Cancer: Cell Structures, Carcinogens and Genomic Instability
Edited by Leon P. Bignold

Molecular mechanisms of human carcinogenesis

William B. Coleman[1] and Gregory J. Tsongalis[2]

[1] *Department of Pathology and Laboratory Medicine, Curriculum in Toxicology, UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill NC, 27599, USA*
[2] *Laboratory of Molecular Pathology, Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, USA*

Abstract. Intensive research efforts during the last several decades have increased our understanding of carcinogenesis, and have identified a genetic basis for the multi-step process of cancer development. Tumors grow through a process of clonal expansion driven by mutation. Several forms of molecular alteration have been described in human cancers, and these can be generally classified as chromosomal abnormalities and nucleotide sequence abnormalities. Most cancer cells display a phenotype characterized by genomic hypermutability, suggesting that genomic instability may precede the acquisition of transforming mutations in critical target genes. Reduced to its essence, cancer is a disease of abnormal gene expression, and these genetic abnormalities contribute to cancer pathogenesis through inactivation of negative mediators of cell proliferation (including tumor suppressor genes) and activation of positive mediators of cell proliferation (including proto-oncogenes). In several human tumor systems, specific genetic alterations have been shown to correlate with well-defined histopathological stages of tumor development and progression. Although the significance of mutations to the etiological mechanisms of tumor development has been debated, a causal role for such genetic lesions is now commonly accepted for most human cancers. Thus, genetic lesions represent an integral part of the processes of neoplastic transformation, tumorigenesis, and tumor progression, and as such represent potentially valuable markers for cancer detection and staging.

Keywords: Chromosomal instability, genomic instability, microsatellite instability, tumor suppressor gene, proto-oncogene.

Cancer: a multi-step genetic disease

Cancer development is a multi-step process through which cells acquire increasingly abnormal proliferative and invasive behaviors. Cancer also represents a unique form of genetic disease, characterized by the accumulation of multiple somatic mutations in a population of cells undergoing neoplastic transformation [1, 2]. Genetic lesions represent an integral part of the processes of neoplastic transformation, tumorigenesis, and tumor progression, and as such represent potentially valuable markers for cancer detection and staging [3, 4]. Several forms of molecular alteration have been described in human cancers, including gene amplifications, deletions, insertions, rearrangements, and point mutations [2]. In many cases specific genetic lesions have been identified that are associated with neoplastic transformation and/or tumor progression in a particular tissue or cell type [1]. Statistical analyses of age-specific

mortality rates for different forms of human cancer predict that multiple mutations in specific target genes are required for the genesis and outgrowth of most clinically diagnosable tumors [5]. In accordance with this prediction, it has been suggested that tumors grow through a process of clonal expansion driven by mutation [6], where the first mutation leads to limited expansion of progeny of a single cell, and each subsequent mutation gives rise to a new clonal outgrowth with greater proliferative potential. The idea that carcinogenesis is a multi-step process is supported by morphological observations of the transitions between pre-malignant (benign) cell growth and malignant tumors. In colorectal cancer (and some other tumor systems), the transition from a benign lesion to a malignant neoplasm can be easily documented and occurs in discernible stages, including benign adenoma, carcinoma *in situ*, invasive carcinoma, and eventually local and distant metastasis [7]. Moreover, specific genetic alterations have been shown to correlate with each of these well-defined histopathological stages of tumor development and progression [8]. However, it is important to recognize that it is the accumulation of multiple genetic alterations in affected cells, and not necessarily the order in which these changes accumulate, that determines tumor formation and progression. These observations suggest strongly that the molecular alterations observed in human cancers represent integral (necessary) components of the process of neoplastic transformation and tumor progression.

