Dynamics of Genetic Instability in Sporadic and Familial Colorectal Cancer

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ABSTRACT

Genetic instability is a defining feature of human cancers. In colorectal cancer, two specific types of genetic instabilities have been identified: microsatellite instability (MSI) and loss of microsatellite instability (LOM). MSI leads to a 10,000-fold increase in the rate of stable DNA changes, whereas chromosomal instability (CIN) promotes the rate at which gross chromosomal changes occur during cell division. In this paper, we develop a mathematical model for the dynamics of colon cancer initiation. We outline the parameters and rate constants that determine the fraction of colon cancers when MSI or CIN mutations precede the fixation of the first tumor suppressor gene. For a wide range of parameter values, we find support for the radical hypothesis that genetic instability involves colon heterogeneity. We compare sporadic and familial forms of colorectal cancer.

INTRODUCTION

It is now widely accepted that alterations of at least two types of genes stimulate the pathway to cancer. The first type involves genes that directly control cell birth and death processes. Such alterations are linked to clinical progression and dysfunction growth patterns. The second is genetic instability. The corresponding tumors may not have a direct influence on the progression of the disease, but the increased rate of genetic change enhances the proliferation of further mutations, some of which can be advantageous and start a new wave of clinical expansion. There are at least two major types of genetic instability: microsatellite instability (MSI) and chromosomal instability (CIN). MSI is characterized by an increased rate of point mutations and single base changes of the genome, and CIN is defined as the independent rate of chromosome alterations, such as the rate of gain of whole chromosomes or loss of chromosomes. A correlate of CIN is an increased rate of loss of heterozygosity (LOH).

Colorectal cancer is a major cause of mortality in the Western world. Approximately 5% of the population develop the disease and about 40% of those diagnosed with it die within 5 years. Considerable progress has been made in identifying genetic events leading to colorectal cancer. Systematic examination of the adenomatous polyposis coli (APC) gene has been linked to one of the earlier steps occurring in sporadic colorectal cancer. It has been shown that the frequency of APC mutations is high in small adenomas but is in cancer. Evidence shows that the APC gene plays a critical role in colorectal cancer and also comes from the study of individuals with familial adenomatous polyposis coli (FAP). FAP patients (who are mutation in one of the copies of the APC gene by their parents, have on average 100 adenomatous polyps.

The APC gene is a tumor suppressor gene which contains cell birth and cell death pathways. Inactivation of only one copy of the APC gene does not seem to play any significant role. Inactivation of both copies of this gene appears to result in an increased cell death to death rates in the corresponding cell and leads to cell death and the formation of a dysplastic crypt. In this paper we define a dysplastic crypt as a crypt that contains cells with only one copy of the APC gene inactivated. Dysplastic crypts are at risk of developing further somatic mutations which will eventually lead to cancer. Dysplastic crypts are different from aberrant crypt foci, which consists of normal, but unnecessary crypts. Aberrant crypt foci often contain KRAS mutations and yet are not believed to progress to neoplasia. In this paper we model the evolution of APC and therefore consider dysplastic crypts. The typical manner is that an average 70-year-old has about 10-15 dysplastic crypts, but precancerous colon have never been published.
Table 1A PARAMETERS OF THE MODEL AND THEIR POSSIBLE NUMERICAL VALUES

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Number of cryps in a colony</td>
<td>10^3</td>
</tr>
<tr>
<td>N</td>
<td>Effective number of cells in a crypt</td>
<td>10^4-10^5</td>
</tr>
<tr>
<td>k</td>
<td>Effective rate of cell life cycle, days</td>
<td>10^3</td>
</tr>
<tr>
<td>u</td>
<td>Probability of mutation in normal (non-MIN) cells</td>
<td>10^-7</td>
</tr>
<tr>
<td>d</td>
<td>Probability of mutation in MIN cells</td>
<td>10^-7</td>
</tr>
<tr>
<td>p</td>
<td>Rate of MIN in normal (non-MIN) cells</td>
<td>10^-7</td>
</tr>
<tr>
<td>P</td>
<td>Rate of MIN in MIN colonies</td>
<td>10^-7</td>
</tr>
<tr>
<td>n_m</td>
<td>Total number of MIN genes</td>
<td>2-4</td>
</tr>
<tr>
<td>n_p</td>
<td>Total number of CN genes</td>
<td>10^3</td>
</tr>
</tbody>
</table>

