

Genetic instabilities in human cancers

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Whether and how human tumours are genetically unstable has been debated for decades. There is now evidence that most cancers may indeed be genetically unstable, but that the instability exists at two distinct levels. In a small subset of tumours, the instability is observed at the nucleotide level and results in base substitutions or deletions or insertions of a few nucleotides. In most other cancers, the instability is observed at the chromosome level, resulting in losses and gains of whole chromosomes or large portions thereof. Recognition and comparison of these instabilities are leading to new insights into tumour pathogenesis.

It is now widely accepted that cancer results from the accumulation of mutations in the genes that directly control cell birth or cell death. But the mechanisms through which these mutations are generated are the subject of continuing debate. It has been argued that an underlying genetic instability is absolutely required for the generation of the multiple mutations that underlie cancer^{1,2}. However, it has also been suggested that normal rates of mutation, coupled with waves of clonal expansion, are sufficient for the process to occur in humans³. Here we review observations relating to the stability of the genome of human cancer cells. Although these observations do not prove that genetic instability is necessary for a tumour to develop, they strongly support the existence of two levels of instability, one or the other of which is affected in the vast majority of cancers.

Types of genetic alterations in tumours

Numerous genetic alterations that affect growth-controlling genes have been identified in neoplastic cells over the past 15 years, providing persuasive evidence for the genetic basis of human cancer. The alterations can be divided into four major categories (Fig. 1).

Subtle sequence changes. These changes involve base substitutions or deletions or insertions of a few nucleotides (Fig. 1a, b), and, unlike the alterations described below, they cannot be detected through cytogenetic analysis. For example, missense mutations in the *K-ras* gene occur in over 80% of pancreatic cancers⁴.

Alterations in chromosome number. Alterations in chromosome number involve losses or gains of whole chromosomes (aneuploidy; Fig. 1c). Such changes are found in nearly all major human tumour types⁵. Examples include the loss of chromosome 10 in glioblastomas, often reflecting the inactivation of the tumour-suppressor gene *PTEN*⁶, and the gain of chromosome 7 in papillary renal carcinomas, reflecting a duplication of a mutant *MET* oncogene⁷.

Chromosome translocations. These alterations can be detected cytogenetically as fusions of different chromosomes or of normally non-contiguous segments of a single chromosome (Fig. 1d). At the molecular level, such translocations can give rise to fusions between two different genes, endowing the fused transcript with tumorigenic properties. An example is provided by the Philadelphia chromosome in chronic myelogenous leukaemias; the carboxy terminus of the *c-abl* gene on chromosome 9 is joined to the amino terminus of the *BCR* gene on chromosome 22 (ref. 8).

Gene amplifications. Gene amplifications are seen at the cytogenetic level as homogeneously stained regions or double minutes (Fig. 1e). At the molecular level, multiple copies of an 'amplicon' containing a growth-promoting gene(s) can be seen. The amplicons contain 0.5–10 megabases of DNA, and are different from the duplications of much larger chromosome regions that result from aneuploidy and translocations⁹. An example is the amplification of

N-myc that occurs in ~30% of advanced neuroblastomas¹⁰.

Differences between 'state' and 'rate'

All four of the alterations described above occur commonly in specific tumour types and are rarely or never observed in normal cells. But the existence of genetic alterations in a tumour, even when frequent, does not mean that the tumour is genetically unstable. Instability is, by definition, a matter of rate, and the existence of a mutation (state) provides no information about the rate of its occurrence. Several factors in addition to a true instability could explain a higher prevalence of mutations in tumours compared with normal cells. The selective conditions in the tumour environment, including aberrant humoral, cell–substratum and cell–cell interactions, are different than those in the environment of normal cells. As a result, mutations in growth-controlling genes could occur at the same rate in tumour cells as in normal cells, but the selective advantage posed by such a mutation in the tumour cells' environment would give rise to clonal expansion, allowing the tumour cell with the mutation to overtake its sister cells. The same mutation occurring in a normal cell, in the absence of clonal expansion, would be undetectable, as it would be masked by millions of sister cells without the mutation. It is also possible that normal cells undergoing mutations are destroyed by apoptosis, a pathway leading to programmed cell death that may protect the organism from the tumorigenic consequences of such mutations. Indeed, mutations in several oncogenes lead to growth stimulation under some circumstances but to apoptosis under others (see, for example, ref. 11).

Microorganisms can be used to illustrate the differences between mutation rate and state. Consider two yeast strains, A and B, each of which initially contains a wild-type gene, *X*. A and B are grown separately in different media. After growth for an equivalent number of generations, it is found that a mutation of gene *X* is present only in A cells. It cannot be concluded that the rate of mutation of gene *X* is higher in A cells than in B cells. The selective conditions operative in the media in which A cells were grown might simply have favoured growth of cells with mutations in gene *X*, even though such mutations arose at equal rates in both cell types. Another alternative is that strain A had a pre-existing mutation in another gene that allowed it to survive in the presence of mutant gene *X*, just as the multiple mutations found in tumours can function cooperatively.

A further complication results from the dynamic nature of tumours. Cancers generally take decades to develop, and it is important to determine whether a genetic instability, even if present at some stage of tumorigenesis, persists. As it is nearly impossible to exclude the occurrence of a transient state of instability during an undefined period of a tumour's history, we will restrict our discussion of instabilities to those that endure throughout the lifetime of

the tumour cell and which can therefore be analysed experimentally.

Subtle sequence instabilities

The first type of instability to be quantitatively assessed in neoplastic cells involved subtle sequence changes that alter one or a few base pairs. Instabilities of this type are uncommon in human cancers, but, when present, they cause dramatic phenotypes.

The molecular mechanisms underlying the fidelity of DNA replication have been summarized^{12,13}. Two separate processes control the error rate—DNA polymerization (and associated proof-reading by the polymerases) and repair. The repair machinery operates on sequence errors generated by polymerases or by mutagens. No consistent pattern of defects in polymerases has been seen in tumours. However, defects in two of the major repair systems—nucleotide-excision repair and mismatch repair—have been well documented.

Nucleotide-excision repair. Nucleotide-excision repair (NER) is responsible for repairing damage caused by many exogenous mutagens. The role of NER in cancer first came to light through

the study of xeroderma pigmentosum and related disorders¹⁴. Patients with these autosomal recessive, inherited diseases develop numerous skin tumours in sun-exposed areas. Fibroblasts from xeroderma pigmentosum patients are sensitive to ultraviolet light, and the patterns of such sensitivity, as well as the sensitivities found in fusions of cells from different patients, indicated that several different genes were likely to be involved¹⁵. This prediction was confirmed through biochemical and genetic experiments which eventually led to the identification of the NER genes, mutations in which result in these disorders¹⁶.

