of growth signals, increased proliferation, evasion of apoptosis, insensitivity to anti-growth signals, and the abilities to promote angiogenesis and metastasize [18]. In addition, genetic instability is believed to be widespread in various cancers [10].

To examine the initiation of cancer, three mutations are presently considered. The D mutation decreases the percentage of stem cells that go through apoptosis and decreases the maximum death rate of non-stem cells. The G mutation increases the rate at which subsequent mutations are acquired. The R mutation alters cell proliferation, by either increasing the rate of proliferation or shifting the balance of stem-cell division to favor symmetric self-renewal. A cell is considered to be healthy and normal if it does not have any mutations and assumed to be cancerous once it has acquired all three mutations. For model simulations, all mutations are one-hit, though mutations requiring two genetic events could easily be incorporated simply by increasing the number of mutations that must occur to malignantly transform a cell. Mutations enabling angiogenesis and metastasis are not considered because model simulations are of the hematopoietic system. However, if one wished to incorporate these mutations, the model could be extended easily in order to accommodate additional mutations.

The order in which mutations are acquired is noted by the order in which D, G, and R are listed. There are six possible sequences in which the mutations accumulate:

- $D \Rightarrow G \Rightarrow R$
- $D \Rightarrow R \Rightarrow G$
- $G \Rightarrow D \Rightarrow R$
- $G \Rightarrow R \Rightarrow D$
- $R \Rightarrow D \Rightarrow G$
- $R \Rightarrow G \Rightarrow D$

Tumor dynamics are compared and contrasted for all six pathways. Note that each pathway produces cancer cells that have acquired the same three mutations, but each pathway is different in the order in which mutations occur. Because a specific cancer is not being modeled, it is assumed that for each D, G, and R mutation in the model, there are approximately 100 genes that may cause transformation [32]. As a result, the mutation rate is one hundred times the suggested mutation rate of 10^{-8} per division [24, 32].

It is assumed that the hierarchical tissue begins in the healthy steady state determined by the model in Equation set 2.1. As a result, the number of stem cells and the maturity distribution of differentiating cells for healthy tissue in homeostasis is the initial condition for simulations of tumorigenesis. Parameter values used to simulate healthy and mutated cells are presented in Table 2.

Because cancer stem cells are believed to drive tumor growth, the emergence of the first cancer stem cell establishes the onset of malignancy. As a result, the time required to generate the first cancer stem cell is recorded for each mutation pathway in order to determine which is the fastest

Parameters Used			
Parameter	Biological Meaning	Normal Value	Mutated Value
S_0	Normal stem-cell homeostasis	18,000 (cells) [24]	18,000 (cells)
α_S	Probability of SSR	0.20 [33]	0.40
α_A	Probability of ASR	0.60 [33]	0.425
α_D	Probability of SD	0.15 [33]	0.15
δ_S	Probability of stem-cell death	0.05 [24]	0.025
k	Stem-cell proliferation rate	0.2471 (weeks ⁻¹) [12]	0.4942 (weeks ⁻¹)
m	Mutation rate of stem cells	10 ⁻⁶ [4, 22, 32]	10 ⁻⁴ [22, 32]
M	Mutation rate of non-stem cells	10 ⁻⁶ [4, 22, 32]	10 ⁻⁴ [22, 32]
b	Max. progenitor proliferation rate	9.7 (weeks ⁻¹)	19.4 (weeks ⁻¹)
ρ_β	Steepness of prog. prol. switch	2	2
ω_β	Maturity at prol. switch	1.85 (weeks)	2.35 (weeks)
d	Max. differentiated cell death rate	16.8 (weeks ⁻¹) [6, 9]	8.4 (weeks ⁻¹)
ρ_μ	Steepness of diff. cell death switch	10	10
ω_μ	Maturity at death switch	3.20 (weeks) [15]	3.70 (weeks)

in cancer development. Four scenarios of mutation acquisition are investigated. In the first case, all mutations are advantageous and increase the cell's competitive advantage in some way. In the second case, mutations occurring in cells that have not yet acquired the ability to evade apoptosis are disadvantageous and increase cell death. The third case investigates the effects of a shift in the stem-cell division pattern that increases symmetric self-renewal. Finally, increased expansion in the progenitor pool due to extra divisions is explored in the fourth case.

