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Report

Linear Model of Colon Cancer Initiation

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ABSTRACT

Cancer results if regulatory mechanisms of cell birth and death are disrupted. Colorectal tumorigenesis is initiated by somatic or inherited mutations in the *APC* tumor suppressor gene pathway. Several additional genetic hits in other tumor suppressor genes and oncogenes drive the progression from polyps to malignant, invasive cancer. The majority of colorectal cancers present chromosomal instability, CIN, which is caused by mutations in genes that are required to maintain chromosomal stability. A major question in cancer genetics is whether CIN is an early event and thus a driving force of tumor progression. We present a new mathematical model of colon cancer initiation assuming a linear flow from stem cells to differentiated cells to apoptosis. We study the consequences of mutations in different cell types and calculate the conditions for CIN to precede *APC* inactivation. We find that early emergence of CIN is very likely in colorectal tumorigenesis.

INTRODUCTION

The mammalian colon is lined with a rapidly proliferating epithelium. This epithelium is organized into compartments of cells called crypts. Intestinal stem cells reside at the base of each crypt.[1] The progeny of stem cells migrate up the crypt, continuing to divide until they reach its mid-portion. Then they stop dividing and differentiate to mature cells. When the cells reach the top of the crypt, they undergo apoptosis and are engulfed by stromal cells or shed into the gut lumen. The cell migration from the base to the top of the crypt takes about 3–6 days.[2] In normal crypts, the rate of cellular death balances the rate of cellular proliferation. If this homeostatic mechanism is shifted toward cellular growth, neoplasia results.

Colorectal tumors progress through four distinct clinical stages that describe dysplastic crypts, small benign tumors, malignant tumors invading surrounding tissues, and finally metastatic cancer. This progression involves several genetic changes such as inactivation of tumor suppressor genes and activation of oncogenes.[3] Mutations of the *adenomatous polyposis coli* (*APC*) gene are considered the earliest[4] and most prevalent genetic changes in colorectal tumorigenesis. More than 85% of colon cancers are estimated to have a somatic mutation of *APC*.[5] *APC* is a tumor suppressor gene on chromosome 5q21. The *APC* gene product plays an important role in modulating the *Wnt*/β-*catenin* signal transduction pathway that regulates, among several other genes, the transcription of the oncogene *c-Myc*.[6,7] *APC* has been shown to reduce net cellular proliferation through an increased rate of apoptosis.[8] Most *APC* mutations found in cancer cells lead to a truncation of the protein, thereby preventing its regulating function. An inherited *APC* germ line mutation leads to the development of hundreds to thousands of benign colorectal tumors, some of which progress to malignancy (familial adenomatous polyposis, FAP).[9]

Genetic instability is a defining characteristic of most human cancers. Two forms of genetic instability have been identified: chromosomal instability, CIN, and microsatellite instability, MIN.[10] More than 80% of colorectal cancers and most other solid tumors have CIN. CIN refers to an increased rate of losing or gaining whole chromosomes or large parts of chromosomes during cell division. It also increases the rate of loss of heterozygosity (LOH). LOH is thought to be an important property of CIN, because tumor suppressor genes have to be inactivated in both alleles. CIN accelerates the rate of inactivation of tumor suppressor genes.

Research into the molecular basis of CIN revealed a large number of genes that trigger CIN when mutated in the yeast *Saccharomyces cerevisiae*.[11,12] These so-called 'CIN genes' are involved in chromosome condensation, sister-chromatid cohesion, kinetochore structure

and function, and microtubule formation as well as in cell cycle checkpoints. So far, only few CIN genes, such as *hBUB1*, *MAD2*, and *BRCA2*, have been identified in human cancer cell lines. By comparison with yeast, we expect several hundred human CIN genes. The classification of CIN genes is based on the mutational events required to engage CIN.[13] Class I CIN genes, such as *MAD2*, trigger CIN if one allele of the gene is mutated or lost. Class II CIN genes, such as *hBUB1*, trigger CIN if one allele is mutated in a dominant negative fashion.[14,15] Class III CIN genes, such as *BRCA2*, trigger CIN if both alleles are mutated. In this paper, we will not consider class III CIN genes. Cell fusion studies provide evidence that CIN can act in a dominant fashion.[16] Thus, in certain cases, one mutational hit is sufficient to trigger CIN.