The biochemistry of NER has been reviewed elsewhere¹⁷, and here we focus on the relationship of NER defects to neoplasia. Skin tumours represent the major tumour type to which patients with NER defects are susceptible; the incidence of internal cancers in patients with NER defects is not raised to the same degree^{18–20}. This is surprising, because NER can correct covalent modifications of DNA resulting from various chemical mutagens, and fibroblasts from xeroderma pigmentosum patients are more sensitive than normal cells to such compounds¹⁶. The simplest explanation for these results is that ultraviolet light is the major mutagen that results in NER-correctable DNA damage to which humans are exposed^{18–20}. The interpretation offers support for the idea that environmental agents (other than ultraviolet light) are not rate-limiting for the development of most common cancers²¹.

It is interesting that xeroderma pigmentosum heterozygotes are at no higher risk for neoplasia than the general population¹⁶. The reason for this is discussed below.

Mismatch repair. Mismatch repair (MMR) was discovered in prokaryotes long ago but has been shown to be involved in cancer only within the past five years. The first clue to the role of MMR in cancer came with the discovery of a group of sporadic (non-familial) colorectal tumours that exhibited widespread alterations of poly(A) tracts in their genomes^{22,23}. Subsequent studies showed that other such 'microsatellites', including poly(CA) repeats, were similarly affected, giving rise to the term 'microsatellite instability' (MIN)^{23,24}. Although it is unusual in sporadic colorectal cancers (CRCs), MIN occurs in most cancers in patients with hereditary non-polyposis colorectal cancer (HNPCC)²⁵. The loci responsible for HNPCC were linkage-mapped to chromosomes 2p16 and 3p21 (refs 26, 27). These results indicated that HNPCC patients might inherit a replication or repair gene that was somatically mutated in the sporadic CRC cases associated with MIN, and that two such genes may reside on human chromosomes 2 and 3 (refs 23, 25–27).

None of the initial reports on MIN proposed a specific repair system that might be responsible for the instability. Strand *et al.*²⁸ were the first to suggest that this phenotype might result from defective mismatch repair: they noted that microsatellite instability had been observed in bacteria with defects in the mismatch-repair genes *mutS* or *mutL*, and showed that *Saccharomyces cerevisiae* with defects in the yeast homologues of either *mutS* or *mutL* exhibited a similar MIN phenotype. This hypothesis²⁸ was confirmed by the identification of the *mutS* homologue *hMSH2* on human chromosome 2 (ref. 29) and the demonstration of inactivating mutations in this gene in chromosome-2-linked HNPCC kindreds³⁰. A *mutL* homologue (*hMLH1*) on chromosome 3 was found to be mutant in the germ line of chromosome-3-linked HNPCC kindreds^{31,32}. This genetic evidence was strongly supported by studies showing that tumours exhibiting MIN lacked detectable MMR activity in biochemical assays^{33,34}. Six human *mutS* or *mutL* homologues are now known which, when inactivated by mutation, lead to a MIN phenotype in cancer patients³⁵.

The biochemistry of MMR has been reviewed^{36,37}, so we focus here on what has been learnt about neoplasia from studies of defects in MMR. Perhaps the most important of these lessons concerns the dominant inheritance of HNPCC. In general, patients with HNPCC have one normal allele of the relevant MMR gene; this wild-type allele is sufficient to maintain normal levels of MMR³³. It is only

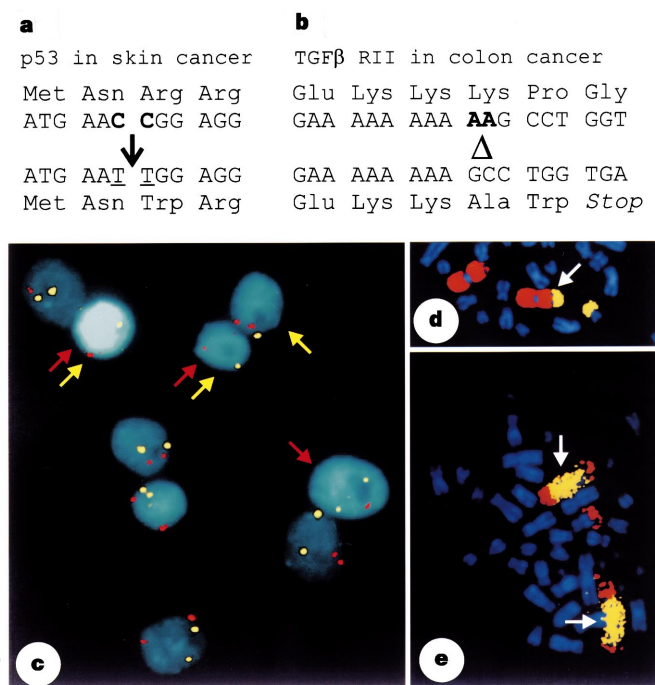


Figure 1 Examples of genetic alterations in cancer. **a**, **b**, Subtle sequence alterations: **a**, mutation at a dipyrimidine site (bold letters) of the *p53* gene (codons 247–248) found in a xeroderma pigmentosum patient with a defect in nucleotide-excision repair (NER)³⁴; **b**, a two-base deletion located within a sequence of ten repeating adenines of the transforming growth factor- β receptor II (*TGF β RII*) gene (codons 125–128) in a colorectal cancer cell line with mismatch-repair (MMR) deficiency³⁵. **c**, Gross chromosomal change. Loss of chromosomes 3 (red arrows) and 12 (yellow arrows) in colorectal cancer (CRC) cells. A clone of the CRC cell line SW837 was expanded through 25 generations before fluorescence *in situ* hybridization (FISH). Interphase nuclei were hybridized with labelled centromeric DNA probes specific for chromosome 3 (red spots) and chromosome 12 (yellow spots). The number of signals detected in SW837 cells was diverse, indicating CIN; normal cells, as well as cancer cells exhibiting MIN, had two red and two yellow signals in nearly every nucleus⁴⁶. **d**, Chromosome translocation. A metaphase plate of the neuroblastoma cell line GIMEN was hybridized by FISH with labelled whole-chromosome-painting probes specific for chromosome 1 (red) and chromosome 17 (yellow), revealing a t(1;17) translocation (arrow). **e**, Gene amplification. FISH with a *N-myc* probe (yellow) and a whole-chromosome-painting probe specific for chromosome 1 (red) revealed an area of *N-myc* amplification (arrow) within the derivative chromosomes 1 of the neuroblastoma cell line Kelly.

when the wild-type allele is inactivated during tumorigenesis—through a gross chromosomal event or a subtle intragenic mutation—that MMR is abolished and mutations accumulate (Fig. 2). The progeny of this cell then accumulate mutations in oncogenes and tumour-suppressor genes, thus resulting in clonal expansion (that is, tumorigenesis). These results and analogous MMR gene mutations in sporadic tumours unambiguously show that somatic mutations in repair genes can be selected for during tumorigenesis, even when such mutations do not directly enhance cell growth.

