Chromosomal instability and human cancer

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Genetic instability is a defining feature of human cancer. The main type of genetic instability, chromosomal instability (CIN), enhances the rate of gross chromosomal changes during cell division. CIN is brought about by mutations of CIN genes, i.e. genes that are involved in maintaining the genomic integrity of the cell. A major question in cancer genetics is whether genetic instability is a cause and hence a driving force of tumorigenesis. A mathematical framework for studying the somatic evolution of cancer sheds light onto the causal relations between CIN and human cancer.

Keywords: genetic instability; tumour suppressor genes; somatic evolution of cancer; mathematical modelling

Human tumorigenesis proceeds by the accumulation of inherited and somatic mutations in gatekeepers and caretakers (Vogelstein & Kinzler 2001). Gatekeepers such as oncogenes and tumour suppressor genes (TSGs) encode proteins that function in growth regulatory and differentiation pathways (Knudson 1971, 1993; Friend *et al.* 1986; Weinberg 1991). Mutations in those genes increase the net reproductive rate of cells and might lead to clonal expansion (Park & Vogelstein 2000). Here, we study cancers that are initiated by the inactivation of one or two TSGs.

Caretakers maintain the genomic integrity of the cell (Lengauer *et al.* 1998; Loeb 2001). Mutations in caretakers lead to genetic instability, which is found in most human cancers (Vogelstein & Kinzler 2001). In a small fraction of colorectal, endometrial, gastric and some other cancers, inactivation of the mismatch repair pathway leads to an elevated point mutation rate and consequent widespread microsatellite instability (Kinzler & Vogelstein 1996; Perucho 1996). The majority of cancers, however, have chromosomal instability (CIN; Rajagopalan *et al.* 2003). CIN refers to an increased rate of losing or gaining whole chromosomes or large parts of chromosomes during cell division (Rajagopalan & Lengauer 2004*a*). The consequence of CIN is an imbalance in chromosome number (aneuploidy) and an increased rate of loss of heterozygosity (LOH). An elevated rate of LOH is an important property of CIN, because it accelerates the inactivation of TSGs.

Note that CIN refers to an enhanced rate of accumulating gross chromosomal changes during cell division; it is not synonymous with the state of aneuploidy that is observed in a static image of the cell's chromosomal content. While CIN is a process that drives most cells to aneuploidy, aneuploidy *per se* does not prove the existence of CIN. There are several ways in which a cell can become aneuploid in the absence of CIN. The cell could have divided many more times than other cells in the tissue; as gross chromosomal changes (rarely) occur in normal cells, this could lead to aneuploidy without CIN. Alternatively, aneuploidy could be caused by exposure to endogenous or exogenous agents that disrupt spindle formation. It is also possible that cancer cells accumulate gross chromosomal changes at the same rate as normal cells, but that these changes are only lethal in normal cells. This hypothesis is strengthened by the knowledge that mutations in cancer cells disrupt the capacity of cells to undergo apoptosis.

Although CIN is defined as an increased rate of losing or gaining parts of chromosomes or whole chromosomes during cell division, CIN has only been formally demonstrated for whole chromosome losses (Lengauer *et al.* 1997). There is no assay at present that can reliably measure the rate of formation of changes at the subchromosomal level, such as deletions, insertions, inversions, rearrangements, amplifications, unequal sister chromatid exchange and gene conversion. These changes are at least as common as losses or gains of whole chromosomes.

A large number of genes have been identified that trigger CIN when mutated in *Saccharomyces cerevisiae* (Shonn *et al.* 2000; Kolodner *et al.* 2002; Nasmyth 2002). These genes are involved in a variety of cellular pathways including chromosome condensation, sister-chromatid cohesion, kinetochore structure and function, microtubule formation and cell-cycle control. Similarly, several genes can cause CIN in *Drosophila melanogaster* when genetically altered (Fung *et al.* 2002; Mihaylov *et al.* 2002). By analogy, we expect hundreds of human CIN genes, but only a few have been identified so far. These genes include hBUB1, MAD2, BRCA1, BRCA2 and hCDC4 (Cahill *et al.* 1998; Wang *et al.* 2004). hBUB1 and MAD2 are required for the proper functioning of the spindle assembly checkpoint (Li & Benezra 1996; Pangilinan *et al.* 1997). This checkpoint modulates the timing of anaphase initiation in mitotic cells containing improperly aligned chromosomes and increases the probability of successful delivery of a correct chromosome set to each daughter cell. BRCA1

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One contribution of 17 to a Discussion Meeting Issue 'Chromosome segregation'.