Aneuploidy refers to the state when a cell no longer has 2n chromosomes. Aneuploidy has been known to be a common feature of most cancer cells for almost a century. We believe that most aneuploidy is a consequence of CIN, but note that aneuploidy by itself is not a proof of CIN. The presence of chromosomal aberrations in a cell does not necessarily imply an increased rate of generating these abnormalities.

The less prevalent form of genetic instability, MIN, is caused by alterations in mismatch repair genes.[10] These alterations lead to an approximately 1000-fold increased point mutation rate in MIN cancer cells. No increased rate of gross chromosomal changes is observed. MIN occurs in about 13% of sporadic colorectal cancers, but seldom happens in other cancer types. CIN and MIN are generally mutually exclusive.

A major question in cancer genetics is to what extent CIN, or any genetic instability, is an early event and thus a driving force of tumorigenesis.[17-23] The precise timing of the emergence of CIN is difficult to measure experimentally, as the molecular basis of CIN is still poorly understood. In this paper, we develop a mathematical approach for the cellular dynamics of colon cancer initiation and determine the importance of early CIN.

In earlier work, we described each colon crypt as a well-mixed pool of (stem) cells that are at risk of receiving *APC* and CIN mutations.[20-24] All cells in this pool were assumed to be in equivalent positions and in direct reproductive competition with each other. There were no spatial effects. All cells in the pool were assumed to have the same rate of cell division and the same probability of mutation. Mutations could only occur in cells within the pool. Mutations in other cells of the colonic crypt were ignored. Here we study a model assuming a simple linear geometry of cellular arrangement, division, differentiation, and death.[25] We allow for the possibility that stem cells and differentiated cells divide at different rates. And we explore the consequences of mutations occurring in stem cells and differentiated cells.

MATERIALS AND METHODS

Mechanism. The human colon is subdivided into $M \approx 10^7$ crypts. Each crypt contains about 1000 to 4000 cells; maybe 1–10 of those cells are stem cells.[26] Stem cells reside at the base of the crypt and divide asymmetrically to produce differentiated offspring (Fig. 1). The differentiated cells migrate toward the top of the crypt, where they eventually undergo apoptosis and are shed into the gut.[27]

In this setting, different scenarios of tumorigenesis are plausible (Fig. 2). The 'traditional' view of colorectal tumor initiation does not involve CIN (Fig. 2A). Initially, the crypt is unmutated and contains only wild type cells, without CIN. The inactivation of the first *APC* allele has to happen in the stem cell in order to take over the whole crypt. If the mutation happens in a differentiated cell, the mutated cell will be pushed to the top of the crypt by the continuous production and migration of differentiated cells and will be 'washed out' of the crypt.[28-30]

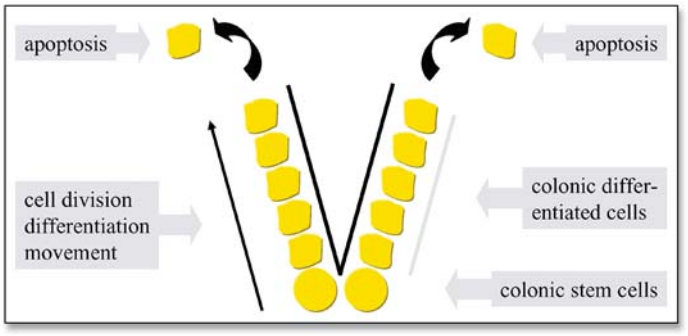

Figure 1. A colonic crypt. Colonic stem cells reside at the base of the crypt and divide asymmetrically to produce differentiated progeny. The progeny migrate toward the top of the crypt where they undergo apoptosis and are shed into the gut lumen or engulfed by stromal cells.

