26

Genome Destabilization and Multistep Progression to Cancer

Neoplasia is a broad group of diseases in which one or more cell lineages have escaped from regulators of cell proliferation as the result of inherited or acquired genetic changes. Neoplasms can be caused by exogenous or endogenous carcinogens. Initially, this leads to benign tumors, such as dysplasia or polyps, with limited growth potential. Further genetic changes, which may take years to occur, lead to escape from additional regulators. The result is tumor progression and finally a cancer that can infiltrate and destroy adjacent normal tissues. Some cancers eventually gain the ability to spread to new sites via blood and lymphatic vessels; that is, they can metastasize.

O. J. Miller et al., *Human Chromosomes*

Immortality of Transformed and Malignant Cells

A key feature of cancer cells and cells transformed by DNA tumor viruses or chemicals is their ability to divide indefinitely (escape senescence): They are immortal. Diploid cells, on the other hand, senesce after a limited number of cell doublings: They are mortal. A consideration of the key regulators of the cell cycle (Chapter 2) suggests two obvious mechanisms for cell senescence: activation of a gene for a CDK inhibitor, such as p21 (*CIP1*) or p16 (*INK4A*) and inactivation of a tumor suppressor gene such as *TP53* or *RB1*. Disruption of the *CIP1* gene by two sequential rounds of targeted homologous recombination made diploid human fibroblasts immortal and prevented the cells from arresting the cell cycle in response to DNA damage (Brown et al., 1997). Escape from senescence might be brought about by mutation or loss of any one of these classes of genes, or by inactivation of the *TP53* or *RB1* gene products by binding to a viral oncoprotein (Chapters 27 and 28). Loss or mutation of a sequence on the long arm of chromosome 6 is sometimes involved, because microcell-mediated introduction of a normal chromosome 6 suppresses growth and causes senescence of SV40-transformed cells (Sandhu et al., 1994). Interestingly, a variety of cancers, including breast cancer, ovarian cancer, and non-Hodgkin's lymphoma, have deletions of 6q26–q27, as shown by loss of heterozygosity (LOH) of genetic markers in the region. This region is also deleted in SV40-transformed cell lines. A gene called *SEN6* (senescence-inducing gene on chromosome 6) is thought to be responsible (Banga et al., 1997).

Another cause of cell senescence is defective telomere function. This can be the result of a deficiency of the TRF2 (telomere-repeat binding) protein. The now unprotected telomeres (chromosome ends) act like double-stranded breaks and trigger the DNA damage checkpoint and cell death by p53-dependent apoptosis (Karlseder et al., 1999). The same endpoint can be reached in cell aging because telomerase activity is low or absent in most somatic cells after embryogenesis is completed. Telomeres thus get shorter with each mitosis, because their synthesis requires telomerase. In contrast, transformed and malignant cells have reactivated their telomerase gene, and telomerase activity is actually elevated in most cancers. Interphase cytogenetic analysis, using comparative genomic hybridization (Chapter 8), has shown that 97% of cancers show a small increase in copy number of the telomerase RNA gene located at 3q26.3. In a few cancers, there is even greater amplification of this gene (Chapter 25).

Genetic Basis of Cancer: Sequential Chromosome or Gene Mutations

It has been known for many years that multiple chromosome changes, both numerical and structural, are extremely common in cancer cells (reviewed by Heim and Mitelman, 1995). Comparative genomic hybridization is a powerful tool for rapidly analyzing these changes (Fig. 26.1; see color insert). These karyotypic changes lead to tumorigenesis and progression by causing gain, activation, loss, or inactivation of specific genes (Chapters 26–28). Mutant genes that cause enhanced susceptibility to malignant disease can also be inherited. Mutations of some genes produce an autosomal dominant pattern of cancer susceptibility. The Li-Fraumeni syndrome is due to a mutation of the *TP53* gene (Malkin et al., 1990). The familial breast and ovarian cancer syndrome is due to a mutation of the *BRCA1* gene (Mik et al., 1994). Inherited mutations of other genes produce an autosomal recessive pattern of tumor susceptibility, as in the chromosome instability syndromes discussed in Chapter 24. More than 20 hereditary cancer susceptibility syndromes are known that are due to specific germline-transmitted mutant genes. These account for only about 1% of all cancers; most of the remaining 99% are due to *somatic mutations* at the chromosome or gene level (Fearon, 1997).