Table 1. Experimental evidence of aneuploidy in early tumours.

(The table shows the percentage of early colorectal adenomas (size 1–3 mm, total number 32) with allelic imbalance in five different chromosome arms. Ninety per cent of the adenomas have allelic imbalance in any one of the five chromosome arms. Data from Shih *et al.* (2001).)

chromosome arm	adenomas with allelic imbalance (%)
1p	10
5q	55
8p	19
15q	28
18q	28
any of these 5	90

and BRCA2 are involved in DNA repair and recombination, checkpoint control of the cell cycle and transcription (Milner *et al.* 1997; Yarden *et al.* 2002). Inherited mutations in BRCA1 and BRCA2 lead to high-grade familial breast cancer (Vogelstein & Kinzler 2001). hCDC4 is an E3 ubiquitin ligase that is involved in regulating the G1–S cell-cycle checkpoint by targeting proteins for destruction (Rajagopalan & Lengauer 2004b; Rajagopalan *et al.* 2004). Inactivation of hCDC4 leads to increased levels of cyclin E, the formation of micronuclei, defects in the execution of anaphase and CIN. Furthermore, the DING gene was found to be somatically mutated in eight out of 23 CIN cancers (Wang *et al.* 2004). DING is a previously uncharacterized gene whose COOH terminus is homologous with the yeast protein Pds1. Pds1 is an anaphase inhibitor in budding yeast and plays a critical role in the control of anaphase (Nasmyth *et al.* 2000; Yanagida 2000). Its human orthologue, hSecurin, is essential for the proper function and processing of the separin protease, for separin-dependent cleavage of the cohesin subunit Scc1, and for maintaining chromosome stability (Jalle-palli *et al.* 2001).

The classification of CIN genes is based on the mutational events required to trigger CIN (Michor *et al.* 2004). Class I CIN genes, such as MAD2, cause 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. Both classes I and II are 'single hit' CIN genes. Class III CIN genes, such as BRCA1 and BRCA2, cause CIN if both alleles are mutated.

A major question in cancer genetics is whether CIN, or any genetic instability, is an early event and hence a driving force of tumorigenesis (Loeb *et al.* 1974; Breivik & Gaudernack 1999; Tomlinson & Bodmer 1999; Shih *et al.* 2001; Marx 2002; Rajagopalan *et al.* 2004). Experimental evidence shows that early colorectal adenomas have allelic imbalance (table 1; Shih *et al.* 2001), but this provides no proof of underlying CIN. hCDC4 mutations have been shown to occur early in colorectal tumorigenesis (Rajagopalan *et al.* 2004). Here, we discuss a mathematical framework for the evolutionary dynamics of tumorigenesis offering insights into the causal relations between CIN and curves initiated by inactivation of one or two TSGs.

