Chapter 1.1

THE MULTIPLICITY OF MUTATIONS IN HUMAN CANCERS

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1. INTRODUCTION

Human cancer cells contain large numbers of mutations. These can be observed as alterations in chromosomal numbers (gains or losses) and structural integrity, by an analysis of the lengths of microsatellite sequences and mutations in oncogenes and tumour-suppressor genes. The question is how and when these mutations originate, what the consequences of these mutations are, and most importantly, whether they drive tumour progression. In order to account for the disparity between the infrequency of spontaneous mutations in normal somatic human cells and the large number of mutations in human cancers, we formulated the hypothesis that cancer cells express a mutator phenotype. The hypothesis states that an increase in mutation rate is an early step during tumorigenesis. As a result, random mutations are generated throughout the genome. Some of these mutations occur in genes that normally function to guarantee the accurate transfer of genetic information during each cell division. Among the many mutations produced, some are ones that impart a growth advantage and result in invasion and metastasis, the hallmarks of cancer. In this chapter we will focus on the multiple mutations in human tumours, postulated sources for these mutations, and the arguments, for and against, the mutator phenotype hypothesis.

2. CHROMOSOME NUMBERS AND CANCER

Changes in chromosome number, aneuploidy, may be a gross manifestation of genetic instability in tumours. During the early part of last

century, using a light microscope and embryos of echinoderms (ascaris and sea urchins), Theodor Boveri made many remarkable observations on the numbers and structures of chromosomes (Boveri, 1902). Boveri postulated that (1) chromosomes are highly organised structures probably involved in heredity, (2) the egg and the sperm contribute equal number of chromosomes to the embryo, and (3) tumour growth may result from aberrant chromosome number or aneuploidy. Technical advances in human clinical cytogenetics led to the verification of Boveri's proposal that cancer cells possess abnormal chromosomal numbers and this has become one marker for grading human tumours. However, even though most solid and some hematopoietic cancers are aneuploid, the fundamental question that has remained unanswered is whether aneuploidy initiates tumorigenesis or is passively acquired during evolution of malignant cells, or simply stated, is aneuploidy the cause or an effect of cancer cell evolution? Duesberg and co-workers have argued that aneuploidy is the somatic event that initiates carcinogenesis (Duesberg et al., 1998). They present evidence that aneuploidy can be induced by treatment of cells with chemical carcinogens and that the induction of aneuploidy precedes the appearance of a transformed phenotype. Recently, Rahman and co-workers provided further evidence supporting the aneuploidy-cancer hypothesis: they reported that biallelic mutations in the spindle checkpoint gene *BUB1B* are associated with aneuploidy in a human disease, MVA (mosaic variegated aneupoidy). MVA is a rare recessive disease characterised by early onset of cancer. Thus in a rare inherited disease, a mutation in a gene that effects chromosome segregation is associated with human cancers (Hanks et al., 2004).

Considering the hundreds of genes encoded in each chromosome, it is difficult to understand how any cell with a different number of chromosomes can possibly maintain viability and how aneuploidy is compatible with live human births such as those seen in Down and Klinefelter syndromes**.** It seems likely that haploinsufficiency, over-expression, squelching and dominant negative interactions would render such cells less fit. Apparently, organisms have evolved buffering systems to tolerate changes in chromosome numbers that we have not even contemplated. Despite the observations that most cancer cells contain aneuploid karyotypes, the timing of acquisition of such events is unknown and hence their direct contribution toward development of a malignant phenotype remains obscure. The generalisation that aneuploidy initiates cancer is difficult to substantiate considering that most tumours are monoclonal and yet not all tumour cells within a tumour mass are aneuploid (Mitelman, 1994). Moreover, premalignant conditions such as Barrett's esophagus (Barratt et al., 1999) and ulcerative colitis exhibit mutations in multiple oncogenes but are not aneuploid (Rabinovitch et al., 1999). Thus, aneuploidy could be one of the manifestations of genetic instability and not causative in initiating carcinogenesis.