Once the mutation has happened in the stem cell, the stem cell produces mutated progeny that eventually populates the whole crypt. The crypt moves from state $APC^{+/+}$ to state $APC^{+/-}$. The inactivation of the remaining *APC* allele might take place in any cell. The inactivation of both *APC* alleles confers a certain probability to the cell to stick on top of the crypt instead of undergoing apoptosis. There are two possibilities:

1. the cell fails to stick on top of the crypt, undergoes apoptosis, and the crypt stays in state $APC^{+/-}$, or
2. the cell succeeds to stick on top of the crypt and initiates dysplastic growth. We call this state $APC^{-/-}$.

CIN can emerge before the inactivation of either *APC* allele. Figures 2B and 2C show two different mutational pathways in which the CIN mutation precedes the inactivation of the second *APC* allele. The cell lineage is in state $APC^{+/-}$ without CIN. Assume the mutation triggering CIN has to happen in the stem cell (Fig. 2B). Then the mutated stem cell produces progeny that eventually populate the whole crypt, and the cell lineage moves to state $APC^{+/-}$ with CIN. The crucial effect of CIN is to increase the rate of LOH, thereby greatly accelerating the inactivation of the second *APC* allele. Thus, the inactivation of the second allele can happen in any cell. If a cell with two inactivated alleles of *APC* succeeds to stick on top of the crypt, dysplastic growth is initiated.

Assume the CIN mutation happens in a differentiated cell (Fig. 2C). The CIN cell produces mutated progeny that populates only part of the crypt. The CIN cells will eventually be washed out of the crypt, unless the second *APC* allele is inactivated in a CIN cell on its way up the crypt. If this cell then succeeds to stick on top of the crypt, it initiates dysplastic growth.

Figure 3 shows all pathways of how a crypt consisting of wild type cells can mutate into a dysplastic crypt. A mathematical analysis of the model reveals the transition rates between different mutational states and the probabilities of *APC* inactivation without and with CIN. These probabilities specify the importance of early CIN and determine the minimum number of CIN genes in the human genome needed to ensure that CIN arises before the inactivation of *APC*.

Mathematical Analysis. The mathematical and statistical analysis of cancer progression using stochastic processes has a long tradition.[31-33] Here we develop a new approach for studying cancer initiation by describing mutational events and cellular reproduction in well-defined populations of stem cells and differentiated cells.

The mutational network of colorectal tumorigenesis is illustrated in Figure 3. First, consider tumor initiation without CIN. The inactivation of the first *APC* allele has to happen in the stem cell. The crypt moves at rate $2u/\tau_0$ from state $APC^{+/+}$ to state $APC^{+/-}$: the stem cell divides every τ_0 days; the mutation rate per *APC* allele per cell division is denoted by u; and there

