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Extra View

Examples of Mathematical Modeling

Tales from the Crypt

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KEY WORDS

age-structure, feedback, mutations, structural stability

ABBREVIATIONS

ODE	ordinary differential equation
PDE	partial differential equation

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ABSTRACT

Mathematical modeling is being increasingly recognized within the biomedical sciences as an important tool that can aid the understanding of biological systems. The heavily regulated cell renewal cycle in the colonic crypt provides a good example of how modeling can be used to find out key features of the system kinetics, and help to explain both the breakdown of homeostasis and the initiation of tumorigenesis.

We use the cell population model by Johnston et al.[5] to illustrate the power of mathematical modeling by considering two key questions about the cell population dynamics in the colonic crypt. We ask: how can a model describe both homeostasis and unregulated growth in tumorigenesis; and to which parameters in the system is the model most sensitive? In order to address these questions, we discuss what type of modeling approach is most appropriate in the crypt.

We use the model to argue why tumorigenesis is observed to occur in stages with long lag phases between periods of rapid growth, and we identify the key parameters.

INTRODUCTION

Mathematical modeling can be a powerful tool for understanding biologically observed phenomena which cannot be understood by verbal reasoning alone.[1] One such example is that of homeostasis in the colonic crypt. The single layer of epithelial cells that line the crypt is renewed every two to three days by a number of long-living stem cells that remain at the bottom of the crypt.[2] As the stem cells divide, their progeny migrate up the crypt wall, and once at the top they are shed into the lumen or undergo apoptosis. This general structure of stem, transit and differentiated cells is also found in many other biological systems, for example the hematopoietic system.

Many modeling studies have tried to capture this tightly regulated system, and three common approaches are to use compartmental, simulation or stochastic models (reviewed in ref. 3). Compartmental models include those by Tomlinson and Bodmer,[4] Johnston et al.,[5] Boman et al.,[6] Hardy and Stark,[7] Wodarz[8] and Paulus et al.,[9] and simulation models have been presented by Loeffler et al.[10,11] Models that capture the stochastic effects of mutational changes include those by Nowak et al.,[12,13] Michor et al.,[14-17] Komarova and Wang[18] and Komarova.[19,20]

In this paper we focus on a compartmental model, and discuss mathematical techniques that can be used to address key questions relating to the population dynamics of the system. We will also show how this mathematical approach can suggest which parameters are most important to determine experimentally, and which it is less important to know accurately. We shall draw some examples from the recent paper by Johnston et al.[5]

We consider two biological questions: (1) how can we formulate a model that will allow for both the tight regulation of the number of cells in a healthy crypt, but also the breakdown of homeostasis when cancer occurs? (2) Which are the important parameters in this system? These questions are addressed in Examples 2 and 3 respectively. Firstly, we need to decide the most appropriate form of mathematical modeling for the cell populations, and this is discussed in Example 1. We begin, however, by summarizing our compartmental model[5] which we will later use to address the key questions relating to colorectal cancer.

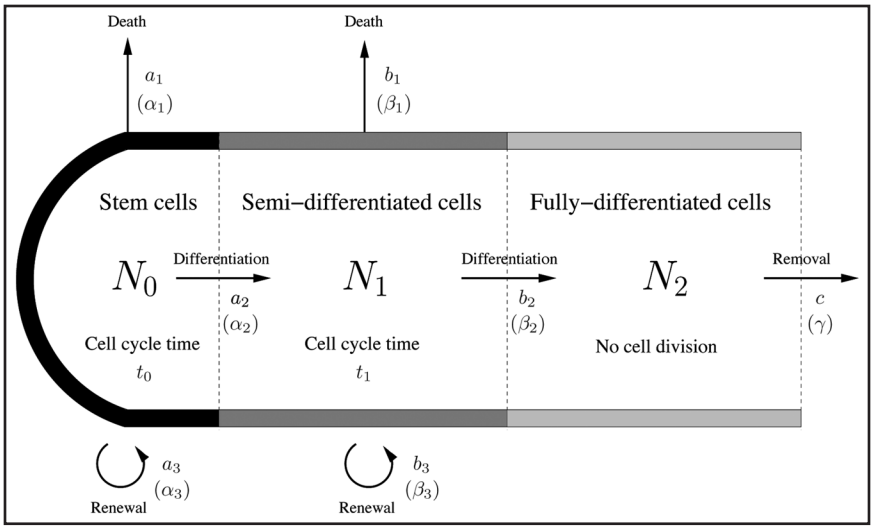

Figure 1. A schematic of a colonic crypt showing the compartmental structure used in the model by Johnston et al.[5] The stem cells differentiate into semi-differentiated cells, which in turn differentiate into fully-differentiated cells. Each cell population can die, and the stem cells and semi-differentiated cells can renew. The parameters for the age-structured model are the proportions of the populations a_i, b_i and c that are leaving the compartments, and the parameters for the continuous model (described in Example 1) are the rates of conversion α_i, β_i and γ measured in hours^{-1}. Note that these compartment sizes are not to scale and that, in reality, the number of stem cells is very much less than the number of transit cells.

A COMPARTMENTAL MODEL

In our compartmental model,[5] which is summarized in Figure 1, we followed Tomlinson and Bodmer[4] who separated the cells in the crypt into populations of stem cells (denoted N_0), fully-differentiated cells (denoted N_2), and a third population for all the transit-amplifying cells that are in the process of differentiating, referred to as semi-differentiated cells (denoted N_1). At the end of each cell cycle the stem cells can die, differentiate into transit cells or renew with probabilities a_1, a_2 and a_3 respectively, whereas transit cells will die, differentiate into fully-differentiated cells or renew with probabilities b_1, b_2 and b_3 respectively. The fully-differentiated cells will be removed from the system with probability c. The probabilities can also be thought of as the proportions of the cell populations that undergo each process. The cell cycle times for the stem and transit cell populations are denoted by t_0 and t_1 respectively and measured in hours.