The number of acquired genetic changes needed to produce cancer was estimated over 30 years ago by mathematical calculations based on the marked age dependence of cancer incidence and mortality. Armitage and Doll, in 1954, arrived at estimates of three to seven for different individual types of cancer, and this was supported by the more extensive analysis of Ashley (1969). These rather accurate estimates strongly suggested that some of the numerous genetic changes seen in cancer were causal. Burch, in 1962, stressed the importance of inherited mutations in accounting for childhood cancers and pointed out that somatic mutations sometimes cause cancers that are usually familial, and vice versa. Knudsen (1971) was the first to present convincing evidence that as few as two mutations were enough to cause at least one type of cancer, the childhood tumor retinoblastoma (Chapter 28). However, most cancers require more genetic changes. Some of these are point mutations of particular genes, but more are chromosome rearrangements, deletions, or amplifications. However, these chromosomal changes are also important because they affect the expression of specific genes.

Three main types of genes play a role in cancer induction. The first are those that block senescence, increase mutation rates, or lead to chromosome instabil-

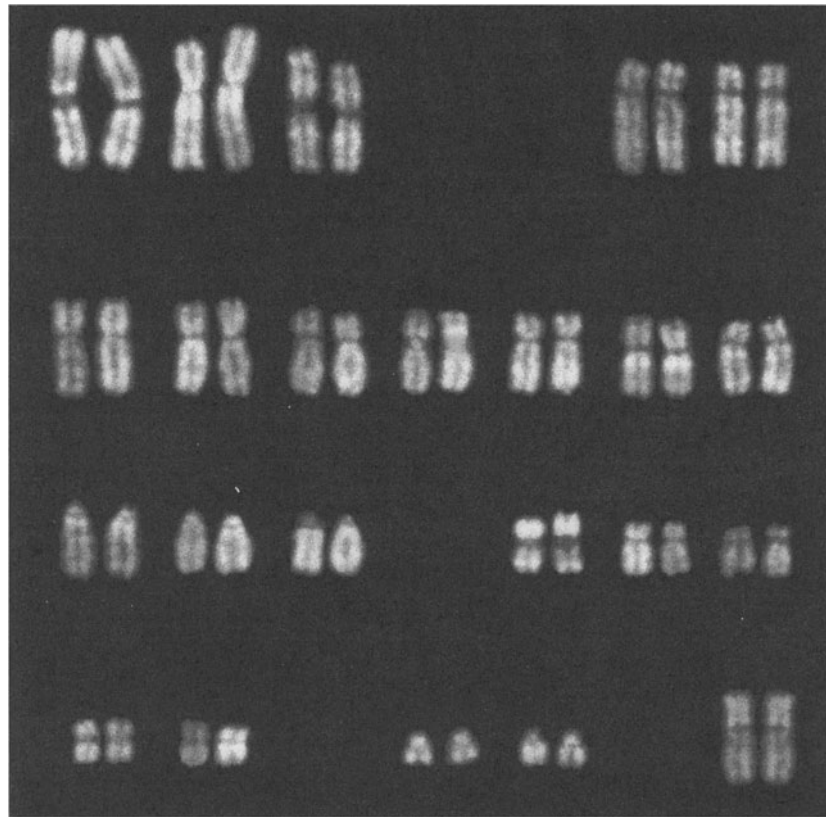

Figure 26.1. Analysis of chromosome changes in a breast cancer by comparative genomic hybridization (CGH). Regions that appear green reflect DNA gains and amplification in the cancer (e.g., 8q, 14, 17q22–q24, and 20q). Regions that appear red reflect DNA losses and deletions (e.g., 1p21–p31, 8p, 11q14–qter, and 16q12–q21) (reprinted from Forozan et al., Trends Genet, v13, Genome screening by comparative genomic hybridization, p 408, copyright 1997, with permission from Elsevier Science) (See color insert).

ity (see DNA Damage Checkpoint and Structural Instability of Chromosomes). The second are growth-stimulating genes, called *cellular oncogenes* or *protooncogenes*, whose normal alleles may be activated or mutate into *oncogenes* (Chapter 27). The third are the *tumor suppressor genes*, sometimes called *antioncogenes*, whose normal function is to regulate cell proliferation or produce differentiation of cells. When these genes are lost or inactivated by mutation, the absence of their products allows malignant growth to occur (Chapter 28).