Table 1B THE THREE MAJOR CLASSES OF HOMOLOGOUS SEGMENTS

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>X_p</td>
<td>X_p, X_p, X_p non-CIN, non-MIN</td>
<td>v_p</td>
</tr>
<tr>
<td>Y_p</td>
<td>Y_p, Y_p, Y_p CIN</td>
<td>v_p</td>
</tr>
<tr>
<td>Z_p</td>
<td>Z_p, Z_p, Z_p MIN</td>
<td>v_p</td>
</tr>
</tbody>
</table>

About 15% of all colorectal cancers have MIN and most of the rest are characterized by CIN. MIN occurs in virtually all human polypoid colorectal cancers (HENPPC), which account for about 5% of all colorectal cancers. The MIN glycogen transformation affects mucin expression. Several genes have been identified whose inactivation leads to an increased rate of late genetic alterations in the main core being MYH2 and SMAD4. Both genes in a single MIN gene must be inactivated in order for any phenotypic change to occur. HENPPC patients inherit a mutation in one of the copies of a MIN gene and often develop colorectal tumors in their fifties. On the other hand, patients with the highest number of polyps, but the rate of progression from polyp to tumor is faster.

Molecular mechanisms leading to CIN in human cancers remain to be understood. If it happens that CIN may be caused by mutations in genes involved in somatic/microsatellite dynamics, or checkpoint genes that maintain the progression of the cell cycle, e.g. p53 or spindle checkpoint genes the DNA damage checkpoint. For example, homologous recombination in the mitotic spindle checkpoint gene BARD1 (Mutations in DNA repair genes have been detected in a small fraction of colorectal cancers with the CIN phenotype). Also, the MAD2 gene seems to be transcriptionally repressed in various solid tumors. Some CIN genes are located in a dominant-negative fashion in one allele in the MIN. If there is an ongoing and related debate about the role of genetic instability in cancer progression. In this paper we attempt to formulate the question with mathematical modeling, and formulate certain conditions under which CIN or MIN precede the acquisition of the full tumor suppressor gene. We compare sporadic colorectal cancer with FAP and HENPPC. We show which processes and state constraints are compatible with existing experimental observations and outline what needs to be measured to improve understanding.

A MODEL FOR THE INITIATION OF SPORADIC COLORECTAL CANCERS

The colorectal epithelium is organized in crypts covered with a self-renewing layer of cells (Fig. 1). The total number of cryps is of the order of 20-25 cryps. Since all cryps go into a single MIN gene, the same rate of MIN must occur in order for any phenotypic change to occur. HENPPC patients inherit a mutation in one of the copies of a MIN gene and often develop colorectal tumors in their fifties. On the other hand, patients with the highest number of polyps, but the rate of progression from polyp to tumor is faster.

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Figure 1. The epithelium of the colon is organized into crypts. Each crypt consists of 1,000-4,000 cells. A small number of stem cells, which are thought to be located at the bottom of the crypt, divide asymmetrically, to replenish the whole crypt. They give rise to differentiated cells which travel within approximately 20 hours to the top of the crypt where they undergo apoptosis. Growth of both crypts of the colon is a balanced process. The mutated cells remain on the top of the crypt, divide and ultimately overtake the crypt. This process gives rise to a dysplastic crypt, which represents the step on the way to colorectal cancer. The number of cryps is of the order of 20-25 cryps. Since all cryps are of the same MIN gene, the same rate of MIN must occur in order for any phenotypic change to occur. HENPPC patients inherit a mutation in one of the copies of a MIN gene and often develop colorectal tumors in their fifties. On the other hand, patients with the highest number of polyps, but the rate of progression from polyp to tumor is faster.

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corresponding (neural) mutant is equal to U/N. (Figures 3A measures that the mutant cells take over the crypt.) Therefore, the rate of change from $X_0$ to $X_0$ is $\frac{1}{b} + b$. Once the first allele of the APC gene has been inactivated, the second allele can be inactivated either by another point mutation or by an LOH event. This process occurs with rate $2 \lambda_2$, where $\lambda_2$ is the rate of LOH in normal (non-CIN) cells. We assume that mutator with both copies of the APC gene inactivated has a large selective advantage, so that once such a mutant is produced, the probability of its fixation is close to one.