3.2.1. All Mutations are Advantageous

Consider a case in which all mutations are advantageous, which for convenience shall be referred to as Case A. In particular, suppose all mutations give the cell a specified advantage over its normal counterpart and do not cause an increase in cell death upon mutation. Specifically, the D mutation decreases the probability of stem-cell death by half and the decreases the death rate of differentiating cells by half. The G mutation augments genetic instability, increasing the rate at which mutations are acquired from 10^{-6} to 10^{-4} . The R mutation doubles the proliferation rate of both stem and progenitor cells. It is worth noting that progenitor cells with the R mutation reach full maturity in half the time of normal cells since the cells are dividing twice as fast but this mutation does not increase the number of divisions they are able to complete.

Under these conditions, genetic instability is the most significant contributor to cancer onset. The GDR and GRD pathways produce the first cancer stem cell in the shortest time, followed by the DGR and RGD pathways, and finally the DRG and RDG pathways. The fastest pathways are those in which G is acquired first, while the slowest acquire G last. Thus, it is evident that the order in which the G mutation is acquired determines the speed of cancer stem cell generation.

The significance of the G mutation may at first seem surprising because it does not increase the cell's fitness as the D and R mutations do. In fact, the G mutation might be thought of as a silent mutation that does not appear to give the mutated cell any advantage. However, the acquisition of G accelerates the rate at which additional mutations are acquired, and therefore decreases the time required to generate the first cancer stem cell.

The sequential order of the G mutation is the most important in determining the fastest pathway, but no such conclusion can be made about the order of D and R mutations. Whether D or R occurs earlier in the fastest pathway depends on the amount of change between normal and mutated proliferation and death rates. For instance, using certain parameter values, GDR could be the fastest, while for others it would predict that GRD is fastest. Over a wide range of parameters, however, the impact of the G mutation is still most significant in determining the time to cancer onset. As a result, the conclusion that genetic instability dictates the time to malignancy is robust.

Although it takes nearly 20 years for the first cancer stem cell to appear in the fastest pathway, cancer differentiating cells are present in the tissue at early times. In fact, all pathways generate a small number of cancer differentiating cells years before any cancer stem cells are formed. In this case it is assumed that no mutation occurs in progenitor populations that arrests differentiation. As a result, mutated differentiating cells may cause hypercellularity in the tissue, but they do not instigate malignancy because they die after completing a prescribed number of divisions. Instead, it is the emergence of the first cancer stem cell that marks the onset of disease because these cells can both self-renew to expand their number and differentiate to form mutated differentiating cells.

We also notice the correlation between the growth dynamics of the stem-cell population with those of the differentiating population. The model predicts that tumor growth is dependent upon the behavior of a small subpopulation of cancer cells. Therefore, under the assumptions of Case A, the model supports the cancer stem cell hypothesis in that a select subgroup of cells promotes tumorigenesis. That is, if differentiating cells do not acquire self-renewal capability, then a small population of mutated stem cells is the driving force in tumor growth.

3.2.2. Lethal Mutations

Because cell division is a tightly regulated process, the presence of mutations can force apoptosis. This defense mechanism prevents the propagation of mutations to progeny, thereby maintaining the genetic integrity of cells in the tissue. As a result, cells that have become transformed are more prone to programmed death unless they have also acquired a mutation that allows them to evade apoptosis. The next case, labeled Case B, investigates the consequences of increased death in cells that have acquired either R or G without obtaining D previously.

Suppose the mutations are defined as in Case A, with the additional condition that cells with either a G or R mutation have a higher death rate if D has not previously been acquired. The DGR and DRG pathways do not change in comparison with Case A since the D mutation is acquired first, but all other pathways are affected. Figure 4 compares the time to first cancer stem cell for each pathway in Cases A and B, as well as Case C, which will be presented shortly. We find that cancer onset is delayed in pathways in which D is not acquired first. Specifically, in sequences where D is acquired second, the first cancer stem cell appears approximately ten years later than it did in case A for parameter values found in Table 1. When D is acquired last, the first cancer stem cell appears approximately twenty years later than it did for the same sequence in Case A. These results suggest that the acquisition of a mutation decreasing cell death is most advantageous in producing cancer cells if mutations are lethal. This follows from the fact that in this simulation, cells with only R and G mutations are suppressed through apoptosis, whereas cells with the D mutation can expand. For that reason, it is not surprising that the DGR pathway is the fastest, with the first cancer stem cell appearing in 25.8 years, while the RGD pathway is slowest and produces the first cancer stem cell in 44.0 years.