We use this model to address the question of modeling homeostasis in Example 2. First, however, we discuss discrete, age-structured and continuous models and their relevance to crypt modeling.

EXAMPLE 1: WHICH IS THE BEST TYPE OF MODELING FOR THE CRYPT?

What is the simplest and most appropriate technique for modeling the cell division process in the colonic crypt? In this section we first compare and contrast the discrete, age-structured and continuous modeling approaches, illustrating them by considering the stem cell population from Figure 1, and then explain when each approach is appropriate.

A discrete modeling approach follows the number of cells in a population at regular time intervals, and does not take into account where the individual cells are in their cell cycle. As in the model by Tomlinson and Bodmer,[4] we count the number of stem cells $N_0(G)$ at each generation G measured in time units of the stem cell division time t_0. The parameter a_3 denotes the proportion of the stem cell population that renews at each division, assuming that all divisions are symmetric (asymmetric divisions can be thought of as half a symmetric renewal and half a symmetric differentiation). We relate the number of stem cells at any generation to the number in the previous generation by the difference equation

$$N_0(G+1) = 2a_3 N_0(G)\,. \tag{1}$$

Assuming an initial number of stem cells, $\hat{n}_0$, this can be solved to find the population at each future G^{th} generation by the formula

$$N_0(G) = (2a_3)^G \hat{n}_0\,. \tag{2}$$

An alternative approach is to use an age-structured model[5] to find the number of stem cells $N_0(t,a)$ at a continuous time point t and age a. In this context, the "age" of a cell refers to the length of time since its last division. This model consists of partial differential equations (PDEs) that quantify the rate of change of N_0 as both time t and age a vary [see equation (3)]. For example, assuming (as we did in ref. 5) that the cells only die, differentiate or renew at the end of their cell cycles, we obtain the PDE

$$\frac{\partial N_0}{\partial t} + \frac{\partial N_0}{\partial a} = 0\,, \tag{3}$$

which is valid for all ages $0<a<t_0$. At the end of each cell cycle we also have that

$$N_0(t,0) = 2a_3 N_0(t,t_0)\,, \tag{4}$$

which is a more specific form of equation (1) relating the numbers of cells being born and those reaching the end of their cell cycle at time t. If an initial distribution of the ages of cells $n_0(a)$ is known, the solution is given by

$$N_0(t,a) = (2a_3)^G n_0(Gt_0 + a - t)\,, \tag{5}$$

where G is the number of times that cells of age a have been through the cell cycle at time t.

A third approach that can be adopted is that of continuum modeling which follows the number of cells $N_0(t)$ at a continuous time t. Whereas in the discrete and age-structured models the cell population renewal, differentiation and death are assumed to occur only at the end of cell cycles, in a continuous model these processes are assumed to occur continuously in time. In the discrete and age-structured models we consider the proportions a_i, b_i and c of the populations that undergo each process at the end of each cell cycle, but in the continuous model we must monitor the rates at which these processes occur in time, which we denote by their Greek equivalents α_i, β_i and γ (measured in hours^{-1}), as shown in Figure 1. Thus, for example, over a small time τ we would expect a proportion $\alpha_2\,\tau$ of stem cells to have differentiated, on average.

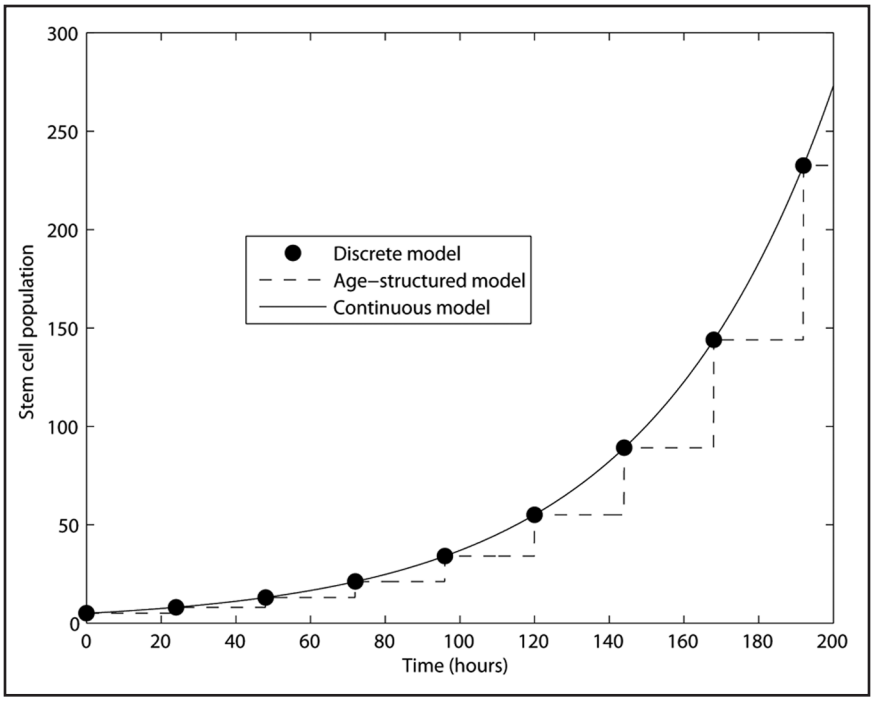

Figure 2. A plot of the stem cell population using the discrete model (equation 2), age-structured model (equation 5) and continuous model (equation 7). The parameters are taken to be α = 0.02 hours^{-1}, t_0 = 24 hours and $\hat{n}_0$ = 5. a_3 is chosen such that $a_3 = e^{\alpha t_0}/2$ (as in ref. 5).

