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ScienceDirect
Physics of Life Reviews 4 (2007) 116–127
PHYSICS of LIFE reviews
www.elsevier.com/locate/plrev

Review

Up a gear? The significance of an elevated mutation rate in tumorigenesis

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Accepted 18 April 2007

Available online 25 April 2007

Communicated by L. Perlovsky

Abstract

Mutations involved in many cancers have been identified, but with some cancers requiring six or more mutations to take on their fully metastatic forms, the question remains whether all of these mutations can be acquired via a process of successive mutation, at a normal rate, and clonal expansion or whether heightened mutation rates are required. This issue has been debated for decades. Recently there has been much interest in forms of genomic instability such as chromosomal instability and microsatellite instability. It remains to definitively show whether or not these instabilities are very early causal events in tumorigenesis. This article reviews the evidence for and against genomic instability being an early causal event in tumorigenesis and surveys the mathematical modelling literature in this area. The focus is on chromosomal instability and microsatellite instability in colorectal cancer.

PACS: 87.10.+e

Keywords: Colorectal cancer; Genomic instability; Tumorigenesis; Mathematical models

Contents

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1571-0645/$ – see front matter
doi:10.1016/j.plrev.2007.04.001

1. Introduction

Cancer is a class of diseases in which diseased cells divide in an uncontrolled manner. The resulting growths, which typically arise locally in a specific tissue, can invade the surrounding healthy tissue and ultimately metastasis (dissemination of cancer cells via the bloodstream) can occur. For an excellent review of the characteristics of cancer see [1].

Within an individual, a cancer can be viewed as a population of evolving cells. These cells acquire mutations which may be selectively advantageous, in the sense that the progeny of those cells increase in frequency within the total cell population. In this evolutionary process, deleterious mutations may also be acquired.

One of the best-studied cancers in terms of its carcinogenic mutations is colon cancer. Vogelstein and colleagues [2] describe a sequence of several mutations in oncogenes (genes typically requiring a mutation in a single allele to have their cancer-promoting effects) and tumour suppressors (genes typically requiring mutations in both alleles) leading to the fully malignant phenotype. Even in this case, however, it remains unclear whether genomic instability precedes the first selectively advantageous mutations. Indeed the earliest selective mutation in many colon cancers is in the APC gene and there is speculation that this gene itself may initiate instability.

In this Review we discuss the hotly contested issues of the timing and significance of genomic instability in tumorigenesis (see, for example, [3–7]). We focus mainly on chromosomal and microsatellite instability (see next section) in colorectal cancer. We describe both experimental evidence and mathematical models designed to address these issues.

The review is structured as follows. We first discuss different types of genomic instability, inherited syndromes leading to genomic instability and ways in which instability can be measured experimentally. We then discuss evidence on the timing and significance of genomic instability in tumorigenesis. Before concluding, we elaborate on the mathematical models that have been applied to studying these issues.

2. Types of genomic instability

2.1. Chromosomal instability (CIN)

Chromosomal instability or CIN is a form of genomic instability in which there is an elevated rate of loss/gain of whole chromosomes or large chromosome fragments or else translocation of parts of one chromosome on to another. This results in cells with unusual karyotypes. For example, around 67% [8,9] of colorectal cancers are aneuploid (they have unequal numbers of different chromosomes). Arguably mutations which allow cells to survive with abnormal karyotypes instead of undergoing apoptosis could be said to induce CIN as well as those which actually raise the rate at which the chromosomal abnormalities occur.

In cells that have CIN, a mutation in a single copy of a tumour suppressor gene may be rapidly followed by loss of heterozygosity, in which the second normal copy of the gene is also lost with a segment or whole chromosome. (This is often followed by reduplication of the remaining chromosome with the mutated form of the tumour suppressor.) These chromosome losses and translocations suggest that mis-segregation of chromosomes at mitosis may be a crucial event in CIN cells.

Genes implicated in CIN include those with roles in the mitotic spindle checkpoint, such as BUB1 [10]. Another possible trigger for CIN is the shortening of telomeres, discussed below.

2.2. Chromosome nondisjunction

The mitotic spindle checkpoint checks that chromosomes are properly aligned in pairs prior to cell division, thus helping to ensure that each daughter cell receives a single chromosome from each pair of copies.