Clonal Origin, Selection, and Multistep Tumor Progression

The extremely wide range of chromosome constitutions found in cancer cells suggests that karyotypic changes occur almost at random and reflect marked genomic instability. Marked variation in nuclear size from cell to cell, a well-known characteristic of many cancers, is a direct result of corresponding variations in chromosome number. Endoreduplication and endomitosis are more frequent in malignant than in normal cells, and the resulting endopolyploidy may reach high levels. Selection favors the fastest-growing cell type, which proliferates into a clone of cells. However, new chromosome constitutions are continually generated in these genomically unstable transformed cells, and continued selection leads to new clones (formerly called stemlines) that have accumulated more cancer-promoting genetic changes. Benign tumors are not always monoclonal. One patient with familial adenomatous polyposis was also an XO/XY mosaic. In situ hybridization with Y-specific probes showed that many of his colon adenomas contained some cells with a Y chromosome and other cells without a Y; that is, they were polyclonal in origin, arising from more than one cell (Novelli et al., 1996). The expectation is that a more rapidly growing (malignant) clone will arise in one adenoma and in turn be supplanted by still more rapidly growing subclones, and the resultant cancer will then be monoclonal.

Despite the bewildering complexity of karyotypic changes seen in cancer cells, it has been shown that the stepwise progression in the degree of malignancy of some tumors is accompanied by specific changes in the karyotype. Figure 26.2 illustrates this in a breast cancer that was analyzed by comparative genomic hybridization. One of the first diseases to show clear-cut sequential changes was chronic myelogenous leukemia (CML), in which the primary aberration is t(9q;22q); other changes appear during the progression of the disease: a second 22q−, trisomy 8, and an i(17q) (Mitelman et al., 1976). Burkitt's lymphoma may require three or four steps, including an infection with Epstein-Barr virus in early childhood and activation of two oncogenes (Land et al., 1983). A particularly complex disease is colon cancer, in which at least seven genetic changes contribute to an increasingly malignant phenotype (Fig. 26.3). The clinical behavior of a cancer reflects these changes; a slow-growing cancer may suddenly become more malignant or, after responding satisfactorily to radiation or chemotherapy, may suddenly become resistant to treatment.

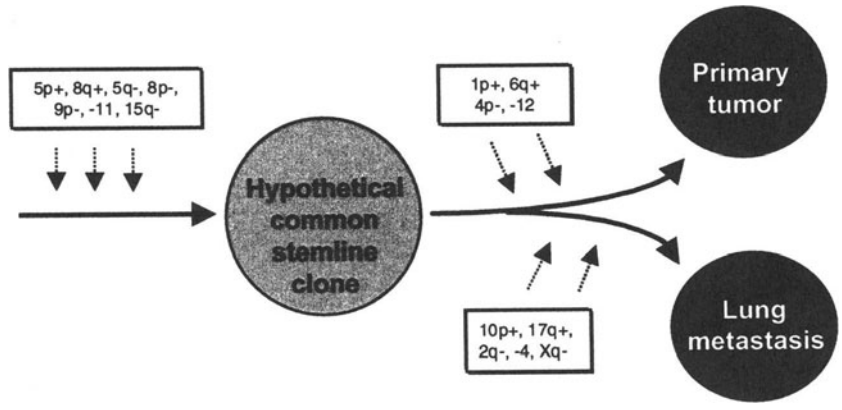

Figure 26.2. CGH analysis comparing chromosome changes in a primary breast cancer with its distant metastasis occurring one year later. Aberrations shared between the two are likely to reflect an early stemline clone from which both primary and metastatic tumors arose by independent clonal evolution. Solid arrows indicate the pathways from normal cell to stemline clone and from the stemline clone to either primary tumor or lung metastasis, short dotted arrows indicate where, in these pathways, various chromosome changes are thought to act (reprinted from Forozan et al., Trends Genet, v13, Genome screening by comparative genomic hybridization, pp 405–409, copyright 1997, with permission from Elsevier Science).

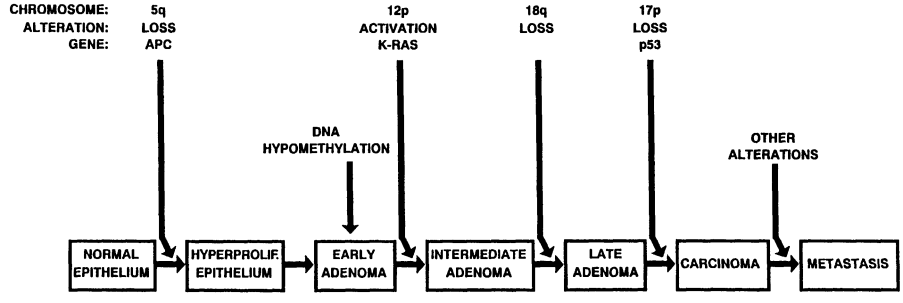

Figure 26.3. Multistep development of colon cancer (Kinzler and Vogelstein, copyright 1995, McGraw-Hill, with permission of the McGraw-Hill Companies).