This continuous approach yields ordinary differential equations (ODEs) that follow the population as it changes in time. The corresponding ODE for the stem cell compartment is

$$\frac{dN_0}{dt} = (\alpha_3 - \alpha_1 - \alpha_2)N_0, \tag{6}$$

which has a solution of the form

$$N_0(t) = \hat{n}_0 e^{\alpha t}, \tag{7}$$

where $\alpha = \alpha_3 - \alpha_1 - \alpha_2$ represents the net per-capita growth rate of stem cells.

Note that in order to compare formula (5) with (2) and (7) it is necessary to integrate the solution over all possible ages to obtain the total numbers for each cell population (as in ref. 5). These solutions are compared in Figure 2, with an initial age profile for (5) where all the cells have the same age. Each of the solutions has the same overall growth behavior, and any of these models could be used to describe the stem cell population. However, which approach is the most appropriate to use when modeling all the cells in the crypt, and which produces the model that is most mathematically tractable?

Let us consider both the stem cells and the transit-amplifying cells, which are assumed to divide on different timescales, t_0 and t_1 respectively. There is an implicit assumption of synchrony of cell division associated with the discrete model, where all the cells divide at the same time and are at the same stage in their cell cycle. Although not biologically realistic, in the case of only one cell type this assumption is at least self-consistent because every cell will divide once every generation. However, the discrete model cannot be used to describe two cell types with different generation times since, except in the special case where the stem cell cycle time is an integer multiple of the transit cell cycle time, $t_0 = pt_1$ (where p is an integer), the cells that are differentiating from the stem cell compartment will enter the transit cell population when the existing transit cells are already partly through their cell cycle, which breaks the synchrony condition.

Hence, in the case of two populations dividing at different rates, it is necessary to use either the age-structured or continuous models. If it is necessary to follow specific variations at times on the order of the cell cycle then the age-structured model is the best choice. The age-structured model is more accurate than the continuous model but produces more complicated solutions, as illustrated in ref. 5 where we solved the age-structured model for two different initial age profiles. Alternatively, when a population changes over a timescale of many cell cycles, the continuous model can capture the same overall behavior with more clarity: ODEs are much easier to solve mathematically than difference equations or PDEs. In ref. 5 we showed that the continuous and age-structured models produced solutions with the same behavior, and we derived conditions relating their parameters.

In conclusion, discrete modeling can be an informative technique when there is only one cell division timescale to consider, but if there is more than one timescale, as is the case with cells in the colonic crypt, then an age-structured or continuous approach must be adopted, and then the choice comes down to the timescale of interest. In Example 2 we investigate the global stability behavior of the system over long timescales, and so we use a continuous model.

EXAMPLE 2: HOW CAN MODELS DESCRIBE BOTH HOMEOSTASIS AND TUMORIGENESIS?

Homeostasis in the crypt is observed to control cell numbers, but this regulation is broken in tumorigenesis where cell populations grow without bound. In this section we discuss the need to model homeostasis, how it can be overcome in tumorigenesis, and how a mathematical model can capture both these processes.

One feature of all of the solutions (2), (5) and (7) to the models in Example 1 is that the stability of the population is critically dependent on the choice of one particular parameter value. The age-structured and discrete model solutions vary in time according to functions of the form $(2a_3)^G$, where G is the number of divisions that the cells have undergone. The continuous model solutions vary with time-dependent functions $e^{\alpha t}$, where α represents the net per-capita growth rate of the stem cells. It is often assumed that stem cells normally divide asymmetrically, producing one daughter stem cell and one daughter transit cell each generation. However it is equally possible that stem cells, sometimes at least, divide symmetrically producing two daughter cells of the same type, either both stem cells or both transit cells. In that case, for the age-structured model, the stem cell population will remain stable if, and only if, exactly half of all symmetric divisions produce two stem cells while the other half produce two transit cells, $a_3 = 1/2$. This is equivalent to the stem cells dividing asymmetrically on average. If $a_3 > 1/2$ then the population will increase exponentially while if $a_3 < 1/2$ then the population will decrease to zero. The same applies in the continuous model, with exponential growth (decay) occurring if α is greater (less) than zero,

and a stable population occurs only if $\alpha = 0$. When the stability of a system is dependent on a specific parameter value being taken a model is said to be structurally unstable. Such models are usually unrealistic since there is often some natural variation in parameter values.

Furthermore, when some stem cells are removed from the crypt, it has been observed that a homeostatic response is induced to create more stem cells.[9] It is not known whether this is due to more stem cells dividing symmetrically, or to de-differentiation of first generation transit cells, but neither scenario can be modeled when these parameters are forced to take fixed values. A dynamic system requires regulation of cell numbers to maintain equilibrium.

One method that captures the homeostatic regulation of the stem cells is to introduce feedback into the system by making the parameter values dependent on the population sizes. In ref. 5 we used the continuous model to include these variable parameters by allowing the rate of stem cell differentiation to depend on the size of the stem cell population. Equally, we could have chosen the feedback to act through the rates of death or renewal instead.