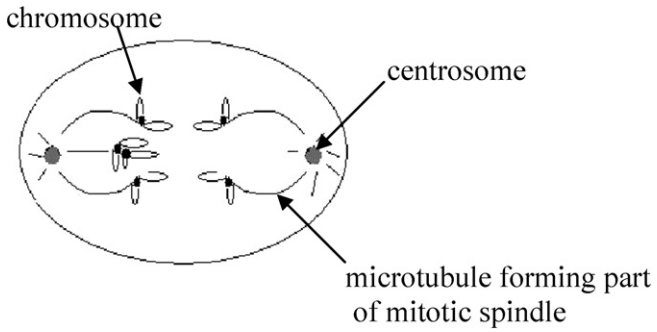

Fig. 1. Diagram of chromosome nondisjunction in a dividing cell. In the dividing cell shown, two pairs of chromosomes are segregating properly (one to each pole) and the other pair is mis-segregating. The chromosomes are moving towards the centrosome at the end to which they are attached by microtubules.

It has been shown in a breast cancer cell line that there is a relationship between variability in chromosome number (taken as evidence of CIN) and the ability of cells to pass through the mitotic spindle checkpoint after the mitotic spindle is disrupted with a drug [11]. Taken together with evidence of mutation of checkpoint genes in CIN, this suggests that CIN may be caused by an impaired mitotic spindle checkpoint allowing cells to divide without properly aligning their copied chromosomes. This results in chromosome nondisjunction, in which two copies of a chromosome end up in one daughter cell, see Fig. 1.

One way in which the mitotic spindle can be impaired is if there are elevated numbers of centrosomes in the cell. These centrosomes set up the spindle from microtubules. There is evidence that their numbers can be elevated if the cell undergoes aborted division. Mutant p53 may be implicated in the failure to kill cells that have undergone aborted division. Most cells with excess centrosomes undergo serious mis-segregation of chromosomes and subsequently die. There is also evidence that APC null cells have elevated centrosome numbers, leading to CIN [12]. APC binds EB1, which associates with the growing ends of microtubules and with centrosomes. Some APC truncations lead to defects in the mitotic spindle and hence instability (generally leading to polyploidy rather than aneuploidy) [12–14]. The protooncogene Myc can also cause genomic instability [15] when transiently activated, seemingly by speeding up the G1/S transition (the transition between the first growth phase and the DNA synthesis phase of the cell cycle) and disabling the checkpoint there. This allows cells with genomic alterations to make the transition.

There is some evidence, however, that if mis-segregation of chromosomes begins to occur then generically furrow regression will result, meaning that the cell does not actually divide. Thus the cell becomes tetraploid (contains four copies of each chromosome) and binuclear [16]. Further division of these tetraploid cells is more subject to mis-segregation, leading to aneuploidy, because these cells have an extra centrosome.

2.3. Microsatellite instability

Microsatellite instability or MSI refers to a state in which cells preserve their normal chromosome content but have greatly elevated mutation rates at the nucleotide level. Particularly susceptible to elevated mutation are certain short repeat sequences in the genome termed microsatellites, hence the name. In DNA replication, the replication machinery may “slip” in these repeating regions and introduce additional repeats (or miss out repeats). Thus microsatellites are subject to changes in length. Approximately 15% of colorectal cancers have MSI [17,18].

Frequently genomic instability is caused by defects in DNA repair genes. These are of different types, including nucleotide excision repair, base excision repair and mismatch repair genes. The last of these are particularly important in preventing MSI. Genomic instability may also be caused by mutations in DNA polymerases or DNA helicases.

In addition to these genetic effects, epigenetic factors can cause MSI (and CIN), e.g., by hypermethylation of genes, leading to gene silencing. Telomere shortening can also lead to instability.

3. Telomere crisis and reduction in genomic instability in mature cancers

Telomeres are repetitive DNA sequences which cap eukaryotic chromosomes. When human cells divide, there is generally a loss of telomere length from the ends of the chromosomes. Normally this leads to senescence, the aging

process that allows cells only to divide a finite number of times. In early tumour progression, some senescence may occur, limiting tumorigenesis. As the telomeres become very short, genomic instability (with the production of dicentric chromosomes and chromosome fragments and with chromosome loss and gain) occurs since uncapped telomeres tend to lead to recombination [19]. This instability causes apoptosis to be induced, for example, by p53. This induction of instability by dysfunctional telomeres and the associated cell death are referred to as *telomere crisis*. In the majority of cancers, as cancer progresses, p53 becomes mutated, allowing tumours with shortened telomeres to avoid senescence. At this stage ("late crisis") there is extreme genomic instability and death is induced by a p53-independent mechanism. Finally there is a re-establishment of telomere-maintenance mechanisms, allowing the cancer cells, which have acquired many chromosomal aberrations, to avoid death [19–22]. With renewed telomere maintenance, the genomic instability of these cancer cells is reduced, although they will retain unusual karyotypes [21].