2.1   Chromosome Instability and Cancer

Chromosomal instability (CIN) results in gains, losses, deletions, insertions, translocations, amplifications, and rearrangements, and is frequently used to grade tumours with respect to prognosis (Lengauer et al., 1998). CIN is characterised by an increased frequency of chromosomal alterations often resulting in loss of heterozygosity (Rajagopalan et al., 2003). The majority of human tumours display the CIN phenotype. However, these tumours may contain larger numbers of other types of mutations that are more difficult to detect. The genes responsible for the maintenance of chromosomal stability in normal cells are beginning to be identified and their function is being delineated. Tumours with the CIN phenotype usually harbour mutations in oncogenes and/or tumour-suppressor genes, many of which are involved in the regulation of transcription. These tumours may display genetic instability as a result of altered global gene expression patterns and global changes in chromatin structure.

Large chromosomal rearrangements, a hallmark of the CIN phenotype, can be visualised by cytogenetic techniques and enhanced visualisation has been provided by spectral karyotyping (SKY) (Bayani et al., 2002). Using gene-specific probes labelled with different coloured fluorochromes, one can map specific segments of chromosomes and demonstrate multiple rearrangements within and between individual chromosomes. There are two widely used molecular techniques that examine populations of molecules at higher resolution. Comparative genomic hybridisation measures differences in hybridisation between fragments of DNA from different sources, each tagged with different fluorescent molecules. Localisation of signal can be achieved by using metaphase chromosomes as a scaffold. Using this technique, a large number of tumours have been shown to exhibit multiple changes in DNA copy number (Iwabuchi et al., 1995; Kallioniemi et al., 1994). The fact that benign tumours also exhibit extensive changes in DNA copy number (El-Rifai et al., 1998) suggests that changes in DNA copy number occur early during tumorigenesis. Loss of heterozygosity (LOH) in tumours permits one to scan small segments of the entire genome using a library of microsatellite markers. The finding that many cancers exhibit multiple changes suggests that in many DNA segments of the tumour genome, there is a modification (gain or loss) of segments of one of the parental alleles. Only a small proportion of the genome is interrogated by this technique since the PCR-amplified segments are about 1000 nucleotides in length. If one assumes that the sampling is representative, then the entire tumour genome may contain thousands of DNA segments that exhibit loss of heterozygosity.

Both comparative genomic hybridisation and measurements of loss of heterozygosity examine populations of DNA molecules and do not score for chromosomal alterations in individual tumour cells. However, both of these techniques have been applied to single metastatic cells in bone marrow and

multiple alterations have been documented (Klein et al., 1999)**.** Moreover, different single cells from the same tumour display alterations in different segments of the same chromosomes.

The majority of human cancers display a CIN phenotype and timing of its expression is presently debated even in the same tumour model, for example, colon cancer. Huang and coworkers reported that mutations in mismatch repair genes occur prior to mutations in the *APC* gene, a frequently mutated gene in colon cancer that is associated with the CIN phenotype. In addition, studies utilising microdissection that trace tumour evolution indicated that microsatellite instability was extensive in early adenomas, and additional instability was observed as adenomas progressed to adenocarcinomas (Shibata et al., 1996). In contrast, others have reported that there is no difference in the frequency and spectrum of mutations in the APC gene in colon tumours that exhibit extensive microsatellite instability versus others that do not (Homfray et al., 1998). These results have suggested that mutations in *APC* initiate carcinogenesis and may occur prior to microsatellite instability (Tomlinson and Bodmer, 1999).