Let us replace α_2 by the variable differentiation rate $R = R(N_0)$, and note that the rates of stem cell death and renewal remain fixed throughout. If the stem cell number N_0 becomes too large then R will increase so that more stem cells differentiate than renew. In practice this might be achieved by producing more transit cells and fewer stem cells, either by changing the proportion of symmetric divisions, or by changing the cell cycle time of the symmetric divisions. Alternatively, if the number of stem cells drops too low then R will decrease and symmetric divisions will produce more stem cells. In order to achieve both these effects, $R(N_0)$ must be an increasing function of N_0. Many functions $R(N_0)$ could be chosen that would have this property, but the different functional forms could produce different qualitative effects on the system.

In our paper,[5] we proposed feedback in the differentiation rate of the form

$$R(N_0) = \alpha_2 + \frac{k_0 N_0}{1 + m_0 N_0}, \tag{8}$$

where $R(N_0)$ replaces α_2 in (6), and k_0 and m_0 are nonnegative constants. The parameter k_0 is measured in hours^{-1} and represents the speed of response of the feedback with large (small) k_0 inducing a fast (slow) feedback response from the system. The parameter m_0 is dimensionless and represents feedback saturation.

When $m_0 > 0$, the differentiation rate cannot increase beyond a maximum size $\alpha_2 + k_0 / m_0$, and there is a nonzero stem-cell steady state of the form

$$N_0^* = \frac{\alpha}{k_0 - m_0 \alpha}, \tag{9}$$

as long as the net per-capita growth rate $\alpha = \alpha_3 - \alpha_1 - \alpha_2$ is in the range $0 < \alpha < k_0 / m_0$. If the growth rate exceeds this value, then the stem cell population grows without bound. This is a saturating feedback because the population dependence in the differentiation rate will regulate the population size if the growth rate is low enough, but once the growth rate passes its saturation limit a steady state can no longer be achieved.

When $m_0 = 0$, the feedback reduces to a linear form and the saturation is switched off. This means that any increase in the size of N_0 will be matched by an equivalent increase in R, and there is a nonzero stable steady state

$$N_0^* = \frac{\alpha}{k_0}, \tag{10}$$

as long as the net per-capita growth rate α is positive, i.e., as long as cells are not removed faster than they are produced. This is the same form as the feedback chosen by Wodarz,[8] who used a logistic form with the un-mutated rate of change of stem cells taken to be $\alpha N_0(1 - N_0/K)$ with a stem-cell carrying capacity $K = \alpha / k_0$. The linear feedback controls the population growth to the extent that unbounded growth is not permitted for any positive parameter values.

Which of these two forms is more appropriate for describing the cell population dynamics in the crypt? Both forms capture homeostatic regulation of stem cells and produce models that are structurally stable. However, in a system with the linear form, unbounded growth can only be achieved by removing the feedback completely (by setting the speed of feedback response k_0 to zero), but with the saturating form unbounded growth can also be achieved by overwhelming the feedback. Therefore, only the saturating form can describe both homeostasis and unbounded growth of cell numbers without removing the feedback. There are two possibilities for how the saturating feedback might occur. The stem and transit populations might both be subject to saturating feedback, and consequently both populations would be able to initiate tumorigenesis. Alternatively, the stem cells may be subject to linear feedback which maintains equilibrium, with the transit cells subject to saturating feedback and possible tumorigenesis. We conclude this section by discussing both possibilities and giving examples of each.

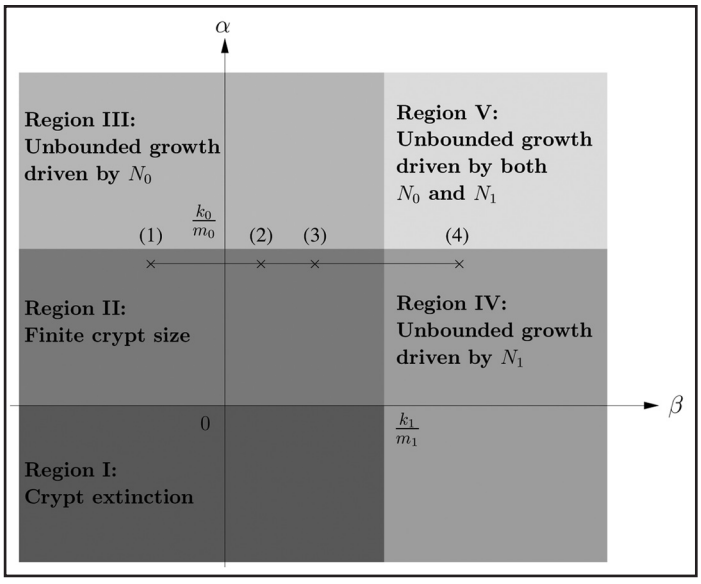

Figure 3. Regions of stability in the (α, β) parameter space for the system with saturating feedback in both stem and transit cell populations. If $\alpha < 0$ and $\beta < k_1 / m_1$ (Region I), then the stem cells cannot sustain their number and the crypt becomes extinct. If $0 < \alpha < k_0 / m_0$ and $\beta < k_1 / m_1$ (Region II), a healthy stable crypt is achieved. In all other regions the growth saturation limit is exceeded and the cell populations grow without bound. In Region III ($\alpha > k_0 / m_0$ and $\beta < k_1 / m_1$) the cancer stem cell driving the unbounded growth is a tissue stem cell, whereas in Region IV ($\beta > k_1 / m_1$ with $\alpha < k_0 / m_0$) the cancer stem cell derives from a transit-amplifying cell. In Region V ($\alpha > k_0 / m_0$ and $\beta > k_1 / m_1$) cancer stem cells originate from both tissue stem cells and transit cells.