4. Syndromes exhibiting genomic instability

Around 5–10% of colon cancers occur in familial patterns [23,24]. Not all of these cancers need be due to inherited mutations, however study of this phenomenon has unearthed genes Msh2 and Mlh1, which are involved in mismatch repair [25–29]. Germline mutations in these genes predispose to hereditary non-polyposis colorectal cancer (HNPCC)[1] with a lifetime probability of acquiring colorectal cancer of roughly 80%. In this cancer there is a high level of slippage in microsatellites. When functioning normally the mismatch repair genes help to repair these slippages.

Bloom's syndrome involves mutations in the BLM gene, a DNA helicase with a probable role in DNA double break repair, untangling of sister chromosomes and suppression of homologous recombination. In the syndrome there are high numbers of chromosome breaks and translocations between sister chromosomes and an elevated predisposition to many cancers including colorectal cancer [30].

Inherited bi-allelic mutations in the MYH gene, which functions in base excision repair cause MYH-associated polyposis. The associated genomic instability causes G:C to T:A transitions in the DNA and so mutations accrue in genes such as APC [31,32].

There are many other syndromes involving genomic instability, which lead to other cancers; two primary examples are xeroderma pigmentosum (XP) and ataxia telangiectasia. Xeroderma pigmentosum is an inherited syndrome, which causes hypersensitivity to UV radiation and results in an elevated level of skin cancer. The gene mutated in XP is involved in nucleotide excision repair (NER), a DNA repair method which recognises abnormal structures where there are alterations in a strand of DNA, cuts out a portion of the strand, including the faulty bit, and employs a polymerase to synthesize a strand with the correct sequence. In XP there is thus NER-related instability. Ataxia telangiectasia is a disorder affecting a DNA damage checkpoint. Normally upon damage the ATM gene induces p53, which is implicated in the G1/S checkpoint. In ataxia telangiectasia, ATM is mutated and the checkpoint is absent, allowing cells with chromosomal abnormalities to propagate. Thus there is hypersensitivity to ionising radiation; DNA breaks and crosslinks may occur and propagate which then predispose to chromosomal deletions, translocations and inversions (and hence CIN).

5. Measuring genomic instability

There are several ways of measuring the presence of extra copies of chromosomes or chromosome portions, the loss of chromosomes/chromosome portions and the presence of chromosome translocations. Particularly worthy of note are the methods of comparative genomic hybridisation (CGH), fluorescence in situ hybridisation (FISH) and spectral karyotyping (SKY).

CGH allows the detection and mapping (finding the location within the genome) of allelic imbalances [33]. DNA from tumour cells is dyed one colour and DNA from normal cells in the same individual is dyed another and the relative intensities of the colours of dye are compared to detect differences in copy number between the cancer and normal cells at various loci. This technique requires fairly large tumours containing few normal cells, but can map differences across the whole genome.

[1] Despite the name, the term HNPCC is often used to refer to the inherited syndrome which confers an increased cancer risk, rather than the cancer itself.

FISH can measure DNA sequence copy number at specific loci in single nuclei. Thus it gives single cell resolution and can be used to look at the diversity in copy number within a tumour [33]. It requires probe sequences for copy number comparison with the DNA in individual cells and so is generally suitable only for looking at specific regions of the genome.

SKY dyes chromosomes from individual nuclei different colours [34]. Each chromosome from a normal diploid cell has a fluorescently labelled probe created by labelling chromosome-specific DNA with a fluorophore. Chromosomes can then be visualised and chromosomal abnormalities in tumour cells can be detected.

Another method of detecting chromosomal abnormalities is flow cytometry to sort the cells according to whether they are dividing and their DNA content [35]. This detects when the cells have non-diploid (in phase G1 of the cell cycle) levels of DNA. A peak of DNA content which is neither at the level of a normal diploid cell in phase G1 nor at twice that level indicates aneuploidy. Another alternative is the direct measurement of loss of heterozygosity at particular loci [35].

Chromosomal instability, of course, implies that chromosome number is changing in time (or translocations, etc. are occurring), not just that cells are aneuploid. Within tumour variance in chromosome copy number may be used to estimate the rate at which chromosomal abnormalities arise, using a mathematical model of the process of chromosome nondisjunction.

6. Evidence

It is undeniable that in many cancers the cells have unusual karyotypes, with loss/gain of whole chromosomes as well as chromosomal translocations. In addition, some cancers have more fine-grained abnormalities and some early-stage tumours have as many as 11,000 mutations throughout their genomes. As mentioned above, the existence of aneuploidy (or indeed fine-grained mutations), however, does not imply genomic instability. It is possible that the chromosomal defects have been acquired at a normal rate and that it is simply the rapid expansion of the tumour cells that means that so many have accrued in individual cells. In addition, the escape from apoptosis evident in many cancer cell lines means that defective cells are less likely to have been eliminated. Evidence for genomic instability means finding that these abnormalities have arisen more rapidly than in normal cells.