Tissues are organized into small compartments of
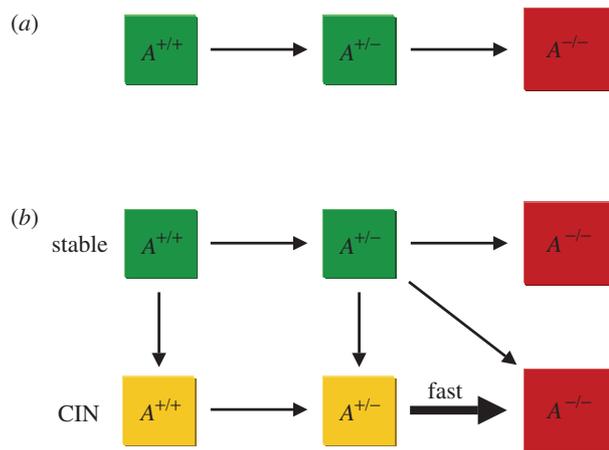
Figure 1. Inactivation dynamics of a tumour suppressor gene (TSG), A, without and with chromosomal instability (CIN) in a compartment of cells. (*a*) Initially, all cells are wild-type with respect to A, $A^{+/+}$. Once a cell with one inactivated allele of A emerges and reaches fixation in the compartment, the compartment moves to the state where all cells have one inactivated allele of A, $A^{+/-}$. Only then does a cell with two inactivated alleles of A emerge and reach fixation in the compartment; the compartment moves to the state where all cells have two inactivated alleles of A, $A^{-/-}$. (*b*) CIN can emerge at any stage of tumorigenesis because of mutation of a CIN gene (single hit CIN gene shown here) and increases the rate at which the second allele of a TSG is inactivated. If an $A^{+/-}$ cell with CIN produces an $A^{-/-}$ cell with CIN before reaching fixation in the compartment, a stochastic tunnel arises (diagonal arrow): the compartment moves from state $A^{+/-}$ without CIN to $A^{-/-}$ with CIN without ever visiting $A^{+/-}$ with CIN.

cells (Mintz 1971; Cairns 1975; Bach *et al.* 2000; Yatabe *et al.* 2001). Not all cells of a compartment need be at risk of becoming cancer cells, however, because certain mutations only have an effect if they occur in stem cells (Michor *et al.* 2003, 2004b). Suppose N_0 cells are at risk in any one compartment. Consider a path to cancer initiated by activation of both alleles of a TSG, A. Initially, all cells of the compartment are wild-type with respect to TSG A, $A^{+/+}$. If the mutation rates inactivating the alleles of A are less than the inverse of N_0, then the approximation of homogeneous compartments holds: a mutated cell will either take over the compartment or go extinct before the next mutation arises (Nowak *et al.* 2002; Komarova *et al.* 2003; Michor *et al.* 2004a). Hence the compartment evolves from the state where all cells have two wild-type alleles, $A^{+/+}$, via the state where all cells have one inactivated allele, $A^{+/-}$, to the state where all cells have two inactivated alleles, $A^{-/-}$ (figure 1a).

CIN can emerge at any stage of tumorigenesis (figure 1b). First, assume that CIN is dominant, i.e. triggered by mutation of a class I or II CIN gene (Nowak *et al.* 2002; Michor *et al.* 2004a). The crucial effect of CIN is to increase the rate of LOH, thereby accelerating the transition from $A^{+/-}$ to $A^{-/-}$. CIN can have a cost for the cell by increasing the rate of accumulating lethal mutations and triggering apoptosis. However, CIN can also be advantageous by reducing the duration of the cell cycle or by providing increased evolvability in detrimental environments (Bardelli *et al.* 2001; Breivik 2001). We therefore
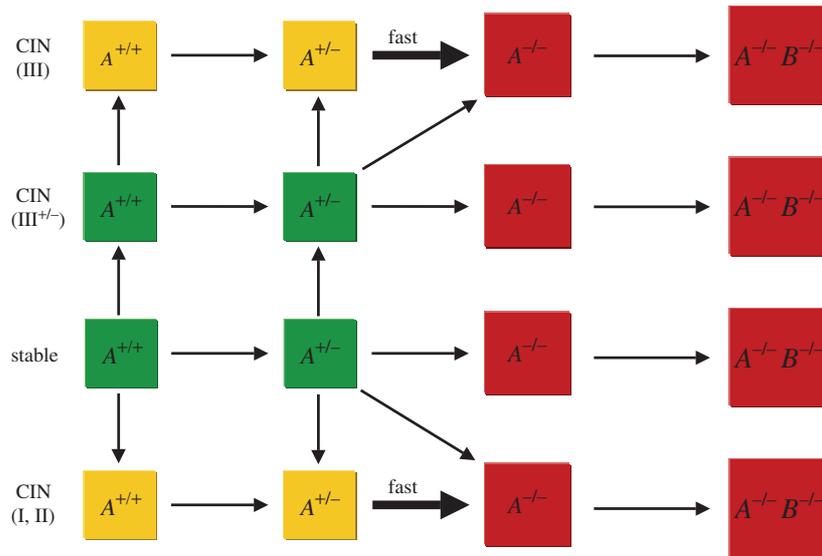
Figure 2. Inactivation dynamics of two tumour suppressor genes (TSGs), A and B, without and with chromosomal instability (CIN) in a compartment of N_0 cells. Initially, all cells are wild-type. Suppose TSG A has to be inactivated first. Then the compartment evolves from the state where all cells have two wild-type alleles of A, $A^{+/+}$, via the state where all cells have one inactivated allele of A, $A^{+/-}$, to the state where all cells have two inactivated alleles of A, $A^{-/-}$. Inactivation of A leads to clonal expansion of the compartment to N_1 cells. It is in this small lesion that TSG B is inactivated, and the compartment moves to the state where all cells have two inactivated alleles of both A and B, $A^{-/-}B^{-/-}$. CIN can emerge at any stage because of mutation of a class I, II or III CIN gene and leads to rapid LOH of the second allele of TSGs A and B.