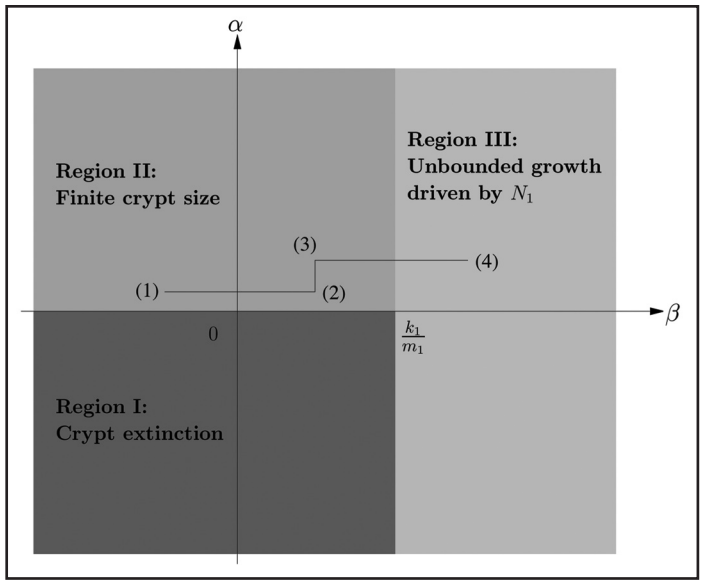

Figure 4. Regions of stability in the (α, β) parameter space for the system with linear feedback for stem cells and saturating feedback for transit cells. If $\alpha < 0$ and $\beta < k_1 / m_1$ (Region I), then the stem cells cannot sustain their number and the crypt becomes extinct. If $\alpha > 0$ and $\beta < k_1 / m_1$ (Region II), a healthy stable crypt is achieved. If $\beta > k_1 / m_1$ (Region III) unbounded growth occurs, and is driven by a cancer stem cell derived from the transit cell population.

Firstly, we consider the case of both the stem and transit cell populations being subject to saturating feedback. We choose the same form of feedback for the transit cells as for the stem cells, replacing the transit cell differentiation rate β_2 by $\beta_2 + k_1 N_1 / (1 + m_1 N_1)$. Since the fully-differentiated cells do not divide and are only removed from the system at a rate γ, there is no need to include feedback in that population. The ODEs and cell population steady states for this system are shown in the Appendix. The stability of the replenishing cycle in the crypt is dependent on just two parameters: the net per-capita growth rates $\alpha = \alpha_3 - \alpha_1 - \alpha_2$ and $\beta = \beta_3 - \beta_1 - \beta_2$ for the stem and transit cell populations respectively. This stability is summarized in Figure 3 for a range of values of the parameters α and β. If $\alpha < 0$ and $\beta < k_1 / m_1$ (Region I), the stem cells cannot sustain their number and consequently the crypt dies out. If $0 < \alpha < k_0 / m_0$ and $\beta < k_1 / m_1$ (Region II), the crypt will be in a normal equilibrium. If either $\alpha > k_0 / m_0$ or $\beta > k_1 / m_1$ (or both) there is unregulated cell population growth in the system. For $\alpha > k_0 / m_0$ and $\beta < k_1 / m_1$ (Region III), the cancer stem cells driving tumorigenesis are the tissue stem cells, whereas for $\alpha < k_0 / m_0$ and $\beta > k_1 / m_1$ (Region IV), the cancer stem cells are derived from transit cells and the stem cell population either remains fixed ($\alpha > 0$) or dies out ($\alpha < 0$). Finally, if both $\alpha > k_0 / m_0$ and $\beta > k_1 / m_1$ (Region V), unbounded growth is driven by cancer stem cells derived from both tissue stem cells and transit cells. So, in summary, the crypt will only maintain a healthy balance of proliferation, differentiation and death if the growth rates satisfy $0 < \alpha < k_0 / m_0$ and $\beta < k_1 / m_1$.

Secondly, we consider the case where the stem cells are now subject to linear feedback and the transit cells are again regulated by saturating feedback. The same form of feedback is chosen for the transit cells as in the last case, and the steady states for this system are shown in the Appendix. The stability of the population numbers is again dependent on the two net per-capita growth rates $\alpha = \alpha_3 - \alpha_1 - \alpha_2$ and $\beta = \beta_3 - \beta_1 - \beta_2$, which is summarized in Figure 4. If $\alpha < 0$ and $\beta < k_1 / m_1$ (Region I), the stem cells cannot sustain their number and consequently the crypt dies out. If $\alpha > 0$ and $\beta < k_1 / m_1$ (Region II), the crypt will be in a healthy equilibrium. If $\beta > k_1 / m_1$ (Region III), the tissue stem cells will either remain fixed ($\alpha > 0$) or die out ($\alpha < 0$), and unbounded growth is initiated from a cancer stem cell derived from the transit cell population. Here, unbounded growth can only be driven by transit cells and not tissue stem cells.

It is assumed that a mutation gives a cell a selective advantage,[4] which would change the parameters associated with that cell and its feedback. This parameter change will increase the size of the steady states unless all the parameters change in combination such that the steady state is unaltered and the mutation is neutral. This increase in steady state could be interpreted as the first stage in the process transforming a normal crypt via an adenomatous polyp to a carcinoma.

It is observed that tumors grow to a certain size and then plateau until another growth spurt occurs. There are several long lag phases between periods of tumor growth, before the cancer finally sets in. Our feedback model can capture this behavior, with each mutation leading to a parameter change and consequently an increase in the steady state size. If any of these mutations result in $\alpha > k_0 / m_0$ or $\beta > k_1 / m_1$, then the saturating feedback is overcome, there is no steady state, and exponential growth is initiated.