Direct evidence for chromosomal instability is difficult to obtain, since there is not yet a good assay. Mathematical models (see sections below) have been used to estimate conditions under which CIN is likely to precede loss of APC in colorectal cancer. Some [36,37] of these indicate that CIN is likely to be the initiating event if more than a threshold number (of the order of 1–10) of CIN genes exist, each of which is dominant and in its mutated form causes CIN.

Shih and co-workers [37,38] have shown that in tiny colon adenomas, with average diameters of 2 mm, 90% had allelic imbalances of one or more of the five chromosomes tested. In many of these tumours, the imbalances occurred in only some of the cells in the tumour, except at the locus of the APC gene (where allelic imbalances, when they occurred, did so in most cells). Their findings are evidence of allelic imbalance (and so perhaps CIN) occurring very early in tumour progression.

It has been claimed that alterations in p53, p16 and increased tetraploidy in Barret's oesophagus precede aneuploidy, whereas in ulcerative colitis, chromosomal instability precedes tumour detection. Thus it may be that, in different types of cancer, early genomic instability has variable significance [39, and references therein].

Evidence suggests that additional silent (fine-grained) mutations in p53 in human tumours are around 20-fold higher than would be expected. These mutations should not be selected for and thus their prevalence suggests that they are occurring at an elevated rate [40].

Other evidence in favour of a role for genomic instability in tumorigenesis comes from data on increased incidence of certain types of cancer in individuals with inherited diseases affecting genomic stability, such as xeroderma pigmentosum and ataxia telangiectasia. The latter, for example, leads to chromosomal instability and greatly increased risk of early-onset cancers, especially leukaemias and lymphomas. HNPCC may account for 1–5% of all colorectal cancers [29] and is characterised by MSI.

On the other hand, it has been found [41] that most sporadic colorectal cancers appear not to show raised levels of mutation at the nucleotide level. This was assessed by measuring the nonsynonymous mutations in approximately 3.2 Mb of coding tumour DNA. In total only three mutations were found. Microsatellite unstable tumour cell lines were not examined in this study and the results indicated a low level of fine-grained mutations, but not of allelic losses. By contrast, Bielas et al. (2006), whose estimates of the frequencies of mutations per nucleotide in cancers were in

broad agreement with those of the aforementioned study, found that the frequencies of random single-nucleotide substitutions in normal tissues (including from the colonic epithelium) were roughly 200-fold lower and thus concluded that the cancers had nucleotide instability.

Epidemiological data on the age-incidence of cancers has been used to try to reconstruct estimates (for example, maximum-likelihood estimates) of the numbers of mutations involved in the progression to cancer and the mutation rates in each step of the multistage carcinogenesis process. Early age-incidence models such as those of Armitage and Doll [42] (and see [43] for a review) suggested that the most likely scenario for multistage tumorigenesis was a sequence of 6 or 7 mutations, with a mutation rate of the order of 10^{-3} per cell per year. This is much higher than normal mutation rates and hence suggests that genomic instability may be a necessary early feature of tumorigenesis. In general, however, more recent studies of this kind do not find it necessary to invoke genomic instability in order to obtain realistic predictions of incidence. Very elegant work on colorectal cancer [44], correcting both for birth cohort and calendar year of diagnosis, suggests a four-stage model with three rare mutational events with rates of the order of 10^{-6} per cell per year, which the authors claim is consistent with the measured locus-specific mutation rates. Thus there is no need to invoke an elevated mutation rate. There is a much more frequent third event which is necessary for transformation. It is possible that this reflects the need for a small time-window of enhanced mutation rate, however the authors postulate an alternative scenario, whereby this event corresponds to the fixation of an initiated cell within the colon crypt (following the first two mutations, stems cells which can clonally expand by symmetric division exist, but the probability of them dying before they can proliferate many times is high, thus the invasion of the crypt by the clone takes some time). Detail on the mathematics of these types of model is given in later sections.

In the next section, evidence on the timing and significance of MSI in colorectal cancer is discussed.

7. Does APC mutation precede MSI in colorectal cancer?

As mentioned approximately 15% of colorectal cancers have MSI. Since this instability results in characteristic tandem repeats, it may be possible to distinguish cancers in which the loss of both APC alleles precedes MSI from those in which the loss follows MSI. There is evidence that the frequency and types of APC mutation in high-level MSI carcinomas are the same as in carcinomas with low levels of MSI or no MSI [45,46].[2] If MSI generically occurred prior to APC mutation and if the APC gene was susceptible to the types of mutations involved in MSI, then one would expect a different spectrum of mutations in high-level MSI carcinomas. Thus it appears that MSI does not play a prior causative role in the mutation of the APC alleles. However, the rate of mutations in the beta-catenin gene is significantly higher in high-level MSI carcinomas [48], suggesting that MSI may cause beta-catenin mutation, which has a very similar phenotypic effect to APC mutation (and is an alternative step in the pathway to tumorigenesis).