Table 2. Minimum number of class I, II and II CIN genes (n_1, n_2 and n_3, respectively) required for emergence of CIN before the inactivation of the first tumour suppressor gene (TSG). (The relative fitness value of CIN cells is denoted by r. Parameter values are $N_0 = 4$, $u = 10^{-7}$, $p_0 = 10^{-6}$, $p = 10^{-2}$, $\tau_0 = 10$ days and $\tau_1 = 1$ day.)

CIN before 1 TSG			CIN before 2 TSGs					
independent of N_1			$N_1 = 10^4$			$N_1 = 10^5$		
r	n_1	n_2	n_3	n_1	n_2	n_3	n_1	n_2	n_3
1.0	2	12	89	1	1	16	1	2	37
0.9	2	14	96	1	1	17	1	3	41
0.5	4	42	169	1	2	35	1	8	74

Table 3. Percentage of tumours initiated by inactivation of one or two tumour suppressor genes (TSGs) in which CIN emerges before the inactivation of the first TSG. (The number of class I, II and III CIN genes is given by n_1, n_2 and n_3, respectively. The relative fitness value of CIN cells is denoted by r. Parameter values are $N_0 = 4$, $N_1 = 10^{-4}$, $u = 10^{-7}$, $p_0 = 10^{-6}$, $p = 10^{-2}$, $\tau_0 = 10$ days and $\tau_1 = 1$ day.)

CIN genes	r	CIN before 1 TSG (%)	CIN before 2 TSGs (%)
$n_1 = 1$	1.0	50	97
	0.9	46	97
	0.5	21	88
$n_2 = 5$	1.0	31	94
	0.9	28	93
	0.5	11	76
$n_3 = 10$	1.0	1.4	31
	0.9	1.2	26
	0.5	0.4	7.7

consider costly, neutral and advantageous CIN phenotypes. If CIN is costly, then the phenomenon of 'stochastic tunnelling' arises (Nowak *et al.* 2002; Komarova *et al.* 2003; Iwasa *et al.* 2004): the compartment moves from $A^{+/-}$ without CIN to $A^{-/-}$ with CIN without ever visiting $A^{+/-}$ with CIN.

Suppose that two TSGs, A and B, have to be inactivated consecutively for tumorigenesis (figure 2). The compartment evolves from $A^{+/+}B^{+/+}$ via $A^{+/-}B^{+/+}$ to $A^{-/-}B^{+/+}$, and subsequently to $A^{-/-}B^{+/-}$ and $A^{-/-}B^{-/-}$. CIN can emerge at any stage of tumorigenesis owing to mutations of class I, II or III CIN genes. Once CIN has emerged, it accelerates the transitions from $A^{+/-}$ to $A^{-/-}$ and from $B^{+/-}$ to $B^{-/-}$. A stochastic tunnel arises if the compartment moves from $A^{+/-}B^{+/+}$ without CIN to $A^{-/-}B^{+/+}$ with CIN without ever visiting $A^{+/-}B^{+/+}$ with CIN. Inactivation of the first TSG can induce neoplastic growth. We assume that the $A^{-/-}$ compartment gives

rise to a small lesion of N_1 cells. In this lesion, TSG B has to be inactivated for further tumour progression. Owing to the increased compartment size, the evolutionary pathway might tunnel from $A^{-/-}B^{+/+}$ directly to $A^{-/-}B^{-/-}$. Mathematical procedures are outlined in Appendix A.