We illustrate this mutational process with two numerical examples. For this purpose, we make the simplifying assumption that once a mutation occurs its selective advantage is instantly conferred to all the other cells. Firstly, we consider the system with saturating feedback in both stem and transit cells, with the initial parameter set $\alpha_1 = 0.1$, $\alpha_2 = 0.3$, $\alpha_3 = 0.69$, $\beta_1 = 0.1$, $\beta_2 = 0.3$, $\beta_3 = 0.397$, $\gamma = 0.139$, $k_0 = m_0 = 0.1$, $k_1 = 0.0003$ and $m_1 = 0.0004$, which gives $\alpha = 0.286$, $\beta = -0.0027$ and produces critical saturation threshold values of $\alpha = k_0 / m_0 = 1$ and $\beta = k_1 / m_1 = 0.75$. Therefore the population is stable with $N_0^* = 4$, $N_1^* = 85$ and $N_2^* = 200$ in Region II of Figure 3. If a mutation occurs (in either β_1 or β_3) that increases β to 0.1, then the stem cells remain at $N_0^* = 4$ and the other population steady states increase to $N_1^* = 410$ and $N_2^* = 1{,}197$. Now suppose a second mutation raises β to 0.2, which further raises the steady states to $N_1^* = 925$ and $N_2^* = 3{,}344$. Finally, a third mutation raises β to 0.8 which is larger than the critical threshold and lies in Region IV of Figure 3. Consequently there can no longer be a steady state, resulting in unregulated growth in the cell populations. Note that the initiating mutation for each of these changes may have occurred in stem cells but a selective advantage is only expressed in transit cells. The effect of this series of mutations is illustrated in Figure 5, where the different parameter regimes corresponding to each phase of growth are labeled (1), (2), (3) and (4) respectively, and these are also marked on the (α, β) parameter space in Figure 3.

For the second numerical example of a sequence of mutations, we consider the system with linear feedback in stem cells and saturating feedback in transit cells. The initial parameter set is $\alpha_1 = 0.1$, $\alpha_2 = 0.3$, $\alpha_3 = 0.69$, $\beta_1 = 0.1$, $\beta_2 = 0.3$, $\beta_3 = 0.388$, $\gamma = 0.1345$, $k_0 = 0.07$, $k_1 = 0.0002$ and $m_1 = 0.0004$, which gives $\alpha = 0.286$, $\beta = -0.0117$ and produces the critical saturation threshold $\beta = k_1 / m_1 = 0.75$. Therefore the population is stable with $N_0^* = 4$, $N_1^* = 85$ and $N_2^* = 200$ in Region II of Figure 4. If a mutation occurs (in either β_1 or β_3) that increases β to 0.1, then the stem cells remain at $N_0^* = 4$ and the other population steady states increase to $N_1^* = 654$ and $N_2^* = 1{,}962$. Now suppose a second mutation raises α to 0.42, which further increases the steady states to $N_0^* = 6$, $N_1^* = 676$ and $N_2^* = 2{,}042$. Finally, a third mutation raises β to 1 which is larger than

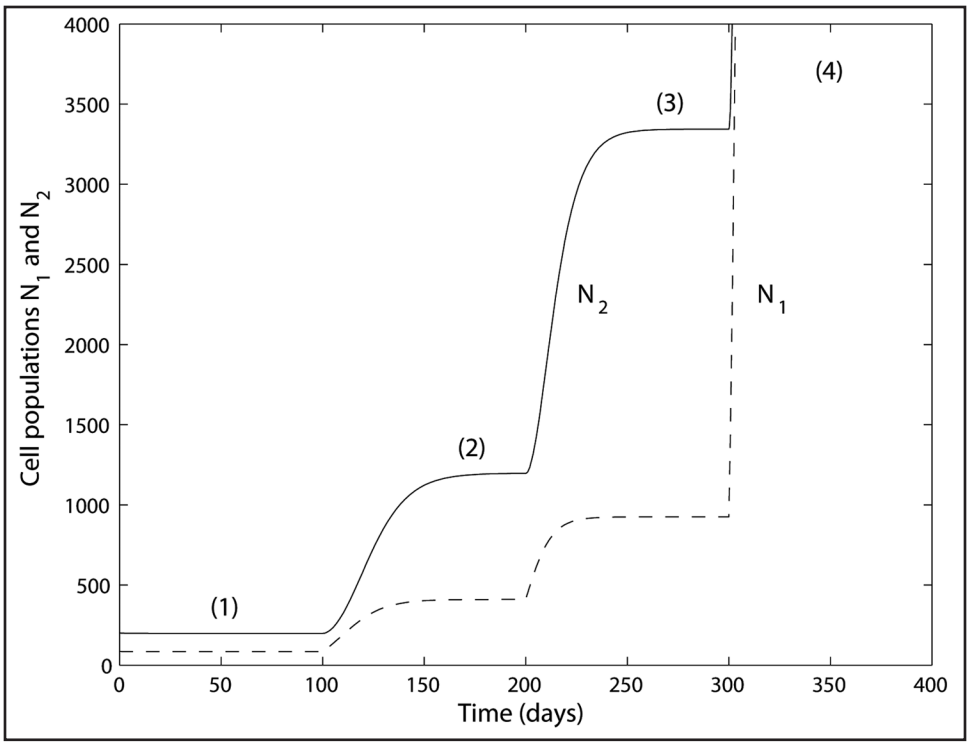

Figure 5. An illustrative sequence of mutations that occur every 100 days. The initial parameters are taken to be $\alpha_1 = 0.1$, $\alpha_2 = 0.3$, $\alpha_3 = 0.69$, $\beta_1 = 0.1$, $\beta_2 = 0.3$, $\beta_3 = 0.397$, $\gamma = 0.139$, $k_0 = m_0 = 0.1$, $k_1 = 0.0003$ and $m_1 = 0.0004$, which gives $\alpha = 0.286$, $\beta = -0.0027$. All the parameters are measured in hours^{-1} apart from m_0 and m_1 which are dimensionless. The mutations cause, successively, $\beta = 0.1$, $\beta = 0.2$ and finally $\beta = 0.8$. After the last mutation, $\beta > k_1 / m_1$ which means there is no steady state and the cell populations grow without bound. The numbers 1–4 correspond to the different points in the parameter space marked on Figure 3.

the critical threshold and lies in Region III of Figure 4. Consequently there can no longer be a steady state resulting in unregulated growth in the cell populations. These different regions of the parameter space are labeled (1), (2), (3) and (4) respectively in Figure 4.