Clearly MSI can play a causative role in tumorigenesis, for example in HNPCC patients. As mentioned previously, these individuals have a lifetime probability of 0.8 of getting colorectal cancer. Curiously this syndrome, which leads to defects in mismatch repair, does not render patients more susceptible to all forms of cancer [49].

8. Age incidence and multistage models

As mentioned above, one method that has been applied to the problem of estimating numbers of mutations in the pathway to cancer and mutation rates involves using age-incidence data. The majority of work in this area employs a multistage model framework in which target cells accrue a sequence of mutations, which may or may not confer selective advantages to those cells. From these models, probability distributions of the time at which a tumour will occur are predicted. Then some inference (usually Bayesian or maximum likelihood) method is applied to estimate parameters of the multistage model from the age incidence data. Perhaps the most likely to give good estimates are studies involving comparative data from sporadic cancers and cancers in people with inherited genetic defects [see, for example, [43,50–52]].

The basic multistage theory [42,53] runs as follows:

We assume that cancer occurs when a cell acquires n specific mutations. These mutations occur independently and the time, T_i, in years to the ith mutation is exponentially distributed with constant rate u_i per annum. The probability

[2] This is, however, contentious (see [47]).

that a given target cell has accrued $n-1$ mutations by time t is given by

$$P(T_{n-1} \leqslant t) = \frac{1}{(n-1)!} \prod_{i=1}^{n-1} \bigl(1 - \exp(-u_i t)\bigr),$$

assuming the mutations must occur in a specific order (if this is not the case then the formula is the same but without the factor $1/(n-1)!$). The rate at which a single target cell initiates cancer is thus this probability multiplied by the probability that the nth mutation has not occurred at time t multiplied by u_n (the rate at which the nth mutation occurs), i.e.

$$\frac{u_n \exp(-u_n t)}{(n-1)!} \prod_{i=1}^{n-1} \bigl(1 - \exp(-u_i t)\bigr).$$

For realistic mutation rates and lifespans, one can assume that $u_i t \ll 1$ and hence that this rate is approximately

$$\frac{\bigl(\prod_{i=1}^{n} u_i\bigr) t^{n-1}}{(n-1)!}.$$

This is the rate of initiation of cancer by one target cell, so to get the rate of incidence of cancer one must multiply by the number of target cells, N.

Thus, from a simple model like this with no selection on intermediate cell types, we get a multiple of t^{n-1} against which to fit the cancer incidence data. Since the incidence goes up like t^5 or t^6, Armitage and Doll [42] concluded that there were six or seven mutations leading to cancer. As mentioned above their estimated mutation rates were higher than those measured in genomically stable cells, but broadly consistent with rates in genomically unstable cells.

Models in which mutant cells can acquire a selective advantage give lower estimated mutation rates. Using a model of this nature, Armitage and Doll [54] predicted two mutations with mutation rates that are plausible for genomically stable cells. A plausible mutation rate for genomically stable cells was also a conclusion of the multistage model of Luebeck and Moolgavkar [44]. They found a four-stage process most likely with estimated mutation rates of the order of 10^{-6} per cell per year.

Calabrese et al. [55] took cancers from individuals with germline mutations in mismatch repair genes. Such patients have a very high penetrance of cancer with microsatellite instability. They noted the age at cancer removal and, assuming a model in which microsatellites, once unstable, mutate (extend/reduce their number of repeats) at a rate of 0.005 per division and assuming cells divide once a day, they estimated the time since mismatch repair function was lost. Subtracting this time from the age at cancer removal, gave the age at loss of mismatch repair (MMR). Then age incidence methods could be applied to the age of loss of mismatch repair to estimate the number of mutations before mismatch repair is lost and the mutation rates of these steps. The authors used a Bayesian inference method with a prior on the number of mutational steps which was uniform on $\{2, 3, \ldots, 10\}$. They found that the most likely values (there was considerable uncertainty since there was only age incidence data from 14 cancers) for the number of mutational steps were 5 and 6, with a 95% credible interval of 4 to 8. The average time since MMR loss in the tumours at removal was estimated to be roughly six years and it was also estimated that clonal expansion started on average a year before tumour removal. Therefore MMR loss preceded clonal expansion by an average (roughly) of only five years. Thus their estimated number of mutational steps until clonal expansion had most likely values which were also 5 and 6. They concluded that MMR loss was a late event in the tumorigenesis process, albeit preceding clonal expansion. (Their model was based on a sequence of occult mutations occurring prior to phenotypic change in the cells.) They also concluded that acquisition of the 5 or 6 mutations prior to MMR loss at a normal mutation rate was plausible.