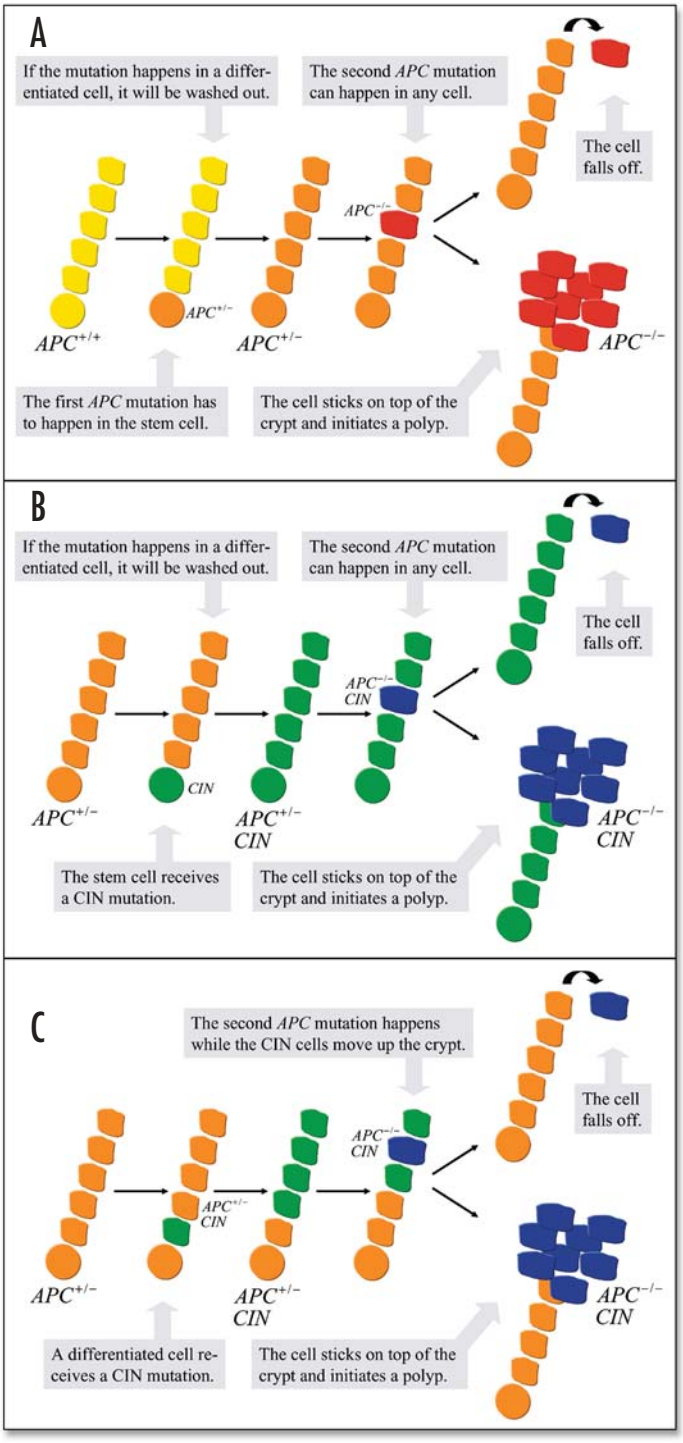

Figure 2. (A) Colorectal tumorigenesis is thought to be initiated by the inactivation of the tumor suppressor gene *APC*. Initially, all cells are wild type, $APC^{+/+}$. The inactivation of the first *APC* allele has to happen in the stem cell; otherwise the mutated cells are washed out of the crypt. The stem cell produces mutated progeny that eventually populate the whole crypt. The crypt moves to state $APC^{+/-}$. The inactivation of the second *APC* allele can happen in any cell. Inactivation of both *APC* alleles might enable the cell to stick on top of the crypt, evade apoptosis, and initiate dysplastic growth. This state is called $APC^{-/-}$. (B) Colorectal tumorigenesis with CIN emerging in the stem cell of an $APC^{+/-}$ cell lineage. The stem cell produces mutated progeny that eventually populate the whole crypt. The crypt moves to state $APC^{+/-}$ with CIN. The inactivation of the second *APC* allele can happen in any cell. This cell might succeed to stick on top of the crypt and initiate a polyp. This state is called $APC^{-/-}$ with CIN. (C) Colorectal tumorigenesis with CIN emerging in a differentiated cell of an $APC^{+/-}$ cell lineage. The mutated cell produces progeny that populate part of the crypt. The CIN cells will eventually be washed out of the crypt, unless the inactivation of the second *APC* allele happens in a CIN cell while it moves up the crypt. This cell might succeed to stick on top of the crypt and initiate a polyp. The rest of the crypt is repopulated by $APC^{+/-}$ cells without CIN. This state is called $APC^{-/-}$ partly with CIN.