2.2   Microsatellite Instability and Cancer

Studies on alterations in microsatellite sequences provided the first and strongest glimpse into the extensiveness of mutations in human cancers. Perucho and associates used oligonucleotides with random sequences as arbitrary primers in PCR-reactions and observed products of different lengths using DNA from human colon tumours compared to those obtained using DNA from adjacent normal tissues (Perucho, 1996). The PCR products contained microsatellite sequences with different numbers of repeats. Subsequent studies established that extensive microsatellite instability (MIN) was associated with hereditary nonpolyposis coli (HNPCC) (Fishel, 2001; Thibodeau et al., 1993), a disease caused by mutations in genes required for the repair of mismatches generated by erroneous DNA synthesis (Kolodner and Marsischky, 1999; Modrich, 1995). Unlike CIN, the MIN phenotype is observed in a small variety of tumours and frequently occurs early during tumorigenesis. The nature of these mutations and their consequences is considered in other chapters in this book. However it is clear that at least in some inherited human tumours (Cleaver and Kraemer, 1989), like HNPCC, MAP (Myh-associated polyposis), XP (Xeroderma pigmentosum) and Bloom's syndrome, the cells are predisposed to genetic instability at the nucleotide level and at least in these tumours, genetic instability clearly causes tumorigenesis. Furthermore, the cancers with a MIN phenotype display tissue specificity (Markowitz, 2000). However, with respect to genomic instability, it should be emphasised that this protocol only analyzes a small percentage of the microsatellite sequences in the genome. If one extrapolates these results and those obtained from studies with other tumours (Stoler et al., 1999) to the whole genome, it can be concluded that some tumours contain as many as

10,000 alterations in the number of repeats within microsatellites. It is usually assumed that microsatellite instability is generated by slippage of DNA polymerases during copying of repeats and thus represents a hot spot for mutagenesis. The extensiveness of microsatellite instability in tumours lacking mutations in mismatch repair genes [listed in (Jackson and Loeb, 2001)] provides an important indicator of the extensiveness of genomic instability in tumours.

2.3   Point Mutations and Cancer

Many agents that damage DNA produce single nucleotide substitutions (Singer, 1996), similar to misincorporations by DNA polymerases (Kunkel and Loeb, 1981). In addition, errors in DNA synthesis by trans-lesion DNA polymerases that copy past bulky adducts in DNA are predominantly single-base substitutions (Kunkel and Bebenek, 2000). The relationship of single base substitutions to gene amplification, large deletions and rearrangements has not been explored. Conceivably, single-base substitutions can initiate many of these events. Single base changes, (point mutations) are likely to be distributed randomly throughout the genome. The random distribution of these mutations renders current methods inadequate for their detection. Conventional DNA sequencing requires multiple copies of each DNA template and the sequences that are obtained score only for the predominant nucleotide at each position; micro-heterogeneity within a population of DNA templates would not be detected (Loeb et al., 2003). In order to detect heterogeneity by DNA sequencing, it is necessary to sequence multiple single DNA molecules obtained from the same tissue sample. As a result, the quantitation of random mutations in tumours and the evaluation of their contribution to a mutator phenotype in cancers are issues that have not been resolved. It should be noted that with each round of clonal selection, the non-selected random mutations are clonally fixed in all progeny derived from that cell (Figure 1). With absolute selection for mutations in an oncogene or a tumour-suppressor gene, the random mutations would also be present among all the progeny cells. In a branched structure for tumour evolution the random mutations would be distributed in groups of different cells throughout the tumour.

2.4   Mutational Requirements for Tumorigenesis

The number of specific mutations required to produce a tumour has been controversial. Based on age-associated increases in cancer incidence, it can be inferred that two mutations are required for tumour induction in retinoblastomas (Knudson, 1971; Knudson, 1985) and as many as 10 mutations in the case of prostate carcinomas (Ware, 1994). In retinoblastoma, the first event can be inherited and the second is a somatic mutation. Based on the transformed phenotype, it has been proposed that at
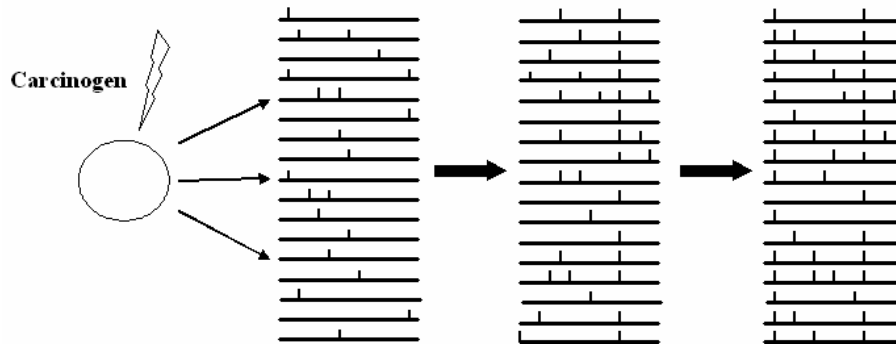
Figure 1. Random mutations are fixed as clonal after selection. Carcinogen or spontaneously-induced expression of a mutator phenotype is an early event generating genome-wide random mutations. The cells harbouring mutation(s) that provide a growth advantage proliferate and expand further, resulting in clonal fixation of that mutation and further generation of new mutations. Selection and clonal proliferation of only one clone is shown for simplicity. The circle represents human tissue, the horizontal lines are individual cellular genomes and vertical lines are random mutations.