The contrasting results from Case A and Case B demonstrate that the fastest pathway is dependent upon the assumptions that are made to characterize mutations. When all mutations are advantageous as in Case A, acquiring the G mutation first leads to the fastest appearance of a cancer stem cell. On the other hand, when mutated cells have increased death without the ability to evade apoptosis as in Case B, acquiring the D mutation gives rise to the first cancer stem cell. In addition, the tissue composition of the fastest pathway from Case A, denoted GDR_A , and the fastest pathway from Case B, denoted DGR_B , are notably different, as illustrated in Figure 3. Following the GDR_A pathway, the majority of cells are normal for the first 32.9 years, after which cancer cells dominate, while cells with one or two mutations remain a small portion of the system, as shown in Figure 3A. Compare this with the tissue composition of the DGR_B pathway plotted in Figure 3B. Normal cells are the majority until 31.1 years, after which cells with one mutation, namely the D mutation, are most numerous. Cells with the D mutation dominate until cancer cells surpass them at 46.8 years. Thus the decline of normal cells in the system is comparable between the two pathways, but the system following the GDR_A pathway is usurped by cells having all three mutations, whereas the tissue following the DGR_B pathway first fills up with cells having only one mutation before being filled with cancer cells. The dominance of cancer is nearly thirteen years faster in GDR_A than in DGR_B , indicating that the disease created through the former is more aggressive than the latter.

Due to cellular machinery that arrests proliferation of mutated cells, it is likely that mutations

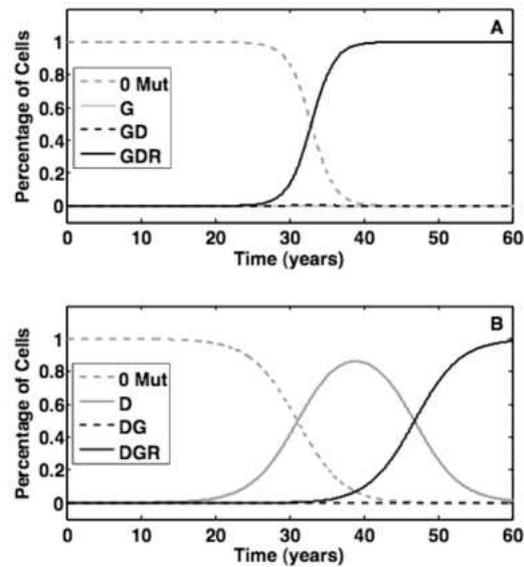


Figure 3: Comparison of tissue composition for fastest paths when all mutations are advantageous versus when some are lethal. (A) The changing tissue composition in the GDR pathway, fastest for Case A in which all mutations are advantageous. Within 33 years, cancer cells dominate the tissue. (B) The changing tissue composition for the DGR pathway, fastest for Case B in which R and G are not advantageous without D first. At approximately 31 years, cells with the D mutation are the majority, but cancer cells increase and take over the tissue in 46 years.

would be detected that would force the cell into apoptosis unless the machinery itself was also erroneously transformed. Consequently, the assumptions of Case B are likely a more realistic depiction of mutation acquisition in human cells. In this case, acquiring the D mutation first bears greatest importance since it ensures the cell's survival, allowing it to accumulate further abnormalities. As in Case A, the population of cancer stem cells drives tumorigenesis and is a very small minority of all tumor cells.

3.2.3. Unbalanced Stem-Cell Division Pattern

Deregulation of cell proliferation can refer either to the alteration of proliferation rate or the transformation of cell division pattern. Both Cases A and B defined the R mutation as an increase in proliferation rate. Now consider Case C in which the R mutation doubles the probability of symmetric self-renewal in stem cells, while the proliferation rate of stem cells and the division properties of progenitors are unaltered. In essence, progenitor cells with the R mutation do not behave differently than those without the R mutation, but stem cells with the R mutation are more likely to symmetrically self-renew than normal stem cells. The G and D mutations are defined as in Case A, and it is assumed that all mutations are advantageous.