Spindle Aberrations and Instability of Chromosome Number

Mitotic spindle abnormalities (Fig. 26.4) were first observed in cancer cells over a hundred years ago by Arnold and others. This observation, plus experimental analysis of the effects of multipolar spindles, led Boveri to suggest in 1914 that multipolar spindles and the resultant large variations in chromosome number among the daughter cells (*mixoploidy*) provide the genetic variation needed for the clonal selection of cells capable of uncontrolled growth. There is overwhelming evidence of abnormal spindles in tumor cells, such as the

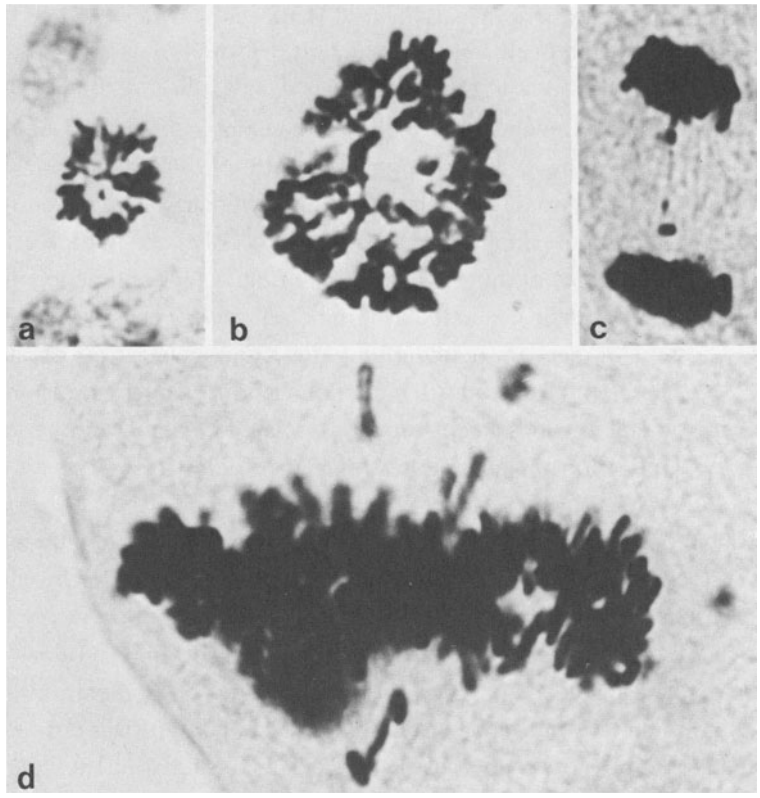

Figure 26.4. Mitotic stages from normal placenta (a) and cervical cancer (b–d). (a) Diploid metaphase (polar view). (b) Metaphase in octaploid range (polar view). (c) Anaphase with a bridge (side view). (d) Giant metaphase (side view) (Therman et al., 1984).

presence of multipolar mitoses (Fig. 26.5), which are practically nonexistent in normal cells (Therman and Kuhn, 1989).

Spindle abnormalities can lead to faulty alignment of the chromosomes in metaphase or anaphase, as seen in most cancers. In *C-mitosis*, named for its resemblance to colchicine-induced metaphase arrest, the chromosomes behave normally through prophase and up until metaphase. A defective or absent mitotic spindle prevents movement of the chromosomes to the metaphase plate or towards the spindle poles. The sister chromatids ultimately separate and form either a tetraploid restitution nucleus or a number of micronuclei of variable sizes. Such micronuclei are usually unable to divide further. C-mitoses and micronuclei are rare in normal cells but occur fairly often in cancer cells, with their abnormal spindles. The induction of micronuclei by spindle poisons such as the colchicine derivative diacetylmethyl (Colcemid) is now widely used for the production of microcell hybrids containing a single human chromosome (Chapter 23).

The centrosome is the chief microtubule organizing center (MTOC) of both interphase and dividing cells (Kellogg et al., 1994), and aberrations of the centrosome are the cause of the enlarged and multipolar spindles seen in many cancer cells. Centrosome duplication usually occurs only once in each cell cycle. However, in the absence of the p53 protein, multiple copies of functionally competent centrosomes can be generated in a single cell cycle (Fukusawa et al., 1996). This may be a major mechanism underlying the carcinogenic effect of mutations in the *TP53* gene, which encodes p53. Amplification or overexpression of the *STK15* gene, which encodes a centrosome-associated kinase, is another cause of centrosome duplication and aneuploidy. It occurs in about 12% of breast cancers and in a wide range of other cancer cell lines (Zhou et al., 1998).