EXAMPLE 3: WHICH PARAMETERS ARE MOST IMPORTANT?

Which parameters are the most influential in a system? This is an important question that mathematical modeling can help to address by using a sensitivity analysis. If it can be shown that some parameters have a strong influence while others have little or no effect, then experiments need only focus (at least initially) on the most influential processes. In this section, we discuss sensitivity coefficients, and how they can be used to ascertain key parameters.

By way of example, let us consider the effect that changes in the net per-capita growth rate α have on the stem cell steady state N_0^*. Suppose we change α by a small amount $\Delta\alpha$, and assume that this induces a small change in N_0^* of ΔN_0^*. The *sensitivity coefficient* of N_0^* with respect to α is defined to be the relative change in N_0^* divided by the relative change in α

$$S = \frac{\Delta N_0^*}{N_0^*} \Big/ \frac{\Delta\alpha}{\alpha} = \frac{\alpha}{N_0^*}\frac{\Delta N_0^*}{\Delta\alpha}. \qquad (11)$$

In the limit as $\Delta\alpha$ tends to zero, this can be expressed in terms of the derivative

$$S = \frac{\alpha}{N_0^*}\frac{\partial N_0^*}{\partial\alpha}. \qquad (12)$$

If S is independent of α then we are effectively finding S such that $N_0^* \propto \alpha^S$, but in general S will be a function of all the parameters in the system, so this relation is only true at the particular point in the parameter space at which S has been calculated. If $S > 1$, a percentage change in α will produce a larger percentage change in N_0^*; if $S = 1$, a percentage change in α causes an equal percentage change in N_0^*; if $0 < S < 1$, then a percentage change in α produces a smaller percentage change in N_0^*; if $S = 0$, a change in α has no effect on N_0^*; and if $S < 0$, then an increase (decrease) in α causes a decrease (increase) in N_0^*.

Applying formula (12) to the stem cell steady state with saturating feedback given in equation (9), the sensitivity coefficient of N_0^* with respect to α is $S_{N_0^*,\alpha} = 1 + m_0 N_0^*$. Since both N_0^* and m_0 are positive, $S_{N_0^*,\alpha} > 1$ which means that an increase in α will produce a greater percentage change in N_0^*. Evaluating the sensitivity coefficient using parameter values $\alpha = 0.286$ and $k_0 = m_0 = 0.1$ gives $S_{N_0^*,\alpha} = 1.4$. Alternatively, if $\alpha = 0.8$, then $S_{N_0^*,\alpha} = 5$ and the dependence of N_0^* on α is much stronger. This illustrates how the dependence on α can vary at different points in the parameter space.

Now let us consider the sensitivity coefficient of the stem cell steady state to the parameter m_0, denoted by $S_{N_0^*,m_0}$. It can be shown that $S_{N_0^*,m_0} = S_{N_0^*,\alpha} - 1$. Since the sensitivity coefficient $S_{N_0^*,m_0}$ will always be less than $S_{N_0^*,\alpha}$, we can say that the stem cell steady state is more sensitive to α than it is to m_0.

By performing this analysis for each parameter to find the effect on each steady state, it can be shown that changes in the parameters α or k_0 have the largest effect on the stem cell number, changes in β or k_1 have the greatest effect on the semi-differentiated cell population, and changes in β, γ or k_1 cause the biggest changes in the fully-differentiated cell population. Changes in m_0 or m_1 produce a less significant change in the steady states. However, we must remember that m_0 and m_1 are crucial in determining the stability boundaries in Figures 3 and 4.

DISCUSSION

The power of mathematical modeling is that it can provide insight into the behavior of complex interacting processes. In this Extra View we have outlined some of the relevant techniques that can be used to model the cell population dynamics in the colonic crypt, and we have illustrated these techniques with three examples.

Firstly, we discussed discrete, age-structured and continuous modeling approaches, why age-structured modeling is necessary to capture the dynamics of more than one population dividing on

different timescales, and how continuum modeling is a more accessible tool.

Secondly, we explained how both homeostasis and tumorigenesis can be modeled by adopting a saturating form of feedback.[5] We presented a scenario for the progression to cancer through a succession of mutations that lead to steady states with greater numbers of cells, until one mutation causes the net per-capita growth rate of stem or transit cells to exceed a critical value and unregulated cell population growth begins. In doing this, we made the simplifying assumption that the selective advantage gained by a mutation will be instantly conferred to the whole population, but a more detailed model might track the progress of healthy and mutant cells separately.

Finally, we discussed how a sensitivity analysis can be used to highlight the key parameters in a model. Modeling can prioritize the list of parameters that is to be measured. Results suggest that the key parameters are the net per-capita growth rates of stem and transit cells, the speed of response of stem and transit cells to changes in their number and the rate at which fully-differentiated cells are removed/die. We have shown how by incorporating the more stringent linear feedback into the stem cells, but not the transit cell compartment, it is then only the transit cells that can become the cancer stem cells. This may well be the best reflection of the actual situation in the large colorectal crypt.