Although patients heterozygous for a MMR-gene mutation are tumour-prone^{30,35}, patients heterozygous for a NER-gene mutation are not¹⁶. One possible explanation for this difference involves the extra step required to lead to the accumulation of mutations in the NER-gene heterozygotes. In a heterozygote with one defective MMR allele, all that is required to begin to accumulate mutations at a high rate is the inactivation of the normal allele inherited from the unaffected parent. This inactivation immediately leads to uncorrected errors during normal DNA replication. In a heterozygote with one defective NER allele, however, inactivation of the normal allele does not always lead to a high mutation rate. Exposure to an environmental agent (that is, ultraviolet light) is required to engender such mutations (Fig. 2).

How frequently do MMR defects occur in human neoplasms? Roughly 13% of colorectal cancers and a similar percentage of endometrial and gastric cancers contain such defects³⁸. Cancers of other types are rarely (<2%) MMR-deficient. Although many cancers may contain a few microsatellite changes, the extent of these changes is much less pronounced than in MMR-deficient cancers. These low levels of microsatellite alterations are likely to reflect near-normal rates of mutation during the multiple rounds of

cellular division that accompany the waves of clonal expansion through which advanced cancers evolve³⁹.

Do cancers that do not exhibit MMR or NER deficiency have other replication or repair abnormalities that render them prone to subtle mutations? The answer is, probably not. Assays that detect such subtle mutations (base substitutions or small deletions or insertions) have been used to study several tumour-cell lines. The mutation rates in such lines have, in general, been found to be roughly equal to those in normal cell lines, with the exception of tumour lines with reported defects in MMR or NER^{1,18}. However, it is difficult to compare mutation rates among tumour and normal cell lines, which grow and age at different rates and are often grown under different conditions. A more rigorous comparison can be made between MMR-proficient and MMR-deficient colorectal cancer cell lines grown under identical conditions. There is a dramatic difference in the mutation rates between these two types of cancer, with the MMR-proficient tumours exhibiting mutation rates similar to those observed in normal cells^{40,41} (Fig. 3).

Chromosome number instabilities

The subtle sequence instabilities represented by NER-associated instability (NIN) and MIN are rare, but another form of instability, involving gains and losses of whole chromosomes, is likely to occur in most human malignancies⁵. Karyotypic studies have shown that the majority of cancers have lost or gained chromosomes, and molecular studies indicate that karyotypic data actually underestimate the true extent of such changes. Losses of heterozygosity, that is, losses of a maternal or paternal allele in a tumour, are widespread and are often accompanied by a gain of the opposite allele. A tumour could lose the maternal chromosome 8, for example, while duplicating the paternal chromosome 8, leaving the cell with a normal chromosome 8 karyotype but an abnormal chromosome 8 'allelotype'⁴². The 'average' cancer of the colon, breast, pancreas or prostate may lose 25% of its alleles^{42–45} and it is not unusual for a tumour to have lost over half of its alleles (Fig. 4).

Such wholesale changes in the genome of cancer cells might be thought to be due to a true chromosomal instability (CIN), but, as mentioned above, static karyotypes and allelotypes cannot be used to determine rates of genetic alteration. Two types of experiments, however, have shown that these cancers do indeed exhibit a true instability that persists throughout the tumour's lifetime. In one study, fluorescence *in situ* hybridization was used to show that losses or gains of multiple chromosomes occurred 10–100 times more often in aneuploid colorectal cancer cell lines than in normal cells or in diploid cancers of the same histological subtype⁴⁶. In another approach, the rate of loss of heterozygosity of markers surrounding a selectable gene was found to be increased tenfold in a MMR-proficient colorectal cancer cell line compared with a MMR-deficient line⁴⁷. The high rates of chromosome losses and gains seen in these quantitative analyses were consistent with previous observations showing that not only are cancers aneuploid, but that the karyotype of a single tumour is often heterogeneous, reflecting the persistent generation of new chromosomal variations⁵. It has also been shown that the high rate of chromosome gains/losses in aneuploid cancers is not due simply to the ability of aneuploid cells to survive such changes in chromosome number⁴⁶.

Relationship between MIN and CIN. In colorectal and endometrial cancers, there is an inverse relationship between CIN and MIN. Cancers showing MMR deficiency are, in general, diploid and exhibit normal rates of gross chromosomal change, whereas MMR-proficient tumours are usually aneuploid and exhibit increased rates of chromosomal change in the same assays⁴⁶ (Fig. 4). Two other comparisons between MIN and CIN tumours are also informative. First, both types of instability seem to occur early during tumour evolution, but the genetic variations resulting from these instabilities increase with tumour progression. In the case of CIN, aneuploidy can often be observed in small benign

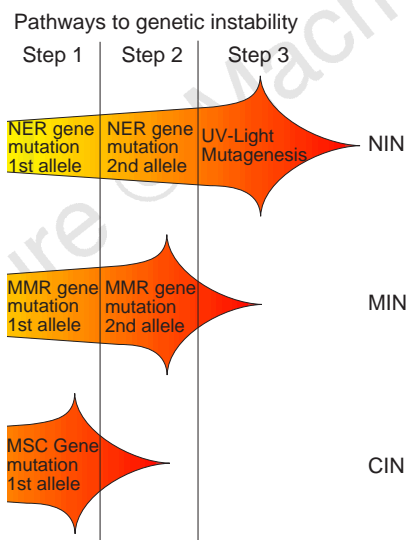


Figure 2 Pathways to genetic instability. Different types of genetic instability require a different number of mutational 'hits' to produce the respective instability phenotype. Top, in a heterozygote with one defective NER allele (step 1), inactivation of the second, normal allele (step 2) does not immediately lead to mutations. Exposure to an environmental agent (ultraviolet light; step 3) is also required to create large numbers of mutations (NER-related instability; NIN). Centre, in contrast, in a heterozygote with one defective MMR allele (step 1), all that is required to begin to develop mutations at a high rate (microsatellite instability, MIN) is the inactivation of the normal allele inherited from the unaffected parent (step 2). Bottom, results of cell fusion and other experiments (see text) indicate that chromosomal instability (CIN) can have a dominant quality. One example of a gene that can be mutated in a dominant-negative manner to cause CIN is *hBUB1*, a component of the mitotic spindle checkpoint (MSC)⁶⁶. Apparently only a single mutational 'hit' of such a gene is required to produce the CIN phenotype.

tumours of the colon or breast, and the deviations from a normal karyotype increase as the tumours enlarge in size and eventually become malignant^{48–50}. Similarly, the microsatellite instability that is the signature of MMR deficiency is seen early during tumorigenesis and increases as the tumours progress in size and disorganization⁵¹. Such increases with progression are expected during clonal expansion, with each new wave of expansion originating in a cell that has divided many times since the last wave.