Mutations and cancer

Mutation is the ultimate source of variability for individual cells (and organisms), and is an essential component of the process of natural selection [9]. Tumorigenesis can be viewed simply as a process of natural selection in which cells develop a growth advantage that allows them to proliferate and invade under conditions where other (normal) cells cannot, and the acquisition of this ability is driven by mutation. In other words, tumor development and progression represents a form of somatic evolution, at the ultimate expense of the host organism [10]. The idea that somatic mutation could significantly contribute to cancer development was suggested by Boveri early in the 20th century [11]. At about the same time, De Vries proposed that certain forms of radiation (Röntgen rays) may be mutagenic [10], suggesting that mutation rates could be influenced by exogenous factors. Evidence from numerous investigations suggests that multiple somatic mutations contribute to the step-wise process of neoplastic transformation and tumorigenesis. In early studies, the nature of these mutations and their contributions to tumorigenesis were not at all clear. Nonetheless, the presence of multiple mutations in cancer cells could be observed in the form of karyotypic alterations and abnormal chromosome numbers in tumor cells. More recent studies utilizing comparative genomic hybridization extended these observations by identifying both gross (cytogenetically detectable) and subtle chromosomal abnormalities in different human

neoplasms [12]. Subsequently, numerous positive and negative mediators (proto-oncogenes and tumor suppressor genes) of cell growth and differentiation have been identified and characterized, defining the basic role for these critical genetic elements in neoplastic transformation and tumorigenesis [1, 13]. Recently, microarray-based gene expression studies have provided definitive evidence that cancer is ultimately a disease of abnormal gene expression [14–16]. Somatic mutations occurring in developing cancers alter gene expression patterns, resulting in significant changes to cellular physiology, including unregulated (or abnormally regulated) cell proliferation and acquisition of invasive behaviors [17, 18]. The gene expression signature of a specific cancer can be used in differential diagnosis, prognostication, and prediction of responses to therapy [19, 20].

The exact number of critical mutations required for neoplastic transformation of normal cells is not known. Investigations involving the statistical analysis of human tumor incidence and natural history in sporadic and inherited human tumors formed the basis for the two-hit model of cancer development [21, 22]. In this model, genetic predisposition for a specific type of neoplasm is conferred on an individual that either inherits or acquires a germline mutation in one allele of a critical target (such as a tumor suppressor gene), constituting the first "hit", and the second "hit" represents an acquired somatic mutation in the remaining normal allele. Accumulation of two hits alters (or eliminates) normal gene function in affected cells, which proliferate to form a tumor. While the kinetics of tumor formation are consistent with this model for some neoplasms, it is now recognized that neoplastic transformation involves the mutational alteration or aberrant expression of multiple genes that function in cell proliferation or differentiation. Furthermore, epigenetic mechanisms can contribute to the multi-hit model of cancer induction through the silencing of critical genes [23–25]. In recent years, a re-examination of the number of critical mutations needed for cancer development led to the suggestion that six to eight mutations may be necessary for progression to an invasive tumor [5, 26]. These analyses provide estimates of the numbers of mutations involving genes that control proliferation and differentiation that are necessary for neoplastic transformation of a specific cell type. However, numerous lines of evidence suggest that tumors are mutation prone and/or accumulate large numbers of mutations [6, 27, 28], and some investigators have estimated that tumor cells may contain thousands or tens of thousands of mutations [29, 30].

Molecular alterations in cancer

The molecular alterations occurring in cancer typically reflect mutations, and can be categorized into two major groups: (i) chromosomal abnormalities, and (ii) nucleotide sequence abnormalities. There has been some debate in the literature as to which forms of mutation are more prevalent in cancer cells and/or constitute the foundations of the molecular mechanism of neoplastic transfor-

mation [31]. However, there is abundant evidence that representations of both of these major categories of genetic abnormalities exist in most tumor cells, and that both significantly contribute to neoplastic transformation.