As modeling and experiments become ever more intertwined, and increasing levels of system complexity are found, we believe that mathematical modeling will play a significant part in future research developments.

APPENDIX

Saturating feedback in the stem and transit cells. The system of ODEs describing the change of the cell populations in the crypt, with saturating feedback in the differentiation rates of stem and transit cells, is

$$\frac{dN_0}{dt} = (\alpha_3 - \alpha_1 - \alpha_2)N_0 - \frac{k_0 N_0^2}{1 + m_0 N_0}, \tag{13}$$

$$\frac{dN_1}{dt} = (\beta_3 - \beta_1 - \beta_2)N_1 - \frac{k_1 N_1^2}{1 + m_1 N_1} + \alpha_2 N_0 + \frac{k_0 N_0^2}{1 + m_0 N_0}, \tag{14}$$

$$\frac{dN_2}{dt} = -\gamma N_2 + \beta_2 N_1 + \frac{k_1 N_1^2}{1 + m_1 N_1}, \tag{15}$$

which gives steady states

$$N_0^* = \frac{\alpha}{k_0 - m_0 \alpha}, \tag{16}$$

$$N_1^* = \frac{1}{2(k_1 - \beta m_1)}\left[\beta + m_1 D + \sqrt{(\beta - m_1 D)^2 + 4Dk_1}\right], \tag{17}$$

$$N_2^* = \frac{N_1^*}{\gamma}\left[\beta_2 + \frac{k_1 N_1^*}{1 + m_1 N_1^*}\right], \tag{18}$$

where $D = \alpha_2 N_0^* + k_0 N_0^{*2}/(1+m_0 N_0^*) = (\alpha_3 - \alpha_1)N_0^*$ is the stem-cell differentiation rate.

Linear feedback in the stem cells and saturating feedback in the transit cells. When the stem cells are subject to linear feedback and there is saturating feedback governing the transit cells, the system of ODEs (13)–(15) and the steady states (16)–(18) remain the same, except now the stem cell saturation is switched off ($m_0 = 0$).

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References

1. Gatenby RA, Maini PK. Cancer summed up. Nature 2003; 421:321.
2. Okamoto R, Watanabe M. Molecular and clinical basis for the regeneration of human gastrointestinal epithelia. J Gastroenterol 2004; 39:1-6.
3. van Leeuwen IMM, Byrne HM, Jensen OE, King JR. Crypt dynamics and colorectal cancer: Advances in mathematical modelling. Cell Prolif 2006; 39:157-81.
4. Tomlinson IPM, Bodmer WF. Failure of programmed cell death and differentiation as causes of tumors: Some simple mathematical models. Proc Natl Acad Sci USA 1995; 92:11130-4.
5. Johnston MD, Edwards CM, Bodmer WF, Maini PK, Chapman SJ. Mathematical modeling of cell population dynamics in the colonic crypt and in colorectal cancer. Proc Natl Acad Sci USA 2007; 104:4008-13.
6. Boman BM, Fields JZ, Bonham-Carter O, Runquist OA. Computer modeling implicates stem cell overproduction in colon cancer initiation. Cancer Res 2001; 61:8408-11.
7. Hardy K, Stark J. Mathematical models of the balance between apoptosis and proliferation. Apoptosis 2002; 7:373-81.
8. Wodarz D. Effect of stem cell turnover rates on protection against cancer and aging. J Theor Biol 2007; 245:449-58.
9. Paulus U, Potten CS, Loeffler M. A model of the control of cellular regeneration in the intestinal crypt after perturbation based solely on local stem cell regulation. Cell Prolif 1992; 25:559-78.
10. Loeffler M, Stein R, Wichmann HE, Potten CS, Kaur P, Chwalinski S. Intestinal cell proliferation. I. A comprehensive model of steady-state proliferation in the crypt. Cell Tissue Kinet 1986; 19:627-45.
11. Loeffler M, Potten CS, Paulus U, Glatzer J, Chwalinski S. Intestinal crypt proliferation. II. Computer modelling of mitotic index data provides further evidence for lateral and vertical cell migration in the absence of mitotic activity. Cell Tissue Kinet 1988; 21:247-58.
12. Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih IM, Vogelstein B, Lengauer C. The role of chromosomal instability in tumour initiation. Proc Natl Acad Sci USA 2002; 99:16226-31.
13. Nowak MA, Michor F, Komarova NL, Iwasa Y. Evolutionary dynamics of tumour suppressor gene inactivation. Proc Natl Acad Sci USA 2004; 101:10635-8.
14. Michor F, Iwasa Y, Nowak MA. Dynamics of cancer progression. Nat Rev Cancer 2004; 4:197-205.
15. Michor F, Iwasa Y, Rajagopalan H, Lengauer C, Nowak MA. Linear model of colon cancer initiation. Cell Cycle 2004; 3:358-62.
16. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, Nowak MA. Dynamics of chronic myeloid leukaemia. Nature 2005; 435:1267-70.
17. Michor F, Iwasa Y, Lengauer C, Nowak MA. Dynamics of colorectal cancer. Semin Cancer Biol 2005; 15:484-93.
18. Komarova NL, Wang L. Initiation of colorectal cancer: Where do the two hits hit? Cell Cycle 2004; 3:1558-65.
19. Komarova NL. Cancer, aging and the optimal tissue design. Semin Cancer Biol 2005; 15:494-505.
20. Komarova NL. Spatial stochastic models for cancer initiation and progression. Bull Math Biol 2006; 68:1573-99.