least six clonal events are required for tumour induction (Hanahan and Weinberg, 2000). In culture, human cells require more mutations for neoplastic transformation than mouse cells (Rangarajan et al., 2004). How many random mutations are required in order to generate a small number of cancer-specific mutations that either inactivate a tumour-suppressor gene or activate an oncogene and hence confer a dominant phenotype for malignant growth is unknown. Thus, the large number of mutations generated by a mutator phenotype is not at variance with the small number of clonal mutations found in tumours.

3.   MUTATOR PHENOTYPE AND CLONAL SELECTION

In order to account for the large numbers of mutations found in human tumours, we have previously advanced the hypothesis that precancer cells must exhibit a mutator phenotype (Loeb et al., 1974). In normal cells, DNA replication is an exceptionally accurate process and spontaneous mutations are very infrequent. As a result, normal mutation rates are insufficient to account for the multiple mutations observed in cancer cells (Jackson and Loeb, 1998). We have hypothesised that cancer cells express a mutator phenotype and that this occurs early during tumorigenesis. An early step in the evolution of a tumour is the introduction of mutations in genes that normally confer genetic stability. These mutant genes induce additional mutations throughout the genome, some of which result in increased fitness

and clonal proliferation. Our initial hypothesis focused on mutations in DNA polymerase and DNA repair genes (Loeb et al., 1974). This hypothesis has been extended as it became apparent that mutations in a wide variety of genes including those involved in checkpoints, chromosome segregation, apoptosis and nucleotide metabolism would also produce a mutator phenotype (Loeb et al., 2003). In this model for tumour evolution, enhanced mutagenesis would be a driving force in promoting mutation accumulation for growth advantage, or simply stated, a mutator phenotype drives selection.

Nowell (Nowell, 1976; Nowell, 1993) proposed that a mutator phenotype could result from repetitive rounds of clonal selection, in which mutations in a single cell impart a proliferative advantage allowing progeny of that cell to repopulate the tumour. Successive rounds of clonal selection would drive tumour progression. With each round of clonal selection, all silent mutations would become clonal (Figure 1). Vogelstein and colleagues, in their description of tumorigenesis of colon cancer, have proposed an ordered succession of mutations in going from a small benign polyp to an adenocarcinoma (Vogelstein et al., 1988). If clonal selection were absolute, then all random mutations would be converted to clonal mutations. In an ordered succession model, each tumour cell would contain all of the mutations, but this has never been demonstrated.

It seems reasonable that both enhanced mutagenesis and repetitive rounds of clonal selection are operative in tumour progression. Miller and co-workers (Miller, 1996) provided evidence that these two processes are linked. They exposed bacteria to a mutagen and then carried out sequential rounds of selection for mutations that rendered the bacteria resistant to different agents. This protocol mimics the requirements for tumour proliferation under different conditions, *i.e.* the need to grow under reduced oxygen, reduced nutrition, ability to preferentially proliferate, etc. They observed that after three successive rounds of selection 100% of the bacteria exhibited a mutator phenotype (Mao et al., 1997). Each round of selection not only selected for mutants that were resistant to the selective agent but also for mutants that increase mutations in genes that render the cells resistant. Thus with successive rounds of selection there is a "piggy-backing" of mutant genetic instability genes.