If it is the case that the majority of mutational steps towards a tumour take place at a normal mutation rate in microsatellite unstable tumours, then this presumably means that genomic instability is not necessary for tumorigenesis. The fact that tumours are commonly chromosomally unstable may either be because this simply speeds up the tumorigenesis process by a small amount or it may be a by-product of other changes involved in tumorigenesis. The fact that very few, if any, tumours are both chromosomally and microsatellite unstable, however, suggests that an instability may be selected for (in the sense that cells which acquire instability have more offspring, and so once one form of instability has arisen, another form confers no further advantage). The results of [55] seem to indicate that MMR loss

and tumour growth often occur following the same rate-limiting (i.e., a step which affects the time taken for tumour initiation) mutation, so perhaps although the majority of rate-limiting steps occur prior to MMR loss, there are several mutational events which occur very rapidly following MMR loss, precipitating tumour growth.

9. Moran process models

Following [56], we first illustrate the Moran process models using a one-hit process model. Then we will discuss what these models have contributed to the understanding of the significance and likely timing of genomic instability in tumorigenesis.

These models assume that the total number of cells in the population (which might correspond to a crypt in a human colon) is constant, say equal to N. In general there are various types of cell and the frequencies of each type can vary as cells divide, die and mutate between the types. In the one-hit model, there are just normal cells, whose number is denoted by a and mutant cells, whose number is denoted by b. (Hence $a + b = N$.) Normal cells have reproductive rate 1 and mutant cells reproductive rate r, which could be greater/less than or equal to 1. If a normal cell divides, then with probability $1 - u$ it will reproduce faithfully and with probability u it will produce a mutant cell. At each time step, one cell divides and, in order to retain a constant cell number, one cell dies. In order that one time unit corresponds roughly to the individual cell cycling time, the time step is set to be $1/N$ (thus in one time unit there are a total of N cell divisions). The probability that the cell chosen to divide is a normal cell is proportional to the number of normal cells and to the reproductive rate of normal cells and hence is $a/(a + rb)$, with $rb/(a + rb)$ being the probability that the cell chosen to divide is a mutant cell. The probability that the cell chosen to die is normal (/mutant) is just proportional to the number of normal (/mutant) cells and hence is a/N ($/b/N$).

This set up describes a Markov process on the states $b = 0, \ldots, N$ with the transition probability, P_{ij}, in a time step of length $1/N$ from a state with $b = i$ to a state with $b = j$ being given by

$$P_{ij} = \begin{cases} \frac{u(N-i)+ri}{N-i+ir}\frac{N-i}{N} & j = i+1, \\ \frac{(1-u)(N-i)}{N-i+ir}\frac{i}{N} & j = i-1, \\ 1 - P_{ii-1} - P_{ii+1} & j = i, \\ 0 & \text{otherwise} \end{cases}$$

for $0 \leqslant i, j \leqslant N$.

The only absorbing state of this biased random walk is $b = N$. Thus one can ask how long it takes from a state in which all cells are normal ($b = 0$) to reach the absorbing state where all cells are mutant.

If t is the actual time, then we set $T = Nt$, so that T enumerates the time steps and hence the cell births and deaths. When u is sufficiently small, most of the time mutations which arise are either eliminated or fixed in the populations before another mutation has had the chance to arise. Thus the rate at which absorption into the state $b = N$ occurs is roughly $Nu * \pi_1$ where π_i is the probability of fixation of the mutant starting from i mutant cells in a process without mutation (in this process with $u = 0$ the state $b = 0$ is also an absorbing state and so $\pi_i \neq 1$ for each i). For conditions on u for this approximation to be valid, see [56]. We can write down a recursion relation for the π_i:

$$\pi_i = \sum_{m=0}^{N} P_{im}(u = 0)\pi_m, \quad 1 < i < N - 1 \text{ and } \pi_0 = 0,\ \pi_N = 1.$$

Substituting in for the transition probabilities we get

$$r(\pi_{i+1} - \pi_i) = \pi_i - \pi_{i-1}, \quad 1 < i < N - 1 \text{ and } \pi_0 = 0,\ \pi_N = 1.$$