Second, when MIN and CIN cells are fused, the resulting hybrids (at least in the two cases studied so far; see ref. 46 and C.L., K.W.K. and B.V., unpublished data) exhibit CIN but not MIN. The complementation of MIN by CIN cells is expected, given that expression of the wild-type MMR gene in the hybrid should restore MMR function⁵². The inability of the MIN cells to complement the CIN phenotype, however, shows that the CIN phenotype has a dominant quality, indicating that it may result from gain of function of an expressed protein rather than from gene inactivation. This, in turn, suggests that only a single mutational 'hit' may be required to produce the CIN phenotype (Fig. 2).

Molecular basis of CIN. The molecular basis of CIN in tumours is just beginning to be explored. Because aneuploidy (and, presumably, an underlying CIN) is nearly ubiquitous in cancers⁵ as well as in transformed cells *in vitro*⁵³, it could be argued that CIN results simply from the abnormal structure and growth properties of the cancer cell. However, the fact that CIN generally does not occur in cancer cells with the MIN phenotype, and that tetraploid cells resulting from the fusion of two MIN cell lines remain chromosomally stable, argues strongly against any nonspecific factors⁴⁶.

One potential explanation for CIN invokes p53 mutation. For example, cells in culture often become grossly aneuploid at the same time that p53 is inactivated⁵⁴, and abnormal spindles and arrest in the G2/M phase of the cell cycle occur in p53-deficient cells^{55,56}. It is unlikely, however, that p53 is generally responsible for CIN, as several cancer lines with p53 mutations are diploid and chromosomally stable (and exhibit MIN because of MMR deficiency)^{46,57}. Furthermore, inactivation of p53 by targeted homologous recombination in a non-CIN cancer line has no measurable effect on chromosomal stability (F. Bunz and C.L., unpublished data). Most important, aneuploidy and CIN appear early during tumorigenesis^{48–50}, whereas p53 mutations do not usually occur until much later^{58,59}. These results indicate that although p53 mutations may exacerbate chromosome instability, they are unlikely to be its primary cause.

The critical clue to understanding the molecular basis of MIN in human cancers was provided by studies of unicellular organisms with MMR deficiency (see above). Analogously, the molecular basis for CIN is likely to be unravelled through studies of chromosome

instabilities in yeast and other organisms. However, in contrast to MIN, where only a few genes give rise to the phenotype, a large number of gene alterations can give rise to CIN in yeast^{60–63}. Genes that, when altered, can lead to CIN include those involved in chromosome condensation, sister-chromatid cohesion, kinetochore structure and function and centrosome/microtubule formation and dynamics, as well as 'checkpoint' genes that monitor the proper progression of the cell cycle^{62–65} (Fig. 5).

One type of checkpoint, the spindle checkpoint, ensures that chromatids do not separate until the chromosomes are aligned appropriately along the mitotic spindle. In yeast, disruption of genes encoding spindle-checkpoint proteins can lead to CIN because checkpoint-defective cells can complete mitosis in the presence of a lagging chromosome, resulting in abnormal chromosome segregation^{62–65}. Two lines of evidence have indicated that abnormal spindle-checkpoint genes might be responsible for CIN in human cancers. First, some aneuploid (CIN) lines, but not diploid (MIN) lines, responded aberrantly to spindle-disrupting agents such as nocodazole and colcemid. Instead of undergoing a relatively long-term arrest in metaphase, the CIN lines appeared to exit mitosis prematurely and begin another round of DNA synthesis⁶⁶. The inability to inhibit entry into S phase when mitosis cannot be completed because of spindle damage is the hallmark of a spindle-checkpoint defect^{62–65}. Second, alterations in the expression or sequence of human mitotic-checkpoint genes have been detected in human cancers. For example, decreased expression of *hMAD2* has been seen in breast cancers and might be important in aneuploidy⁶⁷. A small fraction of colorectal cancers have been shown to contain somatic mutations of either *hBUB1* or *hBUBR1* (ref. 66). Mutations in *BUB1* can function in a dominant-negative manner in both mouse and human cells, conferring an abnormal spindle checkpoint when expressed exogenously^{66,68}. In addition, *hMAD1* is a protein that is targeted by the Tax protein of the human T-cell leukaemia virus type 1; this targeting process inactivates the spindle checkpoint in virally induced leukaemias⁶⁹.

Another type of checkpoint, the DNA-damage checkpoint, prevents cells with DNA damage from entering mitosis. Such DNA damage can result from mistakes made by polymerases during normal DNA replication, from exogenous sources (such as ionizing radiation), from endogenous genotoxins (such as reactive oxygen species), or from incomplete repair. Chromosomes containing damaged DNA could segregate inappropriately because sister chromatids are still connected by DNA or DNA–protein links. Such chromosomes are also susceptible to gross structural alterations because of single-stranded gaps or double-stranded-DNA breaks. Studies of yeast have shown that the chromosome instability arising from failed DNA-damage checkpoints is often associated with

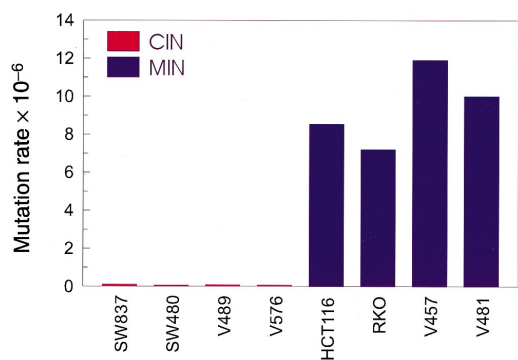


Figure 3 Increased mutation rates at the *HPRT* locus in MIN versus CIN cancer cell lines (ref. 41 and J. Eshleman, M. Veigl, D. Sedwick and S. Markowitz, personal communication). Mutation rates are expressed as 10⁻⁶ mutations per locus per generation.