4. EMERGING MECHANISMS FOR CAUSES OF GENETIC INSTABILITY

There are many pathways that lead to induction of genetic instability and some of these may be tumour-specific. Many inherited diseases associated with a high incidence of cancer, harbour recessive mutations in genes involved in maintenance of genomic integrity in normal cells. Figure 2 lists several genes which when mutated induce genetic instability. The mutations have been detected because they are clonal. Genetic instability can also be

induced by over-expression or inappropriate expression of non-mutated enzymes involved in DNA metabolism.

Mammalian B-cell lymphocytes have the capacity to produce a diverse library of different antibodies. AID (activation-induced cytidine deaminase) has been identified as the key enzyme required for generation of high affinity antibodies. AID is a member of the RNA editing APOBEC-1 family, which is expressed specifically in the germinal centre-B cells (Muramatsu et al., 1999). Ectopic expression of AID and APOBEC-1 family members in *Escherichia coli,* a mouse pre-B cell line, a human B-cell and a non-B cell line resulted in elevation of the mutation frequency of DNA targets, suggesting that deamination by AID may not be restricted to antibody genes (Martin et al., 2002; Petersen-Mahrt et al., 2002). Liver-specific expression of APOBEC-1 in rabbits and mice has been demonstrated to cause dysplasia and hepatocellular carcinoma (Yamanaka et al., 1995). All of the above data suggests that deregulated expression of AID and APOBEC-1 family members may transduce tumorigenesis by induction of genome-wide random somatic mutagenesis.

Postulated pathways for mutation accumulation

DNA Repair:	XPA, XPB, XPC, XPD, ERCC4, ERCC5 MLH1, MSH2, MSH6, MUTYH, PMS1, PMS2 FANC-A, FANC-C, FANC-D2, FANC-E, FANC-F, FANC-G
DNA-damage signaling:	ATM, NBS1
DNA polymerases:	Pol-eta, Pol-beta
DNA recombination:	WRN, BLM, REQ4L, RAD51B
Chromosome segregation:	BUB1B

Figure 2. Pathways and mutant genes found in syndromes with a prevalence of cancers.

The classic replicative DNA polymerases (α, δ, and ϵ) stall upon encountering altered bases (DNA lesions) and it was previously unknown as to how the DNA synthesis could resume after stalling of replication forks at the site of DNA lesions. But, as discussed in detail elsewhere in this book, the recent discovery of a new family of DNA polymerases (Y-family: η, ι, κ, and ζ) that are able to use damaged DNA as a template (bypass) has provided new clues toward understanding the mechanism of bypass of stalled replication forks (Livneh, 2001; Masutani et al., 2000) (Friedberg et

al., 2002; Goodman and Tippin, 2000). Based on accumulated evidence, a model has been proposed that suggests that a Y-family DNA polymerase is recruited to bypass specific DNA lesions, followed by resumption of processive DNA synthesis by replicative DNA polymerases (Friedberg et al., 2002; Haracska et al., 2001). The Y-family DNA polymerases synthesise DNA with very low fidelity on both damaged and undamaged DNA. Their fidelity has been estimated to be ~1000 to 4000-fold lower than the replicative DNA polymerases (Matsuda et al., 2000). Thus the benefits of ensuring continuous DNA synthesis even through the damaged DNA template may also result in generation of spontaneous mutations. The trans-lesion polymerases may be major constitutive sources for the generation of random mutations. It will be important to determine if the expression of some of these enzymes are elevated in specific tumours.

5. ARGUMENTS AGAINST A MUTATOR PHENOTYPE

It is instructive to consider the arguments that have been advanced against a mutator phenotype during tumorigenesis or more specifically, a mutator phenotype at the level of point mutations generated by mutations in DNA polymerases or DNA repair genes.