Chromosomal abnormalities

Chromosomal alterations in cancer include the gain or loss of one or more chromosomes (aneuploidy), chromosomal rearrangements resulting from DNA strand breakage (translocations, inversions, and other rearrangements), and gain or loss of portions of chromosomes (amplification, large-scale deletion). The direct result of chromosomal translocation is the movement of some segment of DNA from its natural location into a new location within the genome, which can result in altered expression of the genes that are contained within the translocated region. If the chromosomal breakpoints utilized in a translocation are located within structural genes, then hybrid (chimeric) genes can be generated. The major consequence of chromosomal deletion (involving a whole chromosome or a large chromosomal region) is the loss of specific genes that are localized to the deleted chromosomal segment, resulting in changes in the copy number of the affected genes. Likewise, gain of chromosome number or amplification of chromosomal regions results in an increase in the copy numbers of genes found in these chromosomal locations.

Nucleotide sequence abnormalities

Nucleotide sequence alterations in cancer include changes in individual genes involving single nucleotide changes (missense and nonsense), and small insertions or deletions (some of which result in frameshift mutations). Single nucleotide alterations that involve a change in the normal coding sequence of the gene (point mutations) can give rise to an alteration in the amino acid sequence of the encoded protein. Missense mutations alter the translation of the affected codon, while nonsense mutations alter codons that encode amino acids to produce stop codons. This results in premature termination of translation and the synthesis of a truncated protein product. Small deletions and insertions are typically classified as frameshift mutations, because deletion or insertion of a single nucleotide (for instance) will alter the reading frame of the gene on the 3' side of the affected site. This alteration can result in the synthesis of a protein that bears very little resemblance to the normal gene product, or production of an abnormal/truncated protein due to the presence of a stop codon in the altered reading frame. In addition, deletion or insertion of one or more groups of three nucleotides will not alter the reading frame of the gene, but will alter the resulting polypeptide product, which will exhibit either loss of specific amino acids or the presence of additional amino acids within its primary structure.

Are cancer cells prone to mutation?

It is widely accepted that cancer cells accumulate numerous genetic abnormalities (consisting of chromosomal alterations and/or nucleotide sequence mutations) during the protracted interval between the initial carcinogenic insult and tumor outgrowth. At least a portion of the genetic changes occurring in neoplasia are related to the underlying molecular mechanism of neoplastic transformation [26, 32, 33]. Nonetheless, whether the myriad of genetic lesions found in cancer cells are the causes or consequences of neoplastic transformation continues to be the subject of debate [34]. Some investigators have suggested that the intrinsic mutation rate in mammalian cells is insufficient to account for the numerous genetic changes observed in cancer cells, leading to the suggestion that an early (essential?) step in neoplastic transformation is the development of a condition of hypermutability or genetic instability [35, 36]. In the past, increased rates of mutation in pre-neoplastic or neoplastic cells would have been attributed to exposure of these cells to exogenous mutagenic agents. However, more recent analyses of the nature and frequency of mutations occurring in human neoplasms suggests that a significant proportion result from spontaneous mutational mechanisms [37]. This observation strengthens the suggestion that cancer cells may exhibit diminished capacities for surveillance and repair of DNA lesions, leading to increased rates of spontaneous mutation and/or increased susceptibility to mutation following exposure to some exogenous carcinogenic agent. An alternative argument suggests that increased rates of mutation are not necessary for accumulation of large numbers of genetic lesions in cancer cells, but that selection of advantageous mutations is a more important feature of the process of tumorigenesis [36, 38].

Spontaneous mutation rates in normal cells

The measured spontaneous mutation rate of mammalian cells depends upon the exact experimental conditions employed and the nature of the cells and target sequence examined [39]. Somatic mutation rates have been determined for a variety of cultured cell types through examination of the spontaneous mutation frequency at one of several specific loci, such as the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) gene, the Na^+-K^+-*ATPase* gene, or the adenine phosphoribosyltransferase (*APRT*) gene. Using the results from several of these studies [40–42], the spontaneous mutation frequency at the HPRT locus can be estimated to be approximately 2.7×10^{-10}–1×10^{-9} mutations/nucleotide/cell generation in untransformed human cells. This mutation rate is sufficient to yield approximately three mutations per cell over the lifespan of an individual, which may be too low to account for the number of mutations thought to be required for carcinogenesis. This observation led to the hypothesis that an early event in neoplastic transformation may involve an increase in the spontaneous mutation rate in cells that are progressing through

this multi-step pathway [42]. Cells expressing the "mutator phenotype" accumulate mutations more rapidly than normal cells, and would therefore be more likely to sustain mutations in critical genes required for enhanced growth and tumorigenesis [43, 44].