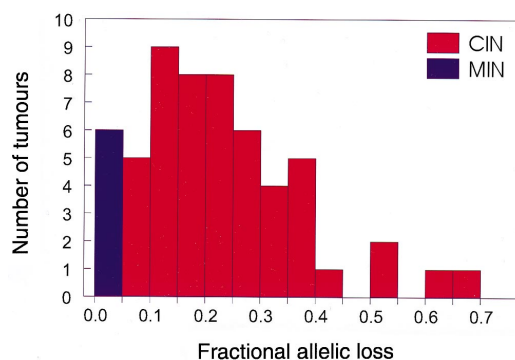


Figure 4 Frequency of allelic losses in MIN versus CIN cancers. Allelic losses were studied by restriction fragment length polymorphisms and Southern blotting. The fractional allelic loss in each tumour is defined as the number of chromosomal arms on which allelic loss was observed divided by the number of chromosomal arms for which allelic markers were informative⁴².

enhanced mitotic recombination as well as with aberrant chromosome segregation, whereas that arising from spindle-checkpoint defects is associated largely with aberrant chromosome segregation⁷⁰. Several genes involved in the DNA-damage checkpoint have been implicated in human tumorigenesis, including *ataxia telangiectasia mutated* (ATM)⁷¹, the ATM-related gene *ATR*⁷², the *BRCA1* and *BRCA2* genes, which interact with the human Rad51 homologue⁷³, and *p53* (ref. 74).

Another potential cause of aneuploidy involves abnormal centrosomes. Multipolar spindles have often been observed in human cancers *in situ* and an abnormal number of centrosomes, detected with specific antibodies, has been observed in breast, lung, prostate, colon, and brain cancers⁷⁵. The abnormal centrosomes in cancers apparently retain the ability to nucleate microtubules *in vitro*. The molecular and genetic bases for the increased number of centrosomes in such cells have not yet been defined. One possibility, however, relates the abnormal centrosomes to a kinase, *aurora2/STK15*, which is involved in centrosome maturation and spindle assembly in *Drosophila*, can affect centrosome number and chromosome segregation when exogenously expressed in mammalian cells, and is highly expressed and occasionally amplified in human cancers^{76,77}. The centrosome-associated kinase PLK1, a homologue of *Drosophila Polo*, has properties similar to those of *aurora2/STK15* and is also highly expressed in a subset of human cancers⁷⁸. Finally, *p53* inactivation can lead to the occurrence of multiple centrosomes in mouse embryonic fibroblasts⁷⁹.

Despite these clues, the molecular basis of CIN remains undefined in most human cancers. The fact that genetic defects of so many genes can lead to CIN, at least in yeast, suggests a heterogeneous basis for CIN in cancers, with many genes each playing a role in a small proportion of the cases (see Supplementary Information). Accordingly, CIN may be so common in cancers precisely

because there are so many genes that, when mutated, can lead to this phenotype and provide the affected cell with the instability required to develop the multiple genetic alterations that lead to malignancy.

Because there are so many genes that may be involved in CIN, it will be essential to use rigorous criteria before concluding that a specific gene is responsible for CIN in a given cancer. In our opinion, these criteria include the following: first, documentation of subtle, intragenic mutations of the gene that alter its function or expression in at least a subset of cases; and second, recapitulation of the CIN phenotype in an immortal diploid line. When the candidate gene is presumed to act in a dominant-negative fashion, recapitulation of the CIN phenotype can be accomplished through exogenous expression of the mutant gene product. When the candidate gene is presumed to act recessively, targeted deletion can be used. In both cases, it is important to do the experiments in immortal, rather than primary, diploid cells to eliminate the possibility that the tested gene is simply immortalizing the cells. Such immortalization, even when spontaneous, may indirectly result in aneuploidy⁵³.

Chromosome translocations

Two patterns of chromosome translocation have been seen in human cancers. The first pattern (complex type) is observed in many solid tumours, but the translocations observed in individual tumours appear nearly random and are usually unrelated to the translocations observed in other tumours of the same histological subtype^{5,80}. Marker chromosomes, containing complicated rearrangements of several chromosomes, are seen frequently in such tumours. Large portions of chromosomal arms are often deleted during the recombinations that lead to the translocations; these deletions are seen as losses of heterozygosity at the molecular level. Losses of heterozygosity can also be generated by mitotic recombinations, which are invisible at the karyotypic level but occur very commonly in most solid tumours (Fig. 4).

The complex type of translocation can result in gains and losses of chromosomal material, much like CIN, as well as in the generation of new gene products, and is likely to be an important component of many common tumours. However, the rate of such translocations in cells cannot be reliably measured, so it is not clear how much more frequently translocations occur in neoplastic cells compared with normal cells. Even the rate of mitotic recombination, which is easy to measure in yeast through analysis of the progeny, cannot be assessed simply in mammalian cells. The molecular basis for the translocations in cancers is not known. However, one attractive possibility is that they arise in cells that enter mitosis before recombination-promoting double-strand breaks (DSBs) are repaired^{64,65}. Good candidates for mediating such 'translocation instability' in human cancers are, therefore, genes such as *ATM*, *ATR*, *BRCA1*, *BRCA2*, *p53* and other components involved in DSB repair or DNA-damage checkpoints (see above). To determine whether these or other candidate genes are actually responsible for translocation instability, we will need convenient assays with which to measure the rate of translocation in somatic cells and more detailed characterizations of the recombination joints. It is not yet known, for example, whether the joints in complex translocations result from homologous or non-homologous recombination. Results from some studies indicate that inactivation of *p53* may increase the rate of homologous recombination of experimental substrates^{81,82}, thus providing yet another link between *p53* and increased genetic instability at late stages of tumorigenesis.

A second pattern of translocation (simple type) is characterized by distinctive rearrangements of chromosomal segments in specific neoplastic diseases. These occur commonly in leukaemias and lymphomas and in a subset of sarcomas and other rare tumours⁸³. The simple type of translocation reproducibly involves the same chromosomal segments, so much so that their karyotypic presence can be used to classify neoplasms and predict therapeutic responses⁸³. Molecular analyses have determined the breakpoints

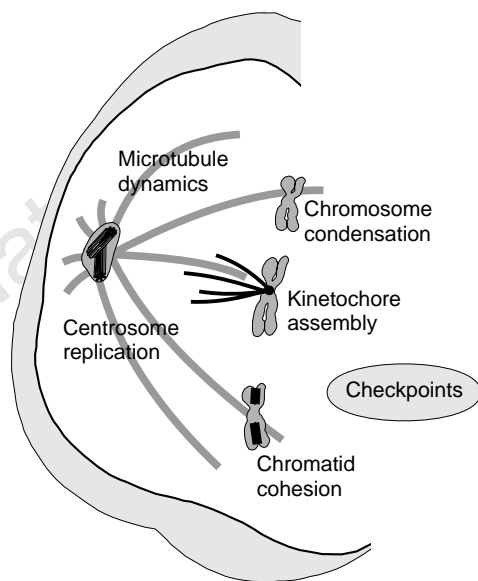


Figure 5 Cellular processes involved in replication and segregation of chromosomes during mitosis. Processes involved include chromosome condensation, cohesion of sister chromatids, and centrosome/microtubule formation and dynamics. Checkpoints that are required in chromosome replication and segregation include the mitotic spindle checkpoint, which ensures that chromosomes are aligned correctly before anaphase, and the DNA-damage checkpoint, which prevents cells with DNA damage from entering prophase. Aberrations in these processes and checkpoints could give rise to the CIN phenotype. Numerous examples of specific genes that could lead to CIN when mutated are listed in Supplementary Information.