First is the concept of negative clonal selection, that is, most mutations result in reduced fitness and as a result, proteins cannot tolerate multiple amino acid substitutions. Recent studies involving random substitutions in a variety of proteins, including DNA polymerases, demonstrate that even these very highly conserved proteins are able to tolerate large numbers of substitutions without impairments in catalytic activity (Patel and Loeb, 2000). Studies from our laboratory have quantitated the probability of enzyme inactivation by single amino acid substitutions (Guo et al., 2004). The average probability of inactivation of human 3-methyladenine glycosylase by any single random amino acid replacement at any position in the protein is 0.34, *i.e* the protein tolerates substitutions in nearly all positions. In addition to mutation tolerance by individual proteins, cells have evolved redundant pathways for preserving vital activities.

Second, mathematical modelling of mutation accumulation in colonic stem cells suggests that one can account for 150,000 mutations per cell in adenocarcinoma of the colon based on normal mutation rates (Tomlinson et al., 1996). The cells that line the crypts of the colon undergo rapid cell divisions starting with the stem cells located near the base of the crypt that divide asymmetrically and give rise to well-differentiated cells that desquamate into the lumen of the intestine. The entire process can occur over thirty-six hours and, as a result, colonic epithelial cells can undergo 5000 divisions during adulthood. Based primarily on this continuous regenerative and replicative process, it has been calculated that normal cells could accumulate large numbers of mutations and thus the expression of a

mutator phenotype in tumours may not be required for tumour progression. These calculations support the concept that colon cancers contain large numbers of random mutations (~10^{13}) and argued that since colonic stem cells undergo large number of cell generations, normal somatic mutations rates are sufficient to generate mutations that drive tumorigenesis. They suggest that premalignant cells need not express the mutator phenotype because increased mutation rates do not confer any advantages for tumour growth but rather natural selection provides the growth advantage (Tomlinson and Bodmer, 1999; Tomlinson et al., 2002). The two major difficulties with this model are that the mutation rate in stem cells is 100-fold lower than that exhibited by somatic cells (Cervantes et al., 2003) and that most tissues that do not shed progeny cells are therefore unlikely to undergo the enormous number of mutations that occur in colonic epithelium. In contrast to this formulation, other mathematical models that compare sporadic and hereditary forms of colorectal cancer indicate that the initial mutations involve mutations in genes associated with genetic instability (Komarova and Wodarz, 2003). It should also be noted that in most tissues other than colon and skin, early tumour cells compete with normal cells within a confined space.

Thirdly, a sequencing study of 3.2 megabases of exonic DNA from 12 tumour cell lines (Wang et al., 2002) revealed only 3 tumour-specific coding mutations. Assuming that this mutation frequency prevails throughout the genome, there would be 3000 mutations in the tumour genome. The authors concluded that because cells lining the intestine rapidly proliferate, the small number of substitutions observed could result from normal mutation rates. Instead, they hypothesise that mutations in genes regulating chromosomal stability are more likely to initiate tumorigenesis. Of great interest would be the frequency of mutations that occur in introns and in non-expressed genes, the mutator phenotype hypothesis would predict that these would accumulate with successive rounds of replication. The limitation of this DNA sequencing protocol is the inability to detect random mutations that have occurred after the last round of clonal selection. Since a patient tumour sample is usually comprised of heterogeneous cellular genomes, only the most frequent mutation at any position would be detected.

Fourth is the lack of preponderance of clonal mutations in tumours that involve genes that regulate genetic stability. Futreal and coworkers have compiled a comprehensive catalogue of genes mutated in human cancer from published literature (Futreal et al., 2004). They reported that only 1% (291) of human genes harbour clonal mutations, predominantly within gene families including protein kinases, transcriptional regulators, and DNA binding proteins. Genes involved directly in maintenance of DNA sequence integrity, like mismatch repair, base excision repair and nucleotide excision repair, only accounted for a very minor fraction. Interestingly, the genes that signal DNA damage, and are involved in DNA repair-transactions also are present in small numbers. Two points emerge from the above study that argues against the mutator phenotype hypothesis: (1) Only a small number

of genes are involved in tumorigenesis and (2) mutations in genes responsible for maintenance of DNA sequence integrity are rarely found in human tumours. One can argue, however, that most tumour cells contain multiple mutations in genetic stability genes and only those that were present in all of the cells in that tumour would be detected.