Lengauer et al. (1997) carried out a molecular analysis of aneuploidy in several cloned colorectal cancer cell lines, using multicolor FISH with nine chromosome-specific probes. Some of the cancers showed dramatic variation in the number of copies of each chromosome from cell to cell within individual clones, with loss or gain occurring at a rate of about 1% per cell generation for each chromosome. This chromosomal instability (CIN) phenotype was not secondary to the malignant phenotype, because other cancers of similar phenotype did not show it. Instead, they were near-diploid, with an invariant chromosome number, but showed a microsatellite instability (MIN) phenotype as the result of defective DNA mismatch repair (see below). Nearly 85% of colorectal cancers have the CIN phenotype and 15% the MIN phenotype. CIN is dominant: Fusion of CIN with non-CIN cells produces hybrids with a CIN phenotype (Cahill et al.,

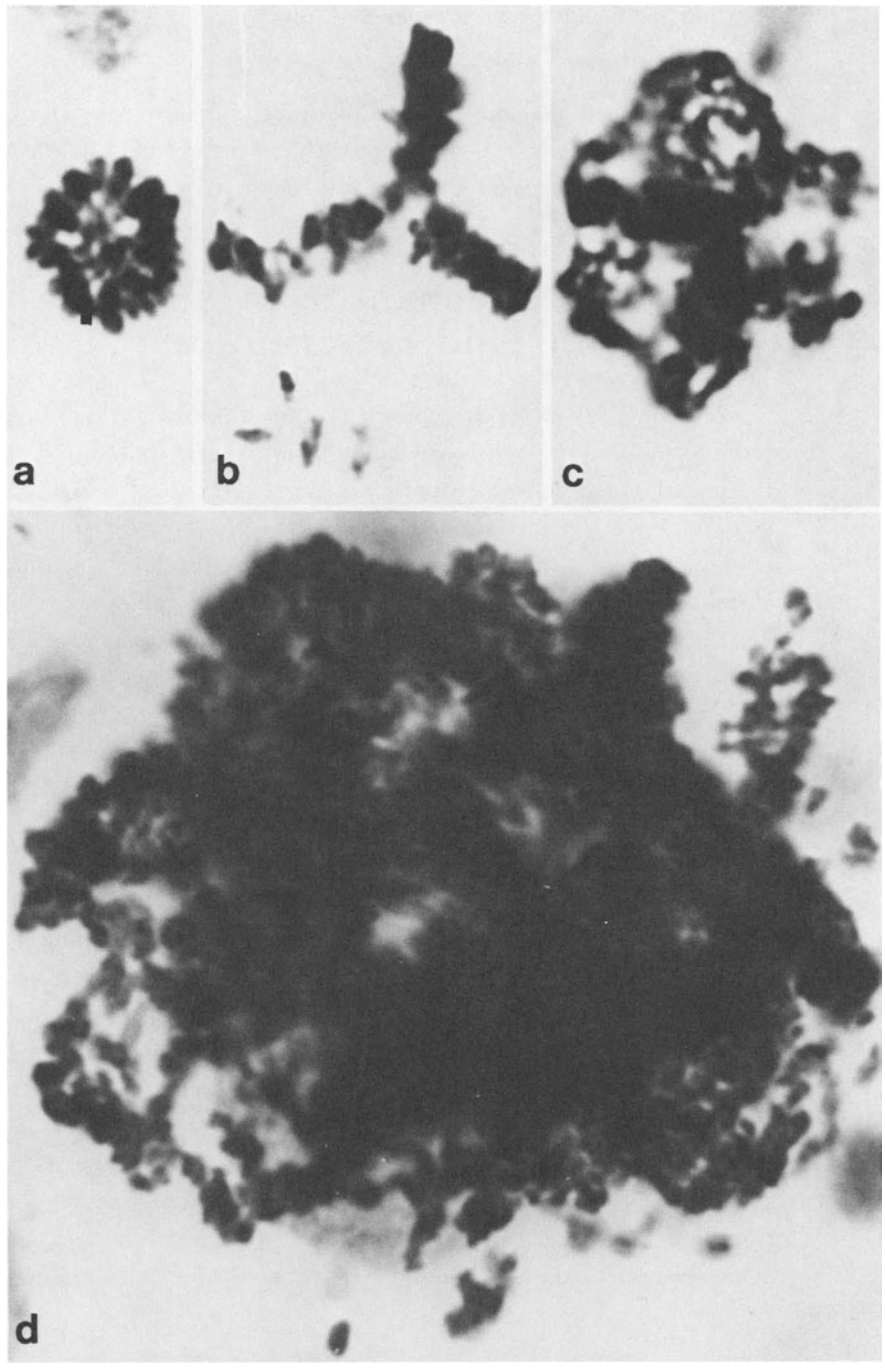

Figure 26.5. Metaphase spreads from normal trophoblast (a) and from cervical cancer (b–d). (a) Diploid metaphase in polar view. (b) Side-view of a tripolar metaphase with laggards. (Therman et al., 1984). (c) Six-polar metaphase. (d) Highly polyploid metaphase with numerous poles (Therman and Kuhn, 1989, reprinted with permission from CRC Press Inc.).

1998). Microcell-mediated transfer of single chromosomes from CIN cells to non-CIN cells might be useful in identifying the chromosome, and ultimately the gene, whose mutation is responsible for the CIN phenotype.

The Major Mechanism of Spindle Aberrations and Heteroploidy

Genome integrity depends upon checkpoint mechanisms that arrest cell cycle progression until damage produced by genotoxic agents or microtubular toxins is repaired or cell death by apoptosis is initiated (Hartwell and Kastan, 1994). In Chapter 2, we discussed the *spindle assembly checkpoint*, which prevents a cell from entering anaphase until a spindle is fully assembled and the kinetochore of every chromosome has a bipolar attachment to the spindle poles. The product of the *TP53* gene, p53, is required for proper function of this checkpoint. Information is growing on just how this works and on the other genes involved. A mutation of *TP53* is present in more than half of all cancers (Hainaut et al., 1997), making it the most common cause of the spindle abnormalities and heteroploidy seen in cancer. The absence of functional p53 protein can be the result of either mutation or loss of both copies of the gene. However, in many cases, a single dominant negative mutation is found in which the abnormal p53 protein from the mutant allele inhibits the product of the normal allele (Gualberto et al., 1998).