There are two steps that require the state $X_0$, from which $X_0$: The expected number of dysplastic crypts in a person of age $t$ is proportional to the product of the two rates and the second power of time, and is given by:

$$M_{t,n}(X_0, X_0) = \frac{1}{b} + b.$$ 

See the Appendix for mathematical details. Some estimates of the expected number of dysplastic crypts, based on Equation 1, are given in Table 2. Note that the quantity $X_0(t)$ is intensively proportional to the square of $t$. Thus it is important for an age-specific analysis to keep the division rate of stem cells low as possible in order to minimize the risk of colon cancer. For example, if the mean rate of dysplastic crypts in a 70-year-old does not exceed 10,000, then it cannot be smaller than about 10 days. This is consistent with the assumption that only the stem cells are at risk of cancer. If ineffective population includes differentiated cells, then the average $t$ is smaller and the expected number of dysplastic crypts is high. Another possibility is that dysplastic crypts can be lost. Our model given the number of dysplastic crypts that are being produced over time, which could be larger than the actual number of dysplastic crypts that patients have at any particular time point. Thus, measurements of the incidence of dysplastic crypts will provide important information about the natural history of colon cancer initiation.

**SPORADIC COLORECTAL CANCERS, CIN AND MIN**

Let us now consider the possibility of developing genetic instability during cancer initiation. Starting from a population of normal cells, there are three different ways to obtain $b$ (i.e., by losing the first copy of the APC gene, by losing the first copy of one of the MIN genes, and by mutating the copy of one of the CIN genes).

We use the notation $X_1$ and $X_2$ respectively for the probability that a crypt contains wild-type cells, CIN cells or cells with a copy of the APC gene inactivated, and Table 2D). Figure 2 shows the mutant selection network of colorectal cancer initiation including CIN and MIN. All the transition rates are calculated using the relevant mutation rates times the probability that the mutant will take over the crypt.

Let us denote the rate of LOH in CIN cells as $\lambda_3$. We assume that the crucial effect of CIN is to increase the rate of LOH, which implies $\lambda_3 < \lambda_2$. Similarly, the advantage of CIN for the cancer cell is to accelerate the loss of the second copy of a tumor suppressor gene. Similarly, the advantage of MIN is to increase the point-mutation rate, which means that it is increased in the probability of finding the crypt that contains $X_0$. $X_0$ or $X_0$ function of $b$. In other words, we want to know the probability for the dysplastic crypt to have CIN ($X_1$), MIN ($X_2$) or no genetic instability ($X_0$) The mutation-selective network of the

<table>
<thead>
<tr>
<th>MIN gene</th>
<th>Total number of dysplastic crypts</th>
<th>Percent of CIN</th>
<th>Percent of MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>mutation</td>
<td>10</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>reversion</td>
<td>100</td>
<td>67</td>
<td>0.001</td>
</tr>
<tr>
<td>deletion</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>rearrangement</td>
<td>100</td>
<td>65</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total number of dysplastic crypts</th>
<th>Percent of CIN</th>
<th>Percent of MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97</td>
<td>0.001</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>0.001</td>
</tr>
<tr>
<td>100</td>
<td>90</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CIN</th>
<th>HNPPCC</th>
<th>total number of dysplastic crypts</th>
<th>Percent of CIN</th>
<th>Percent of MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN</td>
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<td>100</td>
<td>90</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Other possible explanations of the increased numbers of dysplastic crypts in this model include the following: CIN can increase the rate of LOH, which may be correlated with an increase in the rate of LOH in CIN cells. Similarly, the advantage of MIN is to increase the point-mutation rate, which means that it is increased in the probability of finding the crypt that contains $X_0$. $X_0$ or $X_0$ function of $b$. In other words, we want to know the probability for the dysplastic crypt to have CIN ($X_1$), MIN ($X_2$) or no genetic instability ($X_0$) The mutation-selective network of the

Figure 3 is more complicated than the one-dimensional network of Figure 2, but the elimination of $X_0$, $X_1$ and $X_2$ can still be written down. The key is to identify how many different (loss-finding) crypts arise: the initial state ($X_0$) from the state of interest. The flow

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In FAP patients, one allele of the APC gene is inactivated in the germ line. In most of our model, all crypts start in state X. The corresponding mutation-selection network is found in Figure 4A. The solutions are found in Appendix A. X(0) and X(1) are linear functions of time (there is one rate-limiting step), whereas Z(0) grows slower than the second power of time (two rate-limiting steps plus one intermediate step).

Some predictions of the model are shown in Table 2D. The expected number of dysplastic crypts and the fraction of CIN crypts are calculated for n=16 years. As the number of CIN genes, n, increases, we expect more dysplastic crypts, and a larger fraction of crypts with CIN. According to our model, the expected number of dysplastic crypts grows linearly with time, and by the age of 16 years it is expected to be thousands to tens of thousands, see Table 2D. This should be compared with the observation that patients with FAP have hundreds to thousands of polyps by their third decade of life.