Increasing symmetric self-renewal significantly quickens the pace of cancer development. In fact, all pathways have a first cancer stem cell within eight years. The fastest pathway is the GRD pathway, with the first cancer stem cell formed in 5.5 years, but the slowest pathway, DRG, is less than three years slower. Therefore, the difference between the fastest and slowest pathways is relatively insignificant, implying that increased symmetric self-renewal minimizes the impact of other mutations. In other words, when a mutation increases symmetric self-renewal, cancer stem cells rapidly emerge in all pathways so that the order of mutation acquisition does not meaningfully influence the time to first cancer stem cell.

Figure 4 compares the time to first cancer stem cell for each pathway in Cases A, B, and C. Cancer stem cells in Case C emerge 15 to 20 years faster than in Case A and 20 to 40 years earlier than in Case B. The speed of disease onset suggests that aberrant symmetric self-renewal may be a key contributor in aggressive forms of cancer, whereas deregulated cell proliferation may be characteristic of diseases that progress more slowly. Furthermore, increased symmetric self-renewal appears to diminish the impact of genetic instability because the difference between slowest and fastest pathways is relatively insignificant.

3.2.4. Progenitors Complete Additional Divisions

It is unknown if cancer stem cells are mutated stem cells or mutated progenitor cells that have gained stem-cell characteristics, particularly the ability to self-renew. To address this issue, Case D assumes the R mutation affects the proliferation of progenitor cells by increasing the number divisions before terminally differentiating. Stem cells may acquire the R mutation, though it does not alter the stem-cell kinetics and therefore acts as a pre-cancerous mutation that later manifests in progeny that are more differentiated. The proliferation rate of both stem and non-stem cells does not increase with the R mutation so that this mutation only increases the number of progenitor

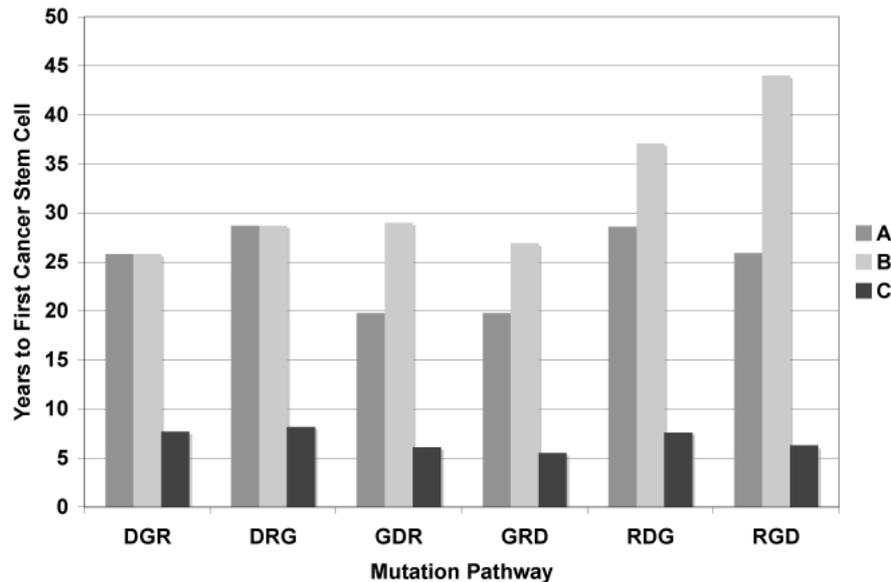


Figure 4: Unbalanced symmetric self-renewal significantly decreases the time to cancer. When stem-cell division pattern is unbalanced with an increase in symmetric self-renewal, cancer stem cells develop more rapidly than when the rate stem cell proliferation is increased.

divisions. As in Cases A, B and C, the D mutation decreases apoptosis, the G mutation increases the mutation rate. In addition, it is assumed that all mutations are advantageous.