Mutation rates in cancer cells

In many studies, the measured mutation rate in malignant cells is significantly higher than that of corresponding normal cells. In some cases the elevated mutation rates were 100-fold higher than in untransformed cells [45, 46]. Tumor cell lines that are deficient for DNA repair exhibit mutation rates that are 750-fold higher than that displayed by DNA repair-proficient tumor cell lines [47]. In addition, the rate of gene amplification in malignant cells is much higher than in normal cells [48]. However, other studies find no difference in the spontaneous mutation rate between normal and malignant cells [40, 41], or suggest that selective pressures associated with clonal expansion of altered cells represent a much more important feature of carcinogenesis than a hypermutational phenotype [38]. Thus, some cancer cells may express a "mutator phenotype" and exhibit an enhanced mutation rate compared to normal cells [28], whereas other cancers may exhibit multiple mutations in the absence of any appreciable increase in mutation frequency. These observations suggest the possibility that multiple molecular mechanisms are needed to reconcile the occurrence of multiple mutations in human cancers and the expression of a mutator phenotype with elevated mutational frequency in only a subset of these tumors.

Genomic instability in human cancer

An appropriate definition of genomic instability is needed before a complete understanding of the interconnecting causes and consequences of genomic instability can be developed, and the contribution of this phenomenon to neoplastic transformation can be appreciated. The observation that most cancer cells contain discernible genetic abnormalities (chromosomal aberrations and/or DNA sequence abnormalities) suggests that all neoplastically transformed cells have sustained genetic damage and may have experienced some form of genomic instability. Normal human cells demonstrate a remarkable degree of genomic integrity, which reflects the combined contributions of high-fidelity DNA replication processes, and the expression of multiple mechanisms that recognize and repair DNA damage. Nonetheless, rare spontaneous mutations can occur in cells that are proficient for both DNA replication and repair. The observation that neoplastic cells contain variable numbers of mutations reflecting specific forms of DNA damage, and that tumors develop over widely variable periods of time, suggests the possible involvement of different

pathogenic mechanisms that may reflect multiple distinct mutagenic pathways to neoplastic transformation. Tumors are highly variable with respect to their growth characteristics; some tumors become clinically evident early in the human lifespan, while others present later in life. This discrepancy could reflect individual differences among tumors with respect to the relative rapidity of their development and progression. Consistent with the proposal that tumors form through clonal expansion driven by mutation [49–51], tumors displaying early onset and rapid progression may accumulate a critical level of genetic damage more quickly than tumors with later onset and more indolent course.

The forms of genetic damage typically displayed by cancer cells (involving chromosomal alterations and/or DNA sequence alterations) are not mutually exclusive. However, different mutagenic mechanisms may be involved in the origins of these genetic abnormalities [52–54]. Nonetheless, it is likely that the same target genes might be involved in tumorigenesis driven by either form of genetic damage. Inactivation of the p53 tumor suppressor gene (loss of function) can be accomplished through point mutation at numerous nucleotide sites [13, 55] or through deletion of the locus on 17p [56]. Likewise, proto-oncogene activation can be accomplished by point mutation, as with the H-*ras* gene [57], or by chromosomal translocation, as with the c-*myc* gene [58].