in many of the translocations and have shown that these usually occur within the same, relatively small segments of DNA. The rearrangements generally lead to the activation of an oncogene through its positioning near a strong promoter or its fusion with another gene. These specific translocations appear to be essential for the development or progression of the neoplasms in which they occur. However, there is no evidence that they are the result of a specific genetic instability that promotes their occurrence at higher frequency than in normal cells. In fact, some of these translocations represent an aberration of the normal recombination processes mediated by RAG proteins that lead to immunoglobulin or T-cell-receptor gene rearrangements, and similar translocations occur in normal cells of lymphoid origin^{84,85}.

Gene amplifications

Amplifications of oncogenes occur in a subset of late-stage cancers of many organs, and amplification of genes involved in metabolism or inactivation of drugs represents a common way for cultured cells to acquire resistance to chemotherapeutic agents⁹. Quantitative measurements have indicated that amplifications may occur at a higher rate in cancer cells than in normal cells⁸⁶. The mechanisms through which amplifications are generated are largely unknown. Further progress in this area might be made if an analogous process could be demonstrated in yeast, an organism that has provided key insights into other forms of genetic change. One potential player in the process is p53, as amplifications occur more easily when p53 is inactivated in mammalian cells^{87,88}. This is not the complete story, however, as amplification can also occur in cancer cells with wild-type p53 genes⁸⁷. One theory is that it is not the rate at which amplification occurs that distinguishes normal cells from cancer cells, but rather the ability of cancer cells exhibiting gene amplification to survive. In normal cells, the presence of an amplicon (the genomic segment that is amplified) may signal that DNA damage has occurred, thus setting off p53-dependent apoptosis⁸⁹. In the absence of normal p53, cells with initial amplifications of a locus may survive and accumulate additional amplicons during further rounds of cellular division. This model would distinguish 'amplification instability' from CIN, in which the actual rate of chromosome-number change, rather than the ability of cells to survive such changes, is altered⁴⁶.

Gene amplification is an important process in human cancers, as it is clearly associated with tumour progression⁹, has prognostic significance¹⁰ and has even provided a target for therapeutics in the case of amplification of HER2/neu in breast cancers⁹⁰. However, it is important to distinguish gene amplification from the other forms of genetic change discussed above. NIN, MIN and CIN occur early in the neoplastic process, affect multiple genes in each cell, and are likely to be essential for development of the sequential mutations that drive the neoplastic process. Although amplifications can significantly affect tumour biology, they affect only a single or a few genes in each cell and, in general, occur late in tumorigenesis.

Conclusions

The data reviewed above lead us to conclude that nearly all solid tumours are genetically unstable. In most cases, the instability is seen at the chromosomal level, with frequent gains and losses of whole chromosomes (CIN). In a few cases, the instability is at the nucleotide level (NIN or MIN) and is the result of faulty DNA repair. Translocations and amplifications add to the chromosomal abnormalities, and may reflect additional mechanisms for generating instability that occur as tumours progress. Instability is the engine of both tumour progression and tumour heterogeneity, guaranteeing that no two tumours are exactly alike and that no single tumour is composed of genetically identical cells. This heterogeneity undermines otherwise sound therapeutic strategies⁹¹.

But there is another side to genetic instability, one that may work to the patient's benefit rather than to the tumour's. Much effort is

currently being expended to target, during treatment, the mutated oncogenes and tumour-suppressor genes that control neoplastic cell growth directly. The genetic instabilities that underlie cancer may provide equally valid therapeutic targets. Although such instabilities are not directly responsible for the cancer's abnormal growth, they are likely to be genetically based and, therefore, permanent components of the cancer cell that unambiguously distinguish it from all normal cells. Because instabilities reflect defects in cellular processes that maintain the integrity of the genome, they can be expected to generate sensitivities to particular chemical agents. For example, cells with defects in nucleotide-excision repair are sensitive to ultraviolet light¹⁶, and cells with defective *BRCA* genes are sensitive to ionizing radiation (reviewed in ref. 92). It can be expected that drugs will be found to which mismatch-repair-deficient cells are particularly sensitive and that some cells with defective mitotic checkpoints will be more sensitive to microtubule-disrupting agents (such as paclitaxel) than normal cells. In fact, one can argue persuasively that all chemotherapeutic compounds used at present are more toxic to cancer cells than to normal cells only and specifically because of the defective checkpoints that occur in the former cells^{2,93}. This line of reasoning suggests that, although instability may be essential for neoplasia to develop, it may also prove to be its Achilles' heel when the tumour is attacked by the right agents. Further research to define the molecular and physiologic bases of instability may, therefore, yield entirely new approaches to treating common forms of cancer. □