The time to first cancer stem cell is slower for all six pathways than in the previous cases because mutated stem cells do not proliferate faster or symmetrically self-renew more than normal stem cells. However, progenitor and differentiated populations expand due to the extra divisions completed by progenitors, as shown in Figure 5A. The percentage of tissue cells having one mutation is plotted in Figure 5B. The DGR and DRG pathways, are the pathways in which a majority of tissue cells have one mutation over time. As demonstrated in Figure 5C, the majority of tissue cells following the RDG pathways have two mutations, R and D. All other pathways are taken over by cells with all three mutations, as shown in Figure 5D. The hypercellularity resulting from the D and R mutations alone could lead to death as the tissue reaches a fatal burden of cells as in the DRG and RDG pathways. However, tissues following these pathways are primarily composed of cells with the D and R mutations that might be more reactive to treatment since genetic instability has not been acquired. If treatment successfully targets cells that have not acquired all three mutations, then pathways generating tumors mainly composed of cells with all three mutations are more problematic.

Unlike Cases A, B, and C, the composition of non-stem cells in the tissue does not mirror the composition of stem cells because the R mutation only manifests itself in progenitor cells. Consider the GDR pathway, which is the first pathway to have a cancer stem cell in this case. Figure 6A plots the composition of the stem-cell pool over time. Normal stem cells dominate for the first forty-nine years, after which the majority of stem cells have the G and D mutations. There

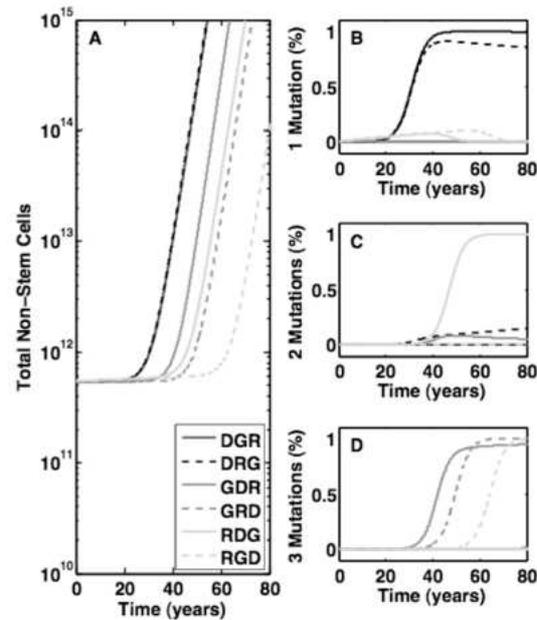


Figure 5: Progenitor and differentiated cells accumulate due to extra progenitor divisions. (A) The total non-stem cell population for each pathway. The DGR and DRG pathways generate hypercellularity the fastest. However, most of these cells only have 1 mutation. (B) The percentage of progenitor and differentiated cells that have one mutation. Tissues following the DGR and DRG pathways are dominated by cells with one mutation. (C) The percentage of progenitor and differentiated cells that have two mutations over time. Tissues following the RDG pathway are mainly composed of cells with both the R and D mutations. (D) The percentage of progenitor and differentiated cells with three mutations over time. Tissues following GDR, GRD, and RGD are eventually taken over by cancer cells with all three mutations.

are a small number of cancer stem cells, and although they exceed normal stem cells in 57 years, they comprise a small percentage of the stem cell population and do not surpass those with only two mutations. In contrast, the composition of differentiated cells is markedly different, as plotted in Figure 6B. Cells with all three mutations take over the non-stem cell pool within 42 years, while cells with one or two mutations remain a small percentage of progenitor and differentiated cells. The contrast between stem-cell and non-stem cell compositions proves that cancer growth is due to expansion in the progenitor pool, not the stem-cell pool.