Based upon these observations, a unifying hypothesis is required to describe the possible mechanisms of genomic instability that account for the disparate numbers of mutations (specific loci *versus* widespread mutation) and diverse nature of genetic damage (types of mutations) that characterize various human cancers. We have proposed that at least two broad categories of genomic instability may exist: (i) progressive (persistent) genomic instability, and (ii) episodic (transient) genomic instability [59]. Evidence supporting the existence of these forms of genetic instability has emerged from studies in bacteria [60], and examples of each form of genomic instability have been described in human cancers. Progressive instability defines an ongoing mutagenic process, with new mutations occurring in each cell generation, and is associated with cells that are compromised in their ability to safeguard genomic integrity. This form of genomic instability would be transmitted from cell generation to cell generation as a heritable trait [60]. For instance, tumor cells from patients with hereditary nonpolyposis colorectal cancer (HNPCC) exhibit progressive genomic instability, which is manifest as alterations in microsatellite sequences [61, 62]. In contrast to progressive instability, episodic instability describes sporadic genetic damage in cells that are proficient in the various pathways that govern genomic homeostasis. This form of instability is associated with tumors that contain specific mutations and/or chromosomal alterations, in the absence of wide-spread damage to the genome. The transient mutator state may account for a large portion of adaptive mutations occurring in cells [60]. For instance, cells exposed to high levels of oxidative stress may incur and accumulate adaptive mutations that enable the altered cells to thrive under highly selective conditions [63, 64]. These mutations can occur in the

absence of cell proliferation [43, 44], but would facilitate clonal expansion of an altered clone in response to subsequent selection pressures [36]. Numerous sporadic tumor types exemplify this form of instability, including sporadic colorectal tumors of the tumor suppressor pathway [65], or the microsatellite mutator pathway [64, 66]. It can be envisioned that both chromosomal abnormalities and DNA sequence abnormalities could result from the expression of either of these forms of genomic instability during neoplastic transformation.

Chromosomal abnormalities in cancer

The majority of human cancers (including solid tumors, leukemias, and lymphomas) contain chromosomal abnormalities, consisting of either numerical changes (aneuploidy) and/or structural aberrations [67, 68]. These types of chromosomal damage may reflect two distinct mechanisms of chromosomal instability [2, 69]: (i) chromosome number instability, and (ii) chromosome structure instability. Recent evidence suggests a genetic basis for chromosomal instability in cancer, involving mutational inactivation of certain types of genes in aneuploid tumors [70].

Detailed karyotypic studies have been performed on a large number of tumor types; many of these studies have examined leukemia and lymphoma, partially reflecting the relative ease with which chromosomes can be prepared from these cancer cells. Traditional cytogenetic analyses of solid tumors are more difficult. Nonetheless, a substantial body of literature on the chromosomal aberrations of solid tumors has emerged [12]. Additional methods have also been applied to examination of chromosomal abnormalities in solid tumors [68]. Numerous studies have investigated allelic loss of heterozygosity (LOH) in various human solid tumors using Southern analysis or PCR [71–75]. While these methods do not provide the same information as karyotypic analysis, large-scale deletions can be inferred from the loss of multiple markers on a specific chromosomal arm [76]. In addition, flow cytometry is now widely employed for determination of tumor ploidy [77], and fluorescence *in situ* hybridization (or derivative methods) is used to examine specific chromosome numbers and alterations [78–80]. A detailed review of chromosomal alterations in human cancer is beyond the scope of this chapter. Several excellent reviews are available [12, 81].

Instability of chromosome number

Numerical alterations of chromosomes can involve both loss of entire chromosomes or allelic losses, which may be accompanied by duplication of the remaining allele. This phenomenon results in the generation of a tumor with normal karyotype, but an abnormal allelotype [71]. Several studies suggest that tumors arising in various tissues share a common chromosome number

instability and may loose a significant number (25–50%) of alleles during neoplastic transformation and tumorigenesis [71, 73, 82, 83]. These large-scale genomic changes may be due to some form of progressive chromosomal instability [84, 85]. Supporting this suggestion, gains and losses of multiple chromosomes occur in aneuploid colorectal cancer cell lines 10- to 100-fold more frequently than in diploid cancer cell lines of the same histological subtype [53, 86]. In other studies, the rate of LOH at marker loci proximal to a selectable gene (*APRT*) was increased 10-fold in colorectal cancer cell lines that exhibit proficiency of mismatch repair (MMR) compared with cell lines that lack MMR [87, 88]. In addition, numerous studies combine to show that aneuploid cancers exhibit highly variable karyotypes [67, 89], suggesting that new chromosomal variations are produced in a progressive manner during tumor outgrowth and evolution.