The number of polyps in FAP patients does not grow linearly with time. Instead, most polyps appear suddenly in the second decade of life. These observations are consistent with the predictions of the model. It is believed that polyps result from dysplastic crypts by means of further somatic mutations and clonal expansions. Therefore, the number of polyps is expected to be a higher than linear power of time, which looks like a step increase in the number of lesions after a relatively non-eventful period. Also, the number of dysplastic crypts (10^3-10^5 in our model) is expected to be much larger than the number of polyps (10^3-10^5) consistent with the expectation that not all dysplastic crypts progress to polyps.

Finally, we note that the logical possibility exists that the second copy of the APC gene in FAP patients may be inactivated by an epigenetic event, just like the second copy of a MIN gene can be silenced. Experimental investigations suggest that this is unlikely in FAP patients, only inactivated epigenetic inactivation of the APC gene.

Figure 4A. Mutation-selection network of FAP initiation. We start with the type X, because the first copy of the APC gene is inactivated in the germ line. Two mutations of the MIN gene are allowed, and because it takes only one step for activation of the second copy of the MIN gene, arrows go through the MIN phenotype.

Table 2D 1. Inactivation of the second copy of a MIN gene happens by means of a point mutation. With the rate u and 2. Inactivation of the second copy of a MIN gene happens by epigenetic silencing.

There is evidence that the second scenario is less likely in the case of HPNCC. In a recent study, DNA methylation of the hMLH1 gene was found in 88% of 40 sporadic MIN cancers but in 15% of 30 cases of HPNCC patients.

Our model predicts that the majority of dysplastic crypts in HPNCC patients are expected to have MIN. However, we do not find that 100% of dysplastic crypts will contain MIN. On the other hand, we note that virtually all tumors in HPNCC patients have MIN. This might suggest that the selection for MIN also happens at later stages of carcinogenesis: dysplastic crypts with MIN might have a faster rate of progression to cancer than dysplastic crypts containing CIN or normal cells.

Finally, we note that the total number of dysplastic crypts in HPNCC patients, as predicted by our model, is of the order 10^10 or 10^11, which is only slightly larger than the expected number of dysplastic crypts in normal individuals and not nearly as high as in the case of FAP (the order 10^20, Table 2D). This is also consistent with observations.

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FAP

MIN Z

u + p

N(G + p)

Z2

2nγu 2u normal X

N(u + p)

X2 CIN Y2

MIN Z0

2u

N(G + p)

Z1

u + p0

2nγu

N(u + p0)

X1
DISCUSSION

Powerfully one of the most fascinating questions in cancer research is to what extent genetic instability is an early event and hence a driving force of tumor progression. The dominant view is that instability is initiated by mutations that increase the neoplastic potential of the cell. These mutations occur in oncogenes and tumor suppressor genes. An alternative possibility is that instability is initiated by mutations in genes that affect the mutation rate and genetic stability of a cell.

In this paper, we have provided a quantitative framework to study the dynamics of colorectal cancer initiation. We calculate the rate of dysplastic crypt formation as a consequence of inactivating both wildtype alleles of the APC tumor suppressor gene. This either induces in normal cells or in cells that have already acquired one or the two genetic instabilities, MIN or CIN. If the rate of triggering genotoxic instability in a cell is high and if the rate of genetic instability is not too large, than inactivation of APC will frequently occur in cells that are genetically unstable. In this case, genetic instability is the first phenotypic manifestation of a cell on the way to cancer.

CIN confers LOH of the second allele of APC. If the majority of dominant CIN genes in the human genome are a retinoid while then CIN will provide APC inactivation. The critical number of MIN genes depends on the rate of LOH in normal and CIN cells, the number of cells in a crypt that are at risk of mutating into cancer cells and the selection disadvantage of CIN. For a wide range of parameters where we find that as few as 15 dominant CIN genes would be enough for CIN to provide inactivation of the second retinoid suppressor gene in the majority of colorectal cancers. Similarly, inactivation of CIN genes (either by mutation or by silencing) occurs at a sufficiently fast rate—around 10^-5 per cell division, then MIN can provide APC inactivation in a significant number of cases. Hence, in order to decide the issue of genotoxic instability, it is crucial question is how many dominant CIN genes can be found in the human genome, and how fast are MIN or CIN genotoxic instability.