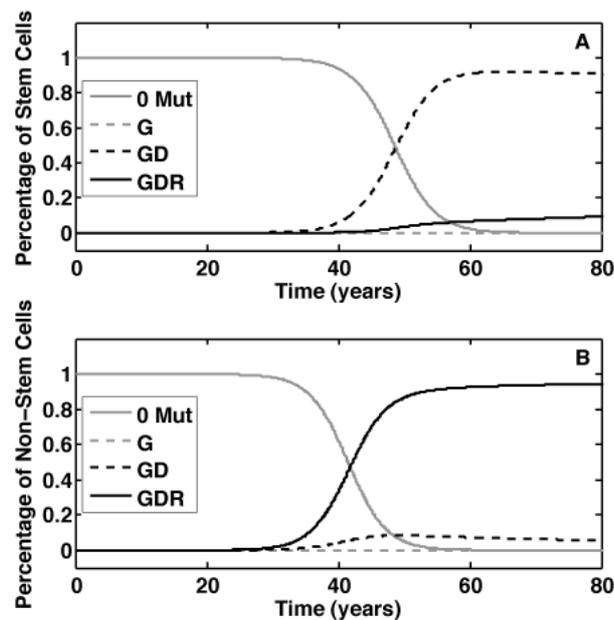


Figure 6: Comparison of stem cell composition and non-stem cell composition for the GDR pathway in case D. (A) The percentage of stem cells with 0, 1, 2, or 3 mutations over time. After 49 years, the majority of stem cells have only the D mutation. (B) The percentage of non-stem cells with 0, 1, 2, or 3 mutations. After 42 years, the majority of non-stem cells have all 3 mutations due to the extra division and amplification of progenitor cells that have acquired the R mutation.

Because mutated differentiating cells continue to mature and have not acquired the ability to limitlessly self-renew, it is still the small percentage of cancer stem cells that drives tumorigenesis. Without the self-renewing cancer stem cell population, mutated differentiating cells would cause hypercellularity but ultimately reach a state of homeostasis, even though elevated. It should be noted that the R mutation is what causes significant expansion in progenitors, and the growth of cell populations with the R mutation may cause proliferative disorders, even if all three mutations have not been acquired.

This case in which R extends proliferation of progenitors without affecting stem cells is unique in that the system may reach a fatal level of cancer cells before any cancer stem cells are formed. By increasing the number of divisions progenitors complete before terminal differentiation, there is a

massive expansion in mutated progenitor and differentiated cell populations and may be indicative of a myeloproliferative disease. However, this type of R mutation is not sufficient in causing cancer without pre-cancerous mutations occurring in the stem-cell pool, which is illustrated in Figure 7. If stem cells are capable of acquiring one, two, or three mutations, then the cancer cell population grows due to the exponential growth of mutated stem cell populations. In contrast, if stem cells do not acquire any mutations, then cancer progenitor and differentiated cells remain at low, undetectable levels because cancer progenitors eventually reach terminal differentiation and die, thereby preventing expansion. Therefore, at least one mutation must occur in stem cells that initiates exponential growth in order to generate malignancy.

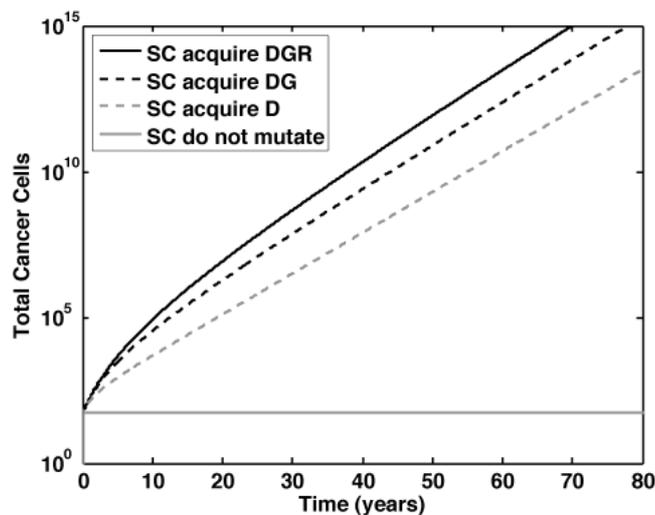


Figure 7: At least one mutation is needed in stem cells for malignant tumor growth. Cancer growth is fastest when stem cells acquire all three mutations, but cancer growth still occurs when one or two mutations may be acquired at the stem cell level. When stem cells do not mutate, increased progenitor expansion alone is not sufficient to cause malignant growth.

These results indicate that unless progenitor cells acquire a mutation that permits them to self-renew and prevent maturation as stem cells normally do, stem-cell mutations are critical in promoting tumorigenesis. However, the model predictions from Case D demonstrate the substantial impact that extra progenitor divisions have on tissue hypercellularity. As a result, it is hypothesized that progenitor self-renewal would generate an even greater increase in tissue mass and would be a more aggressive disease. Such a mutation is believed to facilitate the transition between chronic to blast phase in Chronic Myelogenous Leukemia. The possibility of progenitors gaining limitless self-renewal potential and the impact it has on the progression of this specific disease will be of particular focus in future work.