Several other genes have recently been identified whose products are required for the spindle assembly checkpoint to function. These include *MAD2* and *BUB1*. The expression of *MAD2* is reduced in some breast cancer cells with abnormal checkpoint function. Mutations in *BUB1* are fairly common in some cancers with chromosome instability and aneuploidy due to deficient checkpoint function (Cahill et al., 1998). Sarcomas and other tumors without a *TP53* mutation may show overexpression of the *MDM2* (murine double minute 2) gene. *MDM2* binds to the p53 protein and targets it for rapid degradation. Overexpression, which is sometimes the result of amplification of the *MDM2* gene, may thus be oncogenic because it reduces the level of p53 protein (Momand and Zambetti, 1996).

Structural Chromosome Changes

The presence of large marker chromosomes in some cancer cells was noted many years ago. As cytological methods improved, it became clear that there were

structurally altered chromosomes of all sizes, going all the way down to double minute chromosomes (DMs). The introduction of banding techniques made it possible to identify dozens, and eventually hundreds, of specific translocations, inversions, and deletions, as well as a new type of marker chromosome with a nonbanding, homogeneously staining region (HSR). A single tumor, or a cell line derived from it, usually has several structurally rearranged, or *marker*, chromosomes, indicating a general breakdown of the mechanisms for maintaining chromosome integrity. This is illustrated by the high frequency of ectopic nucleolus organizer regions in cancer cells, the result of frequent recombination events (Atkin and Baker, 1995). Although some of the rearranged chromosomes in cancer cells remain unchanged over many years of growth in culture, as expected if some of the rearrangements play a role in carcinogenesis or tumor progression, cancer cells also continue to generate new marker chromosomes. Miller (1973) developed a simple method to estimate the heterogeneity of any cell population from the karyotype analysis of as few as 5–10 cells. Comparative genomic hybridization and FISH with chromosome painting or microdissection-PCR probes (Chapter 8) are powerful methods for identifying the origin and make-up of rearranged chromosomes.

DNA Damage Checkpoint and Structural Instability of Chromosomes

A breakdown of the G1 DNA damage checkpoint is the major mechanism underlying the structural instability of chromosomes that is so important in tumorigenesis and tumor progression. This checkpoint arrests the cell cycle in G1 when there is DNA damage and blocks entry into S until the DNA damage has been repaired or the cell dies (Chapter 2). This is important because nucleotide substitutions and DNA strand breakage are extremely common events. Spontaneous DNA breaks are induced by the oxidants that are produced in such abundance in our cells by normal metabolic processes (Ames, 1989). The cellular DNA repair machinery normally repairs all but the minute fraction of these that account for most of the background mutation rate. If the damage is not repaired before DNA replication, the result is a mutation or a double-strand break (DSB). DSBs can lead to chromosome rearrangements (Chapter 14); they are also essential for gene amplification to occur (Chapter 25).