6. IMPLICATIONS OF A MUTATOR PHENOTYPE

The presence of large numbers of silent clonal mutations, and random mutations within any tumour can account for the rapid emergence of tumour resistance to chemotherapies. Clonal mutations could result in immediate resistance to chemotherapeutic agents. In contrast, resistance due to the presence of rare mutations within a tumour is likely to take more time to become manifested. Thus, the mutator phenotype hypothesis would predict that amongst the 5×10^8 cells that comprise a clinically detectable tumour, there are cancer cells, which harbour mutant genes rendering them resistant to any chemotherapeutic agent directed against the tumour. While chemotherapy might kill most of the cells within the tumour, the cells harbouring the resistant mutations would be able to proliferate and repopulate the tumour. The simultaneous utilisation of multiple chemotherapeutic agents might offer an advantage in obliterating cells with random mutations, since it would be infrequent for one cell to harbour mutations that render it resistant to two agents.

The overall number of mutations in a tumour may permit a new system of stratification in which the stage of tumour progression and/or the response of tumours to chemotherapy is based on the number of mutations within the tumour. Tumours with large numbers of mutations might be further along in tumour progression, and likely be more drug resistant. The question is whether additional mutagenesis would be likely to result in a more malignant phenotype or would be detrimental by inducing an error catastrophe needs to be explored**.** It is possible that many common cancer therapies are effective in part because they enhance mutagenesis.

The types of mutations that accumulate within a tumour should provide clues to both clonal lineage and to mechanisms for mutation accumulation. While sequential biopsies of human tumours is not feasible, tracing the lineage of animal tumours by mapping mutation frequencies in different genes has been proposed (Shibata et al., 1996). The types and spectra of mutations should provide a footprint of mechanisms that cause mutations in genetic stability genes. The accumulation of single-base substitutions provides a measure of errors during DNA replication that exceed mismatch and base excision repair capacities. Mutations that accumulate preferentially in mitochondrial DNA are likely to result from damage by oxygen reactive species (Fliss et al., 2000). Chromosome rearrangements imply deficits in double-strand break and recombination repair. The accumulation of large

chromosomal gains, losses and duplications provides evidence pointing toward mutations in genes responsible for chromosome segregation.

7.   CONSEQUENCES OF GENETIC INSTABILITY

Human cancers harbour at least two types of genetic instability, CIN and MIN, and can be classified into either category. The concept of genetic instability causing cancer is being debated and proponents of the theory emphasise that premalignant cells must express genetic instability early during tumorigenesis to accumulate advantageous mutations necessary for clonal selection. But what causes genetic instability is presently unknown (Marx, 2002). Tomlinson and Bodmer have argued that premalignant cells need not display genetic instability as an early event to acquire a malignant phenotype. They assert that a combination of normal somatic mutation rates and Darwinian selection is sufficient for normal cells to veer toward a tumorigenic pathway. They propose that since the majority of sporadic human tumours arise from a normal diploid cell with intact DNA repair, cell cycle checkpoints and apoptosis machinery, early expression of genetic instability or hypermutagensis may be detrimental to a cell's viability and not tolerated. Instead the premalignant cells offset the need for early expression of genetic instability by undergoing a large number of cell generations to select for malignant cells. They emphasise that genetic instability may assist tumorigenesis, as in certain inherited cancers such as HNPCC and XP, but may not be a universal prerequisite in all human tumours (Sieber et al., 2003). In contrast to their proposal, we argue that the existence of inherited diseases that arise from mutations in DNA repair genes and display high proclivity toward early onset, as well as the presence of large numbers of DNA alterations observed in tumours that do not arise from actively dividing tissues, provides strong evidence that genetic instability is a primary event in tumorigenesis. In summary, the fundamental question, whether genetic instability is required for tumorigenesis, still needs to be resolved.

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