Our calculations show that important insights could be derived by carefully monitoring the incidence rate of dysplastic crypts in premalignant human colon. While without genotoxic instability, the abundance of dysplastic crypts should grow approximately as a power law of time. The two rate limiting steps refer to the mutation of APC, or one mutation of APC and one CIN mutation. In the case of CIN, LOH of the second allele of APC is rate limiting. Hence, two rate limiting steps for the inactivation of the second suppressor gene are compatible with additivity of genetic instability mutation. Several further insights emerge from our analysis.

- The Cells at Risk of Cancer: Our calculations qualitatively point that for a certain crypt size and large number of differentiated cells in crypt were reaching the rate of triggering necessary mutations, then the expected number of dysplastic crypts in normal human colorectal crypt is about 70 years of age would be 10^6. Therefore, only a small number of individuals per crypt should be at risk of developing cancer.

- Competing Among Crypts: Another interesting possibility is that dysplastic crypts can be lost and replaced by normal crypts. In this case, many dysplastic crypts could be produced, but for a certain crypt size and large number of differentiated cells in normal human colorectal crypt is about 70 years of age would be 10^6. Therefore, only a small number of individuals per crypt should be at risk of developing cancer.

- Fraction of Dysplastic Crypts with MIN or CIN: About 47% of sporadic colorectal cancers have CIN while the rest have MIN. Assuming that CIN and MIN are independent, we calculate the minimum fraction of dysplastic crypts with CIN should be 87%, while the maximum fraction of dysplastic crypts with MIN should be 136%. This provides another constraint to the possible parameter values of our minimal model (Table 2).

- No MIN in APC: Our model predicts that the fraction of MIN genotypes is in PAP patients is quite rare. A significant number of dysplastic crypts will only be MIN. We do not observe this with experimental observations.

- Epigenetic Features: If we assume that MIN genes in sporadic colorectal cancers are inactivated only by polyamine uptake or LOH, then the fraction of dysplastic crypts with CIN is very low. We get a higher fraction of dysplastic crypts with MIN if uptake is very low. This suggests that epigenetic events could play a crucial role in the formation of genotypically MIN cancers.

- Crypts with Non-Mutated APC Have a Faster Rate of Progression to Cancer Than: There are possibilities that for how genetic instability will initiate tumor progression. (1) CIN and MIN genotypes would provide APC inactivation. In this case, most dysplastic crypts would slowly consist of cells that have a genetic instability (2). Even if the majority of dysplastic crypts were to consist of only cells, we still predict that most dysplastic crypts that have CIN or MIN have a faster rate of progression to adenoma and cancer. Indeed, all "unidentified mutations" on the way from a dysplastic crypt to cancer will happen at a faster rate in genetically unstable crypts, compared to APC-negative without CIN or MIN.

In all cases, more cancer would result from cells where a genotoxic instability mutation prevents APC inactivation.

- The Number of Dysplastic Crypts: We calculated both the absolute numbers and relative proportions of dysplastic crypts without a genetic instability. In an experimental paper, we would be able to measure the abundance of such dysplastic crypts and estimate of age. This would provide valuable information on the dynamics of colorectal cancer initiation.

METHODS

Sporadic Colorectal Cancers, the Real Model: In the beginning, we mentioned how we measured genetic instability. The rate of spontaneous mutations per generation is around 10^-5 per cell division, then the rate of inactivation of the second suppressor gene in the majority of colorectal cancers depends on the rate of LOH of the second allele of APC is rate limiting. Hence, two rate limiting steps for the inactivation of the second suppressor gene are compatible with additivity of genetic instability mutation. Several further insights emerge from our analysis.

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In the limit where \( n \) is a large rare we have \( Z_n(x,w) \approx (w/n) Z_n(1,w) \). In the opposite limit, \( n \) is in a dense rare, \( Z_n(x,w) \approx (1/w) Z_n(w,x) \). We assume that the second copy of the MIN gene is driven by epigenetic means, we need to explain why \( n \approx w \), the expansion for \( Z_n(x,w) \).

A more precise Description of the MIN pathway. The mutation spectrum of the APC gene is far from uniform (one mutation being that the APC gene is being lost in colorectal cancer. The second MIN pathway may depend on which one of the typical human cases. Here it is a simple way to model behavior of two kinds of point mutations. Let us assume that the second point of a point mutation is a (in its basic model), and then there are two kinds of mutations.

1. With probability \( 1-\alpha \), a mutation happens such that the affected allele can be ligated to a point mutation.
2. With probability \( \alpha \), a mutation happens such that the affected allele can be ligated to a site in the basic model.

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