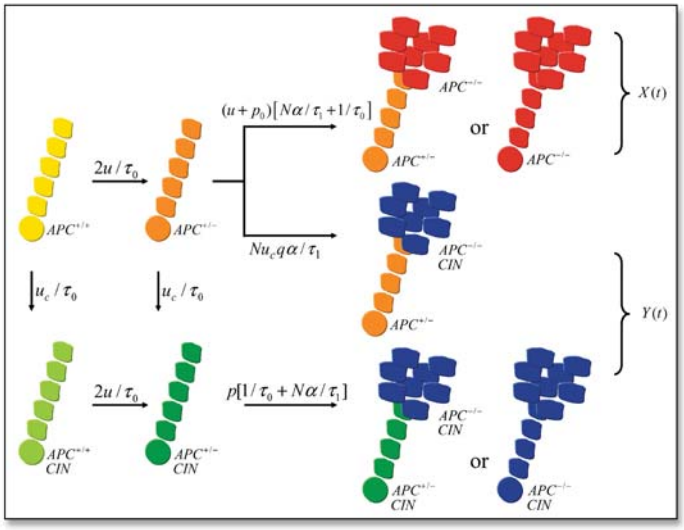

Figure 3. Pathways to dysplasia. Colorectal tumorigenesis might be initiated without CIN. Then the crypt evolves from $APC^{+/+}$ via $APC^{+/-}$ to $APC^{-/-}$ without CIN. CIN can emerge before the inactivation of either *APC* allele. If CIN arises in the stem cell, the crypt evolves from $APC^{+/+}$ either via $APC^{+/+}$ with CIN or via $APC^{+/-}$ without CIN to $APC^{+/-}$ with CIN, and subsequently to $APC^{-/-}$ with CIN. If CIN arises in a differentiated cell, the crypt evolves from $APC^{+/+}$ via $APC^{+/-}$ without CIN to $APC^{-/-}$ partly with CIN. The transition rates are outlined in the text and specify the probabilities of tumor initiation without and with CIN.

are two *APC* alleles. The inactivation of the second *APC* allele can happen either in the stem cell or in a differentiated cell. The crypt moves at rate $(u + p_0)[1/\tau_0 + N\alpha/\tau_1]$ from state $APC^{+/+}$ to state $APC^{+/-}$: the rate of LOH is denoted by p_0; there are N differentiated cells in the crypt; they divide every τ_1 days; and the probability that a cell with two inactivated *APC* alleles sticks on top of the crypt and initiates dysplastic growth is denoted by α. For cell-biological reasons, the second *APC* mutation might have to occur in the stem cell; in that case, we can set $\alpha = 0$. The probability that a crypt is dysplastic without CIN at time t is given by

$$X(t) = (2u/\tau_0)[(u + p_0)/\tau_0 + N(u + p_0)\alpha/\tau_1]t^2/2.$$

CIN can emerge before the inactivation of either *APC* allele. Suppose the mutation triggering CIN has to occur in the stem cell. Then the crypt moves at rate u_c/τ_0 from state $APC^{+/+}$ or $APC^{+/-}$ without CIN to state $APC^{+/+}$ or $APC^{+/-}$ with CIN: the probability that a CIN mutation arises per cell division is denoted by u_c. Both normal and CIN crypts move at rate $2u/\tau_0$ from state $APC^{+/+}$ to state $APC^{+/-}$. The inactivation of the second *APC* allele can happen in any cell, either in the stem cell or in a differentiated cell. The crypt moves at rate $p(1/\tau_0 + N\alpha/\tau_1)$ from state $APC^{+/-}$ to state $APC^{-/-}$: the rate of LOH in CIN cells is denoted by p; we have $p >> p_0$. This rate is fast in the sense that $p(1/\tau_0 + N\alpha/\tau_1) >> 1$. If the second *APC* mutation has to occur in the stem cell, we again set $\alpha = 0$. The probability that a crypt is dysplastic and contains only CIN cells at time t is given by $Y_1(t) = 2uu_c t^2/\tau_0^2$. This probability contains both the case in which the CIN mutation precedes the first *APC* mutation and the case in which the CIN muta-

tion precedes the second *APC* mutation.