Solving the recursion relation yields

$$\pi_1 = \frac{1 - 1/r}{1 - 1/r^N}.$$

Hence the absorption time is roughly

$$t_{\text{abs}} = \frac{1}{Nu}\frac{1 - 1/r^N}{1 - 1/r}.$$

The analysis of these Moran process models is vastly simplified if one assumes that at any given time, with high probability, all cells are of the same type. The system is said to be in a *homogeneous* state. In the one-hit model, this means that the majority of the time either $b = 0$ or $b = N$. This is likely to be true if fixation of a mutation is much faster than the process of mutation. Denote by A the probability that the system is in the state $b = 0$ and by B the probability that it is the state $b = N$. In this approximation $A + B = 1$. If we consider a coarse-grained continuous time approximation then there is a probability $Nu\Delta t * \pi_1$ that, given that the system is in state $b = 0$ at time t, it changes to state $b = N$ at time $t + \Delta t$ (and of course there is no chance that it changes in the opposite direction). Thus we can write down a Kolmogorov forward equation for the probability A (and hence B):

$$\dot{A} = -Nu\pi_1 A \quad \text{with } A(0) = 1.$$

Hence $A = \exp(-Nu\pi_1 t)$.

We can extend the system to a two-hit model (e.g., corresponding to the knocking out of a tumour suppressor). Here we would consider in addition a number of double mutant cells c, with a corresponding homogeneous state with probability C. In the two-hit model there is the possibility of tunnelling (see [56]) where the state with $c = N$ arises without the state $b = N$ ever having arisen. Clearly there must have been cells of the mutant type but these never became fixated in the population and were always very low in abundance.

10. Genomic instability in Moran process models

Again following [56], consider a model of the dynamics of acquisition of mutations in the alleles of the APC gene in a single crypt of a human colon. We assume two hits correspond to a tumour and ask whether tumorigenesis is faster via a route which involves acquiring genomic instability prior to the knockout of both APC alleles or whether it is faster just to mutate the APC alleles. Both MSI and CIN are considered. Since it is assumed that non-stem cells only divide a certain number of times (and it is assumed for now that acquiring two APC mutations does not alter this), mutations acquired by non-stem cells will disappear from the crypt within a few cell divisions. Thus the population of cells considered in this model consists only of the stem cells. It is estimated that there are 1–10 stem cells per crypt (although other authors sometimes assume up to 100). Thus N in the following model is between 1 and 10.

First, neglecting genomic instability, we assume that fixation of mutations is rapid compared to their initial acquisition and let X_0, X_1 and X_2 be the probabilities that the crypt consists of cells with no mutant APC alleles, one mutant allele and two mutant alleles, respectively ($X_0 + X_1 + X_2 = 1$). The probability of mutation at division of a single allele is u and in addition, if one allele is already mutated, there is a probability of loss of the normal allele by loss of heterozygosity of p_0.

Analogously to the equation in the previous section, Kolmogorov forward equations for X_0, X_1 and X_2 can be derived:

$$\dot{X}_0 = -2Nu\pi_1 X_0,$$
$$\dot{X}_1 = 2Nu\pi_1 X_0 - N(u + p_0)\pi_1' X_1,$$

where the factor of 2 comes from the fact that either of the alleles can be mutated in normal cells. Here π_1 is the probability of fixation of a mutation in a single allele (starting in only one cell) and π_1' is the probability of fixation of the second mutant allele.

Further we assume that cells with one mutant allele are neutral with respect to those with no mutant alleles (i.e. $r = 1$ for the single mutant), whereas cells with two mutant alleles have a greatly increased reproductive rate. Thus from the previous section $\pi_1 = 1/N$ (taking the limit as $r \downarrow 1$) and π_1' is close to 1.

The solution is given by

$$X_2(t) \approx 1 + \frac{2u\exp(-N(u + p_0)t) - N(u + p_0)\exp(-2ut)}{(N - 2)u + Np_0},$$

which for $N(u + p_0)t \ll 1$, is approximately

$$X_2(t) = uN(u + p_0)t^2.$$

Reintroducing genomic instability, it is assumed that it takes two mutational hits to induce MSI (although these may be in one of several genes) but only one to induce CIN (again in a choice of several possible genes). Thus cells can be

normal in their genomic instability status, or they can have one or two defective copies of an MMR gene or they can have CIN. In addition cells can have zero, one or two mutant APC alleles. Thus in total there are twelve cell types in the model. Again it is possible to set up a system of Kolmogorov forward equations to describe the probabilities that all the cells in a crypt are of one of the twelve types (assuming once again that fixation is much faster than mutation). This yields 11 ordinary differential equation, since the probabilities sum to one. The analysis is simplified by assuming that t is within a certain range and that all cell types are neutral with respect to normal cells, with the exception of the APC double mutant cells which have very heightened reproductive rates.