The importance of early CIN in tumorigenesis depends on the number of possible CIN mutations, which in turn depends on the number of CIN genes in the human genome. We can calculate the minimum number of CIN genes in the genome that are needed to ensure that in a significant fraction of tumours, a CIN mutation precedes the inactivation of one or two TSGs. Table 2 provides examples for the critical number of class I, II and III CIN genes (n_1, n_2 and n_3, respectively)

depending on the relative fitness value of CIN cells (r) and the compartment size after inactivation of TSG A (N_1). Table 3 shows the fraction of cancers initiated by a CIN mutation depending on r and n_1, n_2 and n_3.

In summary, one or a few neutral CIN genes are enough to ensure that CIN initiates tumour formation in a pathway where one TSG needs to be eliminated in a rate limiting situation. One or a few costly CIN genes are enough to ensure that CIN initiates tumour formation in a pathway where two TSGs need to be eliminated in rate limiting situations.

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APPENDIX A

The stochastic process illustrated in figures 1*b* and 2 can be described by differential equations. Denote by X_0, X_1, X_2 and X_4 the probabilities that a compartment is in state $A^{+/+}B^{+/+}$, $A^{+/-}B^{+/+}$, $A^{-/-}B^{+/+}$ and $A^{-/-}B^{-/-}$, respectively, without chromosomal instability (CIN). Denote by Y_0, Y_1, Y_2 and Y_4 the probabilities that a compartment is in state $A^{+/+}B^{+/+}$, $A^{+/-}B^{+/+}$, $A^{-/-}B^{+/+}$ and $A^{-/-}B^{-/-}$, respectively, with CIN. The differential equations are given by

$$\dot{X}_0 = X_0(-r_0 - c_0) \qquad \dot{Y}_0 = c_0 X_0 - s_0 Y_0$$
$$\dot{X}_1 = r_0 X_0 - X_1(r_1 + c_0 + c_1) \quad \dot{Y}_1 = s_0 Y_0 + c_0 X_1 - s_1 Y_1$$
$$\dot{X}_2 = r_1 X_1 - r_2 X_2 \qquad \dot{Y}_2 = s_1 Y_1 + c_1 X_1 - s_2 Y_2$$
$$\dot{X}_4 = r_2 X_2 \qquad \dot{Y}_4 = s_2 Y_2.$$
$$(A1)$$

The transition rates between the states are given by $r_0 = 2u/\tau_0$, $r_1 = N_0(u + p_0)/\tau_0$, $r_2 = N_1 2u\sqrt{u + p_0}/\tau_1$, $c_0 = N_0 u_c \rho/\tau_0$, $c_1 = [N_0 u_c r/(1 - r)][p/\tau_0]$, $s_0 = r_0$, $s_1 = N_0 p/\tau_0$ and $s_2 = rN_1 2u\sqrt{p}/\tau_1$ (Nowak *et al.* 2002; Komarova *et al.* 2003; Michor *et al.* 2004a). Cells with genotype $A^{+/+}B^{+/+}$ and $A^{+/-}B^{+/+}$ divide every τ_0 days, while cells with genotype $A^{-/-}B^{+/+}$ and $A^{-/-}B^{+/-}$ divide every τ_1 days.

CIN can arise at any stage of tumorigenesis and causes very fast LOH. The mutation rate triggering CIN, u_c, depends on the number of class I, II and III CIN genes in the human genome, n_1, n_2 and n_3, respectively. The mutation rates of class I and II CIN genes are $u_c = 2n_1(u + p_0)$ and $u_c = 2n_2 u$, respectively. The mutation rate of the first allele of class III CIN genes is $2n_3 u$, and the mutation rate of the second allele of class III CIN genes is $u = p_0$. The probability of fixation of a CIN cell depends on its somatic fitness, r, and is given by $\rho = (1 - 1/r)/(1 - 1/r^{N_0})$ (Nowak *et al.* 2003).

Equation (A1) is a system of linear differential equations which can be solved analytically using standard techniques.

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