Suppose the mutation triggering CIN occurs in a differentiated cell. Then the crypt moves at rate $Nu_c q\alpha/\tau_1$ from state $APC^{+/-}$ without CIN to state $APC^{-/-}$ partly with CIN: the expected probability that a CIN cell encounters an LOH event on its way up the crypt is $q = 1 - [1 - 2^n(1 - p)^n]/[(2^n - 1)(1 - 2(1 - p))]$; the maximum number of cell divisions a differentiated cell undergoes before being shed off the top of the crypt is denoted by n. If the cell with two inactivated *APC* alleles succeeds to stick on top of the crypt, the $APC^{+/-}$ stem cell repopulates the remainder of the crypt. The probability that a crypt is dysplastic and partly consists of CIN cells at time t is given by $Y_2(t)=Nuu_c q\alpha t^2/(\tau_0\tau_1)$. The overall probability that a crypt is dysplastic with CIN at time t is given by

$$Y(t) = Y_1(t) + Y_2(t) = (2u/\tau_0)[2u_c/\tau_0 + Nu_c q\alpha/\tau_1]t^2 / 2.$$

CIN precedes the inactivation of *APC* if $Y(t) > X(t)$. The inequality specifies the minimum number of CIN genes needed to make sure CIN emerges before the inactivation of *APC*. Suppose there are n_1 class I genes that trigger CIN if one allele is mutated or lost, and n_2 class II genes that trigger CIN if one allele is mutated; then the probability that a CIN mutation arises per cell division is given by $u_c = 2n_1(u + p_0) + 2n_2u$. Either $n_1 > (N\alpha\tau_0 + \tau_1)[2Nq\alpha\tau_0 + 4\tau_1]^{-1}$ class I CIN genes or $n_2 > (u + p_0)(N\alpha\tau_0 + \tau_1)[2u(Nq\alpha\tau_0 + 2\tau_1)]^{-1}$ class II CIN genes are sufficient to make sure that CIN arises before the inactivation of *APC*.

Numerical Examples. The human colon contains about $M = 10^7$ crypts. Each crypt consists of approximately $N = 1000$ differentiated cells [26] The mutation rate per *APC* allele per cell division is $u = 10^{-7}$: the mutation rate per base per cell division is about 10^{-10} (see ref. 34), and we assume an *APC* allele can be inactivated by point mutations in approximately 1000 bases. The rate of LOH in normal cells, p_0 , might be of the order of u. The rate of LOH in CIN cells is $p = 0.01$ (see ref. 35). Assume stem cells divide every $\tau_0 = 10$ days and differentiated cells divide every $\tau_1 = 1$ day. The maximum number of cell divisions a colonic cell undergoes is $n \approx 10$, because ten cell divisions are sufficient to populate a crypt of about $N = 1000$ cells. Hence, the probability that a CIN cell with one inactivated *APC* allele encounters an LOH event in the second *APC* allele on its way up the crypt is $q \approx 0.01$. Note that the probability α that a cell with two inactivated *APC* alleles sticks on top of the crypt cannot be too high, otherwise too many dysplastic crypts develop.

i. Assume $\alpha = 0.001$. Then a 70-year old has about 14 dysplastic crypts without CIN and 5 dysplastic crypts with CIN if there is $n_1 = 1$ class I CIN gene, or 3 dysplastic crypts with CIN if there is $n_2 = 1$ class II CIN gene.

ii. Assume $\alpha = 0.01$. Then a 70-year old has about 132 dysplastic crypts without CIN and 8 dysplastic crypts with CIN if there is $n_1 = 1$ class I CIN gene, or 4 dysplastic crypts with CIN if there is $n_2 = 1$ class II CIN gene.

iii. We can calculate the minimum number of CIN genes needed to ensure that CIN emerges before the inactivation of *APC*. If $\alpha = 0.01$, then $n_1 = 17$ class I CIN genes or $n_2 = 34$ class II CIN genes are needed. If $\alpha = 0.001$, then $n_1 = 3$ class I CIN genes or $n_2 = 6$ class II CIN genes are needed. If $\alpha = 0$, i.e., if the inactivation of the second *APC* allele has to happen in the stem cell, then $n_1 = 1$ class I CIN gene or $n_2 = 1$ class II CIN gene is needed to ensure that CIN emerges before the inactivation of *APC*. Figure 4 shows the number of class I CIN genes, n_1, needed for a 10%, 50%, and 90% probability that CIN emerges before the inactivation of *APC* in dependence of α.