The absence of chromosomal instability in diploid cancers and/or cancers that exhibit nucleotide sequence alterations, argues against a nonspecific mechanism for chromosomal instability related to abnormal properties of neoplastic cells [2]. Further, the high rates of numerical chromosomal alterations in aneuploid cells do not simply reflect the ability of these cells to survive changes in chromosome number [53]. Tetraploid cells resulting from the fusion of diploid cancer cells retain a stable tetraploid chromosome number [53], suggesting that the presence of a nondiploid chromosome number does not precipitate progressive chromosomal instability. Rather, the evidence supports the existence of a specific form of genetic instability in cancer cells that results from dysfunction of normal chromosomal homeostasis producing numerical chromosomal abnormalities. Several possibilities have been investigated, including the involvement of (i) mutant p53 protein, (ii) abnormal centrosomes, (iii) abnormal mitotic spindle checkpoint function, or (iv) abnormal DNA-damage checkpoint function [2, 85, 90].

Inactivation of the p53 tumor suppressor leads to abnormalities of chromosome number

The p53 tumor suppressor protein has long been suggested to play significant roles in cell cycle progression and cell cycle checkpoint function in response to DNA damage [91, 92]. The *p53* gene is commonly mutated in human cancers [37], and these same cancers frequently exhibit abnormalities of chromosome number [93–95]. Cells in culture often become aneuploid concurrent with mutation or inactivation of *p53* [96, 97], suggesting that loss of p53 function leads to abnormal regulation of mitosis and segregation of chromosomes [98]. However, other lines of evidence do not support a direct role for *p53* mutation in chromosomal instability. For example, aneuploidy occurs very early in the process of neoplastic transformation and tumorigenesis [99], and *p53* mutation typically occurs later in the process [100]. In addition, some diploid tumor cell lines that exhibit a stable karyotype also contain mutant *p53*

[101]. These observations suggest that loss of normal p53 function may contribute significantly to chromosomal instability in certain forms of cancer, but does not represent the primary cause of this form of genomic instability.

Abnormal centrosome function leads to chromosomal abnormalities

Aneuploid tumors demonstrate significant numbers of chromosomal imbalances, whereas such imbalances are rare in diploid tumors. The abnormalities of chromosome number observed in aneuploid tumors are consistent with a mechanism involving dysfunction of chromosome segregation during mitosis. Several lines of evidence support the idea that the integrity of the centrosome plays an integral role in the development of aneuploidy. Human tumors and tumor-derived cell lines have been characterized to contain abnormal numbers of centrosomes, abnormally sized and shaped centrosomes, and multipolar spindles in a number of human neoplasms, including tumors of the breast, lung, prostate, colon, pancreas, head and neck, bile duct, and brain [102, 103]. Aneuploid colorectal carcinoma (CRC) cell lines displayed elevated centrosome numbers compared to diploid tumor cell lines, which displayed normal centrosome numbers [86]. Further, centrosome function was impaired in most aneuploid CRC cell lines examined, whereas centrosome function was found to be intact in all diploid tumor cell lines [86]. These observations suggest that abnormal centrosome number and/or function are common among neoplastic cells that display aneuploidy, and may represent an essential component of chromosome number instability in human cancers.