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- Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res.* **51**, 3075–3079 (1991).
- Hartwell, L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell* **71**, 543–546 (1992).
- Tomlinson, I. P., Novelli, M. R. & Bodmer, W. F. The mutation rate and cancer. *Proc. Natl Acad. Sci. USA* **93**, 14800–14803 (1996).
- Almoguer, C. *et al.* Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* **53**, 549–554 (1988).
- Mitelman, F., Johansson, B. & Mertens, F. *Catalog of Chromosome Aberrations in Cancer* Vol. 2 (Wiley-Liss, New York, 1994).
- Wang, S. L. *et al.* Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res.* **57**, 4183–4186 (1997).
- Zhuang, Z. *et al.* Trisomy 7-harboring non-random duplication of the mutant MET allele in hereditary papillary renal carcinomas. *Nature Genet.* **20**, 66–69 (1998).
- Nowell, P. C. Genetic alterations in leukemias and lymphomas: impressive progress and continuing complexity. *Cancer Genet. Cytogenet.* **94**, 13–19 (1997).
- Brodeur, G. M. & Hogarty, M. D. in *The Genetic Basis of Human Cancer* 1st edn Vol. 1 (eds Kinzler, K. W. & Vogelstein, B.) 161–179 (McGraw-Hill, New York, 1998).
- Seeger, R. C. *et al.* Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N. Engl. J. Med.* **313**, 1111–1116 (1985).
- Kauffmann-Zeh, A. *et al.* Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* **385**, 544–548 (1997).
- Kunkel, T. A. DNA mismatch repair. The intricacies of eukaryotic spell-checking. *Curr. Biol.* **5**, 1091–1094 (1995).
- Sia, E. A., Jinks-Robertson, S. & Petes, T. D. Genetic control of microsatellite stability. *Mutat. Res.* **383**, 61–70 (1997).
- Cleaver, J. E. Defective repair replication of DNA in xeroderma pigmentosum. *Nature* **218**, 652–656 (1968).
- De Weerd-Kastelein, E. A., Keijzer, W. & Bootsma, D. Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. *Nat. New Biol.* **238**, 80–83 (1972).
- Bootsma, D., Kraemer, K. H., Cleaver, J. E. & Hoeijmakers, J. H. J. in *The Genetic Basis of Human Cancer* (eds Kinzler, K. W. & Vogelstein, B.) 245–274 (McGraw-Hill, New York, 1998).
- Wood, R. D. DNA repair in eukaryotes. *Annu. Rev. Biochem.* **65**, 135–167 (1996).
- Cairns, J. The origin of human cancers. *Nature* **289**, 353–357 (1981).
- Feinberg, A. P. & Coffey, D. S. Organ site specificity for cancer in chromosomal instability disorders. *Cancer Res.* **42**, 3252–3254 (1982).
- Kraemer, K. H., Lee, M. M. & Scotto, J. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. *Carcinogenesis* **5**, 511–514 (1984).
- Ames, B. N. & Gold, L. S. Endogenous mutagens and the causes of aging and cancer. *Mutat. Res.* **250**, 3–16 (1991).
- Peinado, M. A., Malkhosyan, S., Velazquez, A. & Perucho, M. Isolation and characterization of allelic losses and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. *Proc. Natl Acad. Sci. USA* **89**, 10065–10069 (1992).
- Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D. & Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* **363**, 558–561 (1993).
- Thibodeau, S. N., Bren, G. & Schaid, D. Microsatellite instability in cancer of the proximal colon. *Science* **260**, 816–819 (1993).
- Aaltonen, L. A. *et al.* Clues to the pathogenesis of familial colorectal cancer. *Science* **260**, 812–816 (1993).
- Peltomäki, P. *et al.* Genetic mapping of a locus predisposing to human colorectal cancer. *Science* **260**, 810–812 (1993).