It is found that, if we assume that the mutations that cause MSI and CIN also occur at a rate u per target allele, CIN cells have a greatly increased rate of heterozygosity p and there are n_c genes any of which when mutated cause CIN, then the probability that all the cells in the crypt have normal genomic stability status and two mutant APC alleles is roughly

$$Nu(u+p_0)t^2 \quad \text{(as before)},$$

whereas the probability that they all have CIN and have two mutant APC alleles is roughly

$$4n_c u^2 t^2$$

if we assume that $N(u+p_0)t \ll 1$ and $Npt \gg 1$. Likewise if we assume that the elevated mutation rate in MSI cells is $\hat{u}$ with $\hat{u}t \gg 1$ then the probability that they all have MSI and have two mutant APC alleles is roughly

$$n_m u(u+p_0)t^2.$$

The limits in which $pt \ll 1$ and $\hat{u}t \ll 1$ lead to probabilities for unstable APC double mutant crypts proportional to t^3 and t^4, respectively. Therefore in these limits the most likely tumorigenesis is without instability.

Thus in the time range $1/\hat{u}$, $1/(Np) \ll t \ll 1/(N(u+p_0))$, the most likely type of APC double mutant cell to occur depends on the relative sizes of $N(u+p_0)$, $4n_c u$ and $n_m(u+p_0)$. It is assumed that p_0 and u take roughly the same value. Thus the most likely type of APC double mutant cell depends on the relative sizes of N, $2n_c$ and n_m. Suppose the number of stem cells in a crypt is 5, then chromosomal instability in the first APC double mutant is favoured if there are three or more genes which when knocked out yield CIN and MSI is favoured if there are more than five genes which when knocked out yield MSI. $n_c = 8$, $n_m = 4$ and $N = 5$ could yield roughly 65% CIN adenomas, 15% MSI adenomas and 20% genomically stable adenomas.

These authors have published a number of papers on this topic, investigating the likely significance of genomic instability in tumorigenesis under a variety of assumptions. For further details see for example [57,58].

11. Conclusions

Pre-tumour progression models suggest that, in HNPCC, loss of the second allele of the mismatch repair gene may be a relatively late event in tumour progression albeit prior to significant clonal expansion. Presumably this is also true in sporadic replication error positive (RER+) colorectal tumours. However cancer penetrance in HNPCC is very high so clearly loss of MMR is an important event in these tumours and may trigger several rapid mutations in genes, which lead to clonal expansion and other features of cancer.

Patients inheriting a defective APC allele are almost certain to develop colorectal cancers and APC is mutated in most sporadic colorectal cancers. Thus it seems that losing both APC alleles (or some phenotypically almost equivalent mutation, such as in beta-catenin) is key in developing colorectal cancer. A crucial question is thus whether genomic instability precedes, or succeeds the loss of the second APC allele. It seems that MSI may cause mutations in beta-catenin but not in APC. Since in high-level MSI carcinomas, approximately 50% have APC mutations and approximately 27% have beta-catenin mutations, this suggests that clonal expansion may occur prior to MSI in 50–73% of cases.

Chromosomal instability is prevalent in colorectal cancer but it is still not clear how early it occurs. As mentioned above, the crucial question may be whether it precedes or succeeds loss of the second APC allele, and this issue has been the subject of a longstanding debate between the groups of Loeb and Vogelstein/Lengauer on the one hand and Tomlinson/Bodmer on the other [3,5,39]. A third possibility, for which there is quite good evidence, is that APC truncations cause chromosomal instability by failure of the mutated form to properly regulate microtubule end attachments in the formation of the mitotic spindle [12,14]. It appears that a single mutated allele may be sufficient to

cause mitotic defects. However mutations in checkpoint genes may also be required to give rise to the chromosomal instability seen in colorectal cancer. Statistics vary on the level of aneuploidy and chromosomal instability in *early* tumours. One study, using comparative genomic hybridisation showed that roughly 60% of FAP polyps show aneuploid changes [31]. Others showed that 25–33% of sporadic adenomas were aneuploid by flow cytometry ([59,60] respectively). However association between APC mutation status and CIN was only found for specific APC truncations. Thus not all APC mutations, sufficient for clonal expansion, appear to cause CIN. Some studies show that loss of heterozygosity in APC only occurs in a minority of colorectal cancers [61,62]. This may indicate that CIN is unlikely to precede the second APC mutation. It appears that CIN may commence at around the time of initiation of small adenomas and may sometimes be caused (at least in part) by APC truncations.

The existence of microsatellite stable diploid tumours [17] suggests that while genomic instability may aid tumorigenesis, it may not be a necessary requirement. It is possible, however, that these tumours may have other types of instability.

Acknowledgements

KMP would like to thank Ian Tomlinson, Trevor Graham and Chris Hornsby for helpful discussions and the Joint Research Councils (EPSRC, BBSRC and MRC) for funding under grant GR/R47455.

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