The G1 DNA damage checkpoint function requires the normal protein products of the *ATM* (ataxia telangiectasia) and *TP53* genes (Chapter 2). *ATM* muta-

tions are carcinogenic, though rare (Chapter 24), but *TP53* mutations are present in more than half of all cancers, as noted above. *TP53* mutations act as a "mutator phenotype" by allowing cells to live (since apoptosis requires p53) and the S phase to proceed despite DNA damage. This leaves unrepaired DNA and leads to chromosome breaks and point mutations. Because they disable the checkpoint and are so abundant, *TP53* mutations are the major cause of the structural as well as the numerical chromosome changes seen in cancer. This includes the gene amplifications responsible for DMs and HSRs, because of the role DSBs play in their origin. The introduction of wild-type p53 restores cell cycle checkpoint control and inhibits gene amplification in cells with nonfunctional mutant *TP53* alleles (Yin et al., 1992).

Why should *TP53* mutations be so prevalent in cancer cells? One reason is that many of the *TP53* mutations produce a dominant negative phenotype; that is, a *single* mutant allele results in the absence of any functional p53 protein in the cell (Gualberto et al., 1998). For other tumor suppressor genes, mutation or loss of *both* copies of the gene is required, and this is much less common (the probability of two independent mutations versus the probability of one mutation). Another reason is that *TP53* has several mutational hotspots. Of more than 5000 somatic mutations of *TP53*, nearly 20% occurred in only five codons: those for amino acids 175, 245, 248, 249, and 273 in the p53 protein (Hainaut et al., 1997).

A striking feature of these hotspots is that they vary from cancer to cancer, reflecting the different environmental agents involved in their causation. For example, in skin cancers, where exposure to sunlight is the carcinogenic agent, the hotspots are adjacent pyrimidines, because ultraviolet light induces the cyclobutane ring type of pyrimidine dimers from adjacent TT, TC, and CC pairs. *TP53* mutations are seen in 90% of squamous cell carcinomas but in only 50% of basal cell carcinomas, which arise from a deeper layer of the skin, although they still show the mutation signature of UV damage. In contrast, in lung cancer, there are three different hotspots, at the codons for amino acid positions 157, 248, and 273 in the p53 protein. These are the sites of selective strong binding of the metabolic product of benzpyrene, the major carcinogen in tobacco smoke, providing a direct etiologic link between a carcinogen in tobacco smoke and lung cancer. All three codons contain a methylated CpG dinucleotide, and the benzpyrene metabolite does not bind if these sites are experimentally demethylated (Denissenko et al., 1997).

Other Causes of Structural Instability of Chromosomes

Mutations of several poorly characterized genes that are involved in spindle assembly and DNA damage checkpoints account for some cases of chromosome instability. One of these genes, *RAD1*, is an essential component of the cell cycle checkpoint that is activated by DNA damage. *RAD1* maps to 5p13.2, a region that is frequently altered in small cell lung cancer, bladder cancer, squamous cell carcinoma, and adenocarcinoma (Marathi et al., 1998). Malfunction of the DNA damage checkpoint is not the only cause of structural instability. Failure to repair damaged DNA can also lead to chromosome changes and an increased risk of cancer.

There are multiple DNA repair pathways, each of them dependent upon the normal function of dozens of genes. Mutations of some of these occur, but are rare (Chapter 24). However, some are considerably more common, for example, genes in the DNA mismatch repair pathway that is initiated when a nucleotide sequence in one strand of the DNA ends up with an illegitimate pairing partner. The altered conformation triggers a repair pathway that involves nicking of one strand about 100 bp both 5′ and 3′ of the mismatch, excising the mismatched region and filling in the gap by using the other strand as template. Mutations in four of the five genes known to be required for this *long patch repair* have been seen in hereditary nonpolyposis colon cancer (HNPCC), a family of autosomal dominant disorders (Kolodner, 1995). Female carriers of a mutant *MSH6* gene are even more likely to develop uterine endometrial hyperplasia and carcinoma than colon carcinoma (Wijnen et al., 1999). Mutations in the related genes *MSH2*, *MLH1*, *PMS2*, and *PMS1* produce a mutator phenotype, with elevated frequencies of mutations of many other genes and of microsatellite repeats, such as dinucleotide or trinucleotide repeats. Methylation of the *MLH1* promoter silences the gene and is the primary cause of the microsatellite instability seen in sporadic endometrial cancers (Simkins et al., 1999).