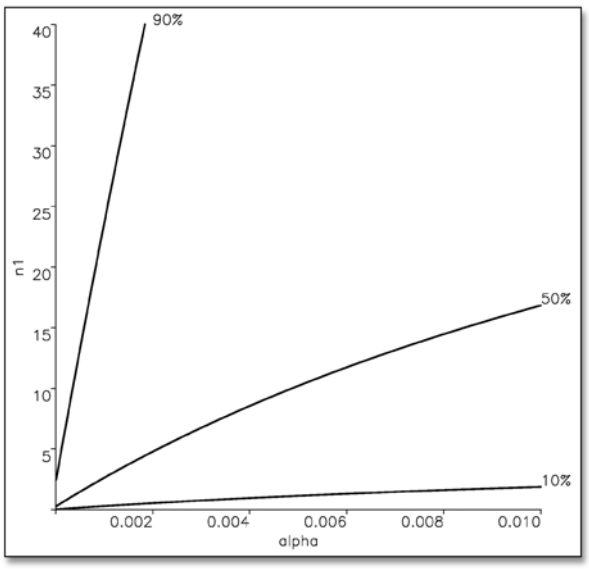

Figure 4. Number of class I CIN genes, n_1, needed for a 10%, 50%, and 90% probability that CIN emerges before the inactivation of *APC*, in dependence of the probability α that a cell with two inactivated *APC* alleles, $APC^{-/-}$, sticks on top of the crypt and initiates dysplastic growth. Parameter values are $N = 1000$, $\tau_0 = 1$, $\tau_1 = 1$, $q = 0.01$, and $t = 70$ years.

RESULTS AND DISCUSSION

In this paper, we develop a new mathematical approach for cellular dynamics, tissue architecture, and differentiation patterns of colonic crypts. The model is based on simple genetic and mechanical assumptions. A colonic stem cell gives rise to a lineage of differentiated cells that move up the crypt and undergo apoptosis on top of the crypt. Stem cells are assumed not to be subject to somatic selection; each stem cell and its offspring can be described as an independent cell lineage. All of these cells can receive mutations inactivating one or both alleles of the tumor suppressor gene *APC*, which is thought to initiate tumorigenesis. The cells can also receive mutations in genes that give rise to chromosomal instability, CIN. CIN might have a cost as it increases the chance of lethal mutations and apoptosis. If CIN causes the stem cell to divide more slowly, the stem cell still populates the crypt with mutated progeny. If CIN causes the stem cell to undergo apoptosis, the crypt dies out and might be replaced by a neighbor crypt.

We study the effect of various mutations that arise in stem cells or differentiated cells. Some mutations, such as the inactivation of the first *APC* allele, have to happen in the stem cell; otherwise the mutated cells are pushed to the top of the crypt and 'washed out' by the continuous production and migration of wild type differentiated cells. Other mutations, such as the inactivation of the second *APC* allele, can also happen in a differentiated cell. The inactivation of both *APC* alleles enables the cell to stick on top of the crypt, evade apoptosis, and initiate dysplastic growth.

A major question in cancer genetics is whether genetic instability is an early event and thus a driving force of tumorigenesis. The present paper shows how to calculate the rate of colon cancer initiation and the conditions for chromosomal instability to precede the inactivation of *APC*. For plausible parameter values, we obtain that a small number of CIN genes is sufficient to ensure that CIN emerges before the inactivation of *APC*. CIN, however, accelerates the rate of inactivation of every successive tumor suppressor gene needed for further tumor progression. If colorectal tumorigenesis requires the inactivation of two or more tumor suppressor genes in rate limiting situations, then even fewer CIN genes are needed to ensure that CIN arises before the inactivation of the first tumor suppressor gene.[36] It is therefore very likely that a CIN mutation initiates colorectal tumorigenesis.

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