4. Conclusions

Although many types of mutations have been identified in cancer cells, it is difficult to determine the order in which they were acquired that led to malignancy. We have examined mutation acquisition in hierarchical tissue with a maturity-structured mathematical model in order to investigate the impact that mutation order has on tumor dynamics. In particular, the sequential accumulation of somatic mutations was modeled to examine the multi-step process that initiates cancer. Importantly, it was concluded that the order in which mutations are acquired does affect the tempo of tumorigenesis. In addition, tumor composition varies for different mutation pathways, so that some sequences generate tumors that are dominated by cancerous cells, while others are primarily comprised of cells with only one or two mutations.

For each mutation pathway considered, the time to first cancer stem cell determined the onset of malignancy, so that the fastest pathway could be established. If all mutations are advantageous, genetic instability is the key determining factor for the emergence of cancer stem cells, and this result is robust for a wide range of parameters. The fastest pathways acquired genetic instability first, which agrees with the results of Michor *et al.*, who predicted that chromosomal instability was an early event in colon cancer [25]. This result differs from the work by Spencer *et al.*, who predicted that the fastest pathway to cancer ends with genetic instability [32]. Rather than following the particular order in which mutations accumulate, however, Spencer *et al.* did not distinguish the chronological order of mutations that generated cells with a particular phenotype. In contrast, the predictions resulting from our work suggest that the specific sequential order of mutation acquisition decisively influences tumor dynamics.

In addition to the importance of mutation sequence, model predictions indicate that certain types of mutations are more significant than others in dictating cancer onset. For instance, when all mutations are advantageous, acquiring genetic instability first leads to the fastest path. In contrast, if mutations are lethal when evasion of apoptosis has not yet been acquired, then the fastest pathways are initiated with mutations decreasing cell death. Particularly significant are mutations that cause the stem-cell division pattern to be unbalanced in the favor of symmetric self-renewal. Increased symmetric self-renewal significantly quickens cancer onset and progression because it rapidly expands the cancer stem cell population. Furthermore, it diminishes the importance of all other mutations in that cancer stem cells emerge in all pathways within a relatively short time of each other.

When mutations affect stem and progenitor cells similarly, the model predicts that the dynamics of the differentiating-cell population are dictated by the dynamics of the stem-cell population. As a result, the cancer stem cell population is the driving force of tumor growth. However, if a mutation is acquired in stem cells, but is not manifested until inherited in a progenitor, then the dynamics of stem cells and non-stem cells do not closely correlate. For example, a mutation that increases the number of progenitor divisions contributes to hypercellularity and tumorigenesis, even before the formation of cancer stem cells. Yet, if progenitor and differentiated cells do not acquire limitless self-renewal potential, malignancy does not form unless some initial mutations occur in the stem-cell population. This result demonstrates the driving force of cancer stem cells in tumor formation and disease progression. Furthermore, the model predicts that the cancer stem cell population is

a small minority of tumor cells for all cases discussed here. Because differentiation pathways are not disabled, mutated progeny continue to expand and mature, forming cancerous differentiated cells that significantly outnumber stem cells. According to model results, it is predicted that the percentage of tumor cells that are cancer stem cells significantly increases only if differentiation is somehow inhibited.

The mathematical model we have presented here provides a general framework that could be used to investigate tumorigenesis in any hierarchical tissue. To demonstrate the usefulness of this model, simulations of mutation acquisition in hematopoietic cells were conducted, but the model structure is general enough to be adapted to other tissues and include any number of mutations. The maturity structure offers unique insight into the maturity distribution of tumor cells, which may be beneficial for studying malignancies in which the distribution of stem, progenitor, and differentiated cells is significantly altered. Another novel feature of this model is the incorporation of all three modes of stem-cell division, which makes it possible to determine the effects on tumor dynamics when the balance is deregulated. In this model, it is assumed the probabilities of symmetric self-renewal, asymmetric self-renewal, and symmetric commitment differentiation are constant and regulatory mechanisms governing stem-cell division pattern are not incorporated. There are many factors that influence self-renewal and differentiation, however. Apart from the current discussion, we have investigated the role of regulatory mechanisms in maintaining tissue homeostasis. In the future, we will discuss consequences that follow when these mechanisms become deregulated and examine the effects on tumorigenesis.

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