Double-strand breaks in DNA are repaired by a pathway that involves using an undamaged homologous DNA molecule as the template for repair and is called *recombinational DNA repair* (Chapter 14). Two of the proteins in the DSB repair complex are RAD50 and MRE11. *RAD50* maps to 5q31.1, the region most often deleted in acute myeloid leukemia, and *MRE11* maps to 11q21, a region frequently altered in various cancers (Dolganov et al., 1996). The two major

breast cancer susceptibility genes, *BRCA1* and *BRCA2*, are probably also involved in DSB repair. Their products are associated with the RAD51 protein, another member of the DSB repair complex (Patel et al., 1998).

Changes in DNA methylation may be an important cause of structural changes, as many cancers show genome-wide demethylation. Demethylation affects all classes of transposable elements, including the very abundant L1 LINE retrotransposons, which are the major sites of methylation throughout the genome. Demethylation of their genes, including the reverse transcriptase gene, may be the reason for the much greater abundance of their transcripts in cancer cells than in normal cells (Jürgens et al., 1996). If this leads to increased transpositional activity, it increases genome instability, because movement of transposons is associated with chromosome breakage, as pointed out many years ago by McClintock. The induction of multiradials by demethylation of satellite DNAs (Chapter 22) may be due to the activation of L1 LINE retrotransposons in heterochromatin. The generally high level of methylation of transposable elements, coupled with the well-known silencing of gene expression by methylation, has led to the hypothesis that DNA methylation developed as a mechanism for inactivating incoming DNA sequences, such as retroviruses, that integrate into the host's genome (Yoder et al., 1997).

Demethylation can lead to the relaxation or loss of imprinting of specific genes frequently seen in cancers. Two-thirds of Wilms tumors that are not associated with LOH of markers on 11p show biallelic expression (loss of imprinting) of the *H19* and *IGF2* (insulin-like growth factor 2) genes at 11p15.5, leading to overexpression of the proliferation-promoting *IGF2* product (Rainier et al., 1993).

Environmental Causes of Cancer

The increasing molecular evidence of the important role mutations play in tumorigenesis and progression comes as no surprise to those who learned long ago of the importance of environmental agents as causes of cancer, because most carcinogens are mutagens, such as X-rays, other types of radiation, chemicals, and viruses. A few, such as the phorbol esters, are not. Instead, they act as tumor promoters, primarily by stimulating cell proliferation. Many mutations arise as errors of DNA replication, so tumor promoters increase the chance that another mutation can arise in a cell lineage that already harbors one or more relevant mutations. DNA tumor viruses such as polyoma, SV40, human papilloma (HPV),

and adenovirus, can transform diploid fibroblasts into immortal cells that can ultimately become malignant.

Virus-induced cell transformation is a multistep process that requires the continued presence of a single viral protein: large T for polyoma or SV40, E1A for adenovirus, or E7 for HPV. These proteins bind, and inactivate, the RB1 tumor suppressor protein (Ko and Prives, 1996); HPV E7 also binds the p53 tumor suppressor protein and induces its degradation (White et al., 1994). The normal p53 protein is polymorphic, sometimes with proline in position 72 and sometimes with arginine. Although both alleles are functional, arg72-p53 is more readily degraded than pro72-p53. Patients with HPV-associated cervical cancers are much more likely to be homozygous for the arginine-encoding allele than expected from the allele frequencies. In fact, individuals homozygous for the arg-encoding allele have a seven-fold greater risk of developing cervical cancer than heterozygotes do but no increased risk for developing non-HPV-associated cancers (Storey et al., 1998).

Just how important are environmental mutagens and tumor promoters in comparison to inherited mutations or other endogenous risk factors? They may be extremely important. Different populations show striking differences, up to 30-fold or greater, in the incidence of many different types of cancer. This is far more than could be accounted for by genetic differences between the populations. Furthermore, a specific environmental factor has been demonstrated in some cases, such as tobacco smoking in lung cancer, Epstein-Barr virus in nasopharyngeal carcinoma and Burkitt lymphoma, papilloma virus in cervical cancer, and sunlight (UV) exposure in skin cancer. As many as 15% of all cancers in developed countries may be virus-related and 25% in developing countries (Zur Hausen, 1997).

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