27. Lindblom, A., Tannergard, P., Werelius, B. & Nordenskjold, M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. *Nature Genet.* **5**, 279–282 (1993).
28. Strand, M., Prolla, T. A., Liskay, R. M. & Petes, T. D. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365**, 274–276 (1993).
29. Fishel, R. *et al.* The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* **75**, 1027–1038 (1993).
30. Leach, F. S. *et al.* Mutations of a mutL homolog in hereditary nonpolyposis colorectal cancer. *Cell* **75**, 1215–1225 (1993).
31. Bronner, C. E. *et al.* Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* **368**, 258–261 (1994).
32. Papadopoulos, N. *et al.* Mutation of a mutL homolog in hereditary colon cancer. *Science* **263**, 1625–1629 (1994).
33. Parsons, R. *et al.* Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* **75**, 1227–1236 (1993).
34. Umar, A. *et al.* Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. *J. Biol. Chem.* **269**, 14367–14370 (1994).
35. Peltomaki, P. & de la Chapelle, A. Mutations predisposing to hereditary nonpolyposis colorectal cancer. *Adv. Cancer Res.* **71**, 93–119 (1997).
36. Modrich, P. Mismatch repair, genetic stability and tumour avoidance. *Phil. Trans. R. Soc. Lond. B* **347**, 89–95 (1995).
37. Kolodner, R. Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev.* **10**, 1433–1442 (1996).
38. Perucho, M. Cancer of the microsatellite mutator phenotype. *Biol. Chem.* **377**, 675–684 (1996).
39. Dams, E., Van de Kelft, E. J., Martin, J. J., Verlooy, J. & Willems, P. J. Instability of microsatellites in human gliomas. *Cancer Res.* **55**, 1547–1549 (1995).
40. Bhattacharya, N. P., Skandalis, A., Ganesh, A., Groden, J. & Meuth, M. Mutator phenotypes in human colorectal carcinoma cell lines. *Proc. Natl Acad. Sci. USA* **91**, 6319–6323 (1994).
41. Eshleman, J. R. *et al.* Increased mutation rate at the hprt locus accompanies microsatellite instability in colon cancer. *Oncogene* **10**, 33–37 (1995).
42. Vogelstein, B. *et al.* Allelotype of colorectal carcinomas. *Science* **244**, 207–211 (1989).
43. Boige, V. *et al.* Concerted nonsyntenic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelotype. *Cancer Res.* **57**, 1986–1990 (1997).
44. Seymour, A. B. *et al.* Allelotype of pancreatic adenocarcinoma. *Cancer Res.* **54**, 2761–2764 (1994).
45. Radford, D. M. *et al.* Allelotyping of ductal carcinoma *in situ* of the breast: deletion of loci on 8p, 13q, 16q, 17p and 17q. *Cancer Res.* **55**, 3399–3405 (1995).
46. Lengauer, C., Kinzler, K. W. & Vogelstein, B. Genetic instability in colorectal cancers. *Nature* **386**, 623–627 (1997).
47. Phear, G., Bhattacharya, N. P. & Meuth, M. Loss of heterozygosity and base substitution at the APRT locus in mismatch-repair-proficient and -deficient colorectal carcinoma cell lines. *Mol. Cell. Biol.* **16**, 6516–6523 (1996).
48. Bardi, G. *et al.* Cytogenetic comparisons of synchronous carcinomas and polyps in patients with colorectal cancer. *Br. J. Cancer.* **76**, 765–769 (1997).
49. Silverstein, M. J. *Ductal Carcinoma in situ of the Breast* (Williams & Wilkins, Baltimore, 1997).
50. Bomme, L. *et al.* Cytogenetic analysis of colorectal adenomas: karyotypic comparisons of synchronous tumors. *Cancer Genet. Cytogenet.* **106**, 66–71 (1998).
51. Aaltonen, L. A. *et al.* Replication errors in benign and malignant tumors from hereditary non-polyposis colorectal cancer patients. *Cancer Res.* **54**, 1645–1648 (1994).
52. Koi, M. *et al.* Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-methyl-N'-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. *Cancer Res.* **54**, 4308–4312 (1994).
53. Li, R. *et al.* Aneuploidy correlated 100% with chemical transformation of Chinese hamster cells. *Proc. Natl Acad. Sci. USA* **94**, 14506–14511 (1997).
54. Harvey, M. *et al.* *In vitro* growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* **8**, 2457–2467 (1993).
55. Cross, S. M. *et al.* A p53-dependent mouse spindle checkpoint. *Science* **267**, 1353–1356 (1995).
56. Lanni, J. S. & Jacks, T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. *Mol. Cell. Biol.* **18**, 1055–1064 (1998).
57. Eshleman, J. R. *et al.* Chromosome number and structure both are markedly stable in RER colorectal cancers and are not destabilized by mutation of p53. *Oncogene* **17**, 719–725 (1998).
58. Baker, S. J. *et al.* p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res.* **50**, 7717–7722 (1990).
59. Auer, G. U., Heselmeyer, K. M., Steinbeck, R. G., Munck-Wikland, E. & Zetterberg, A. D. The relationship between aneuploidy and p53 overexpression during genesis of colorectal adenocarcinoma. *Virchows Arch.* **424**, 343–347 (1994).
60. Hoyt, M. A., Stearns, T. & Botstein, D. Chromosome instability mutants of *Saccharomyces cerevisiae* that are defective in microtubule-mediated processes. *Mol. Cell. Biol.* **10**, 223–234 (1990).
61. Spencer, F., Gerring, S. L., Connelly, C. & Hieter, P. Mitotic chromosome transmission fidelity mutants in *Saccharomyces cerevisiae*. *Genetics* **124**, 237–249 (1990).
62. Murray, A. W. The genetics of cell cycle checkpoints. *Curr. Opin. Genet. Dev.* **5**, 5–11 (1995).
63. Nasmyth, K. At the heart of the budding yeast cell cycle. *Trends Genet.* **12**, 405–412 (1996).
64. Elledge, S. J. Cell cycle checkpoints: preventing an identity crisis. *Science* **274**, 1664–1672 (1996).
65. Paulovich, A. G., Toczyski, D. P. & Hartwell, L. H. When checkpoints fail. *Cell* **88**, 315–321 (1997).
66. Cahill, D. P. *et al.* Mutations of mitotic checkpoint genes in human cancers. *Nature* **392**, 300–303 (1998).
67. Li, Y. & Benezra, R. Identification of a human mitotic checkpoint gene: hSMAD2. *Science* **274**, 246–248 (1996).
68. Taylor, S. S. & McKeon, F. Kenetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* **89**, 727–735 (1997).
69. Jin, D. Y., Spencer, F. & Jeang, K. T. Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. *Cell* **93**, 81–91 (1998).
70. Hartwell, L. H. & Smith, D. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. *Genetics* **110**, 381–395 (1985).
71. Rotman, G. & Shiloh, Y. ATM: from gene to function. *Hum. Mol. Genet.* **7**, 1555–1563 (1998).
72. Smith, L. *et al.* Duplication of ATR inhibits MyoD, induces aneuploidy and eliminates radiation-induced G1 arrest. *Nature Genet.* **19**, 39–46 (1998).
73. Zhang, H., Tomblin, G. & Weber, B. L. BRCA1, BRCA2, and DNA damage response: collision or collusion? *Cell* **92**, 433–436 (1998).
74. Lane, D. Awakening angels. *Nature* **394**, 616–617 (1998).
75. Dooxey, S. The centrosome—a tiny organelle with big potential. *Nature Genet.* **20**, 104–106 (1998).
76. Zhou, H. *et al.* Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nature Genet.* **20**, 189–193 (1998).
77. Bischoff, J. R. *et al.* A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J.* **17**, 3052–3065 (1998).
78. Wolf, G. *et al.* Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. *Oncogene* **14**, 543–549 (1997).
79. Fukasawa, K., Choi, T., Kuriyama, R., Rulong, S. & Vande Woude, G. F. Abnormal centrosome amplification in the absence of p53. *Science* **271**, 1744–1747 (1996).
80. Johansson, B., Mertens, F. & Mitelman, F. Primary vs. secondary neoplasia-associated chromosomal abnormalities—balanced rearrangements vs. genomic imbalances? *Genes Chromosomes Cancer* **16**, 155–163 (1996).
81. Sturzbecher, H. W., Donzelmann, B., Henning, W., Knipschild, U. & Buchhop, S. p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *EMBO J.* **15**, 1992–2002 (1996).
82. Meekel, K. L. *et al.* Inactivation of p53 results in high rates of homologous recombination. *Oncogene* **14**, 1847–1857 (1997).
83. Le Beau, M. M. & Rowley, J. D. Chromosomal abnormalities in leukemia and lymphoma: clinical and biological significance. *Adv. Hum. Genet.* **15**, 1–54 (1986).
84. Tycko, B., Palmer, J. D. & Sklar, J. T cell receptor gene trans-rearrangements: chimeric gamma-delta genes in normal lymphoid tissues. *Science* **245**, 1242–1246 (1989).
85. Hiom, K., Melek, M. & Gellert, M. DNA transposition by the RAG1 and RAG2 proteins: a possible source of oncogenic translocations. *Cell* **94**, 463–470 (1998).
86. Tlsty, T. D., Margolin, B. H. & Lum, K. Differences in the rates of gene amplification in nontumorigenic and tumorigenic cell lines as measured by Luria-Delbruck fluctuation analysis. *Proc. Natl Acad. Sci. USA* **86**, 9441–9445 (1989).
87. Livingston, L. R. *et al.* Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* **70**, 923–935 (1992).
88. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C. & Wahl, G. M. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell* **70**, 937–948 (1992).
89. Oren, M. Relationship of p53 to the control of apoptotic cell death. *Semin. Cancer Biol.* **5**, 221–227 (1994).
90. Pegram, M. D. *et al.* Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.* **16**, 2659–2671 (1998).
91. Owens, A. H., Coffey, D. S. & Baylin, S. B. *Tumor Cell Heterogeneity* (Academic, New York, 1982).
92. Abbott, D. W., Freeman, M. L. & Holt, J. T. Double-strand break repair deficiency and radiation sensitivity in BRCA2 mutant cancer cells. *J. Natl Cancer Inst.* **90**, 978–985 (1998).
93. Waldman, T., Lengauer, C., Kinzler, K. W. & Vogelstein, B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. *Nature* **381**, 713–716 (1996).
94. Williams, C. *et al.* Clones of normal keratinocytes and a variety of simultaneously present epidermal neoplastic lesions contain a multitude of p53 gene mutations in a xeroderma pigmentosum patient. *Cancer Res.* **58**, 2449–2455 (1998).
95. Markowitz, S. *et al.* Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* **268**, 1336–1338 (1995).

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