The mechanism leading to formation of increased numbers of centrosomes in cancer cells remains undefined. However, abnormal centrosome number and function has been linked to the STK15 kinase in some cancers [104, 105], and to a related kinase (PLK1) in others [106]. The *STK15* gene was found to be amplified in approximately 12% of primary breast cancers, and in cell lines derived from neuroblastoma and tumors of the breast, ovary, colon, prostate, and cervix [105]. Overexpression of STK15 was detected (by immunostaining) in 94% of invasive ductal carcinomas of the breast irrespective of histopathological subtype, suggesting that overexpression of this centrosome-associated kinase may be a common feature of breast cancers [107]. In addition, overexpression of STK15 was found in cell lines that lacked evidence of gene amplification, and ectopic expression of STK15 in near diploid human breast epithelial cells produced centrosome abnormality accompanied by induction of aneuploidy [105]. An alternative mechanism suggests that mutational inactivation of *p53* or functional inactivation of p53 through binding by mdm2 results in abnormal centrosome numbers and induction of chromosomal instability [108, 109]. Furthermore, there is evidence that loss of *BRCA1* or *BRCA2* can lead to centrosome amplification and chromosome segregation dysfunction [110, 111]. These studies combine to suggest that a number of different genes may contribute to centrosome function and homeostasis in normal

cells, and that inactivation or dysregulation of one or more of them can lead to abnormal centrosome number/function.

Aberrant mitotic spindle checkpoint function leads to aneuploidy

The mitotic spindle checkpoint governs proper chromosome segregation by ensuring that chromatid separation does not occur prior to alignment of all chromosomes along the mitotic spindle [112]. It follows that if the mitotic spindle checkpoint is defective, chromosome segregation during mitosis will occur asynchronously, potentially producing an unequal distribution of chromatids between the daughter cells [112]. Evidence supporting a role for aberrant mitotic spindle checkpoint function in the development of aneuploidy includes the observation that aneuploid cells respond inappropriately to agents that disrupt the spindle apparatus, such as colcemid. Normal cells respond to colcemid treatment by arresting in metaphase, whereas cells that display instability of chromosome numbers prematurely exit mitosis and initiate another round of DNA synthesis [54]. The hallmark of mitotic spindle checkpoint defect is the inability to inhibit entry into S phase when mitosis cannot be completed due to damage to the mitotic spindle [113]. Mutation or aberrant expression of genes that encode proteins involved in mitotic spindle checkpoint function can eliminate proper checkpoint function, contributing to the development of aneuploidy. A number of these genes have now been identified [114]. Alterations in mitotic spindle checkpoint genes have been documented in several human cancers, including decreased expression of *hMAD2* in breast cancer [115], and mutations in the *hBUB1* gene in CRC [54, 116]. However, these mitotic spindle checkpoint genes are not implicated in all aneuploid cancers. Some aneuploid breast cancers lack mutations in *hBUB1* and exhibit normal mRNA expression levels [117]. Likewise, cancers of the respiratory tract, including head/neck cancers, small cell lung carcinoma, and non-small lung carcinoma, have not been shown to have significant numbers of mutations in *hBUB1* [116, 118, 119], and sporadic tumors of the digestive tract rarely contain mutations of *hBUB1* or *hsMAD2* [120]. The absence of mutations or significant alterations in expression of mitotic spindle checkpoint genes in aneuploid cells suggests that additional genes and/or mechanisms of checkpoint inactivation are operational in the majority of cancers that demonstrate chromosomal instability. Certain *p53* mutations have been described that are associated with gain-of-function and relaxed spindle checkpoint function in response to mitotic inhibitors, suggesting that both mutational inactivation of *p53* and dominant gain-of-function mutations in *p53* can contribute to genomic instability and aberrant chromosome segregation [121]. In addition, defective checkpoint function has been demonstrated in patients with ataxia telangiectasia who carry mutations of the *ATM* gene [122]. These studies combine to suggest that a variety of genes may function in normal control of the mitotic spindle checkpoint, and when mutated or aberrantly expressed could con-

tribute to chromosomal instability through inactivation of the mitotic spindle checkpoint.

Abnormal DNA damage checkpoint function leads to aneuploidy

The DNA damage checkpoint represents the major cellular mechanism that guards against the replication of damaged DNA or entry of cells with DNA damage into mitosis. The types of DNA damage that elicit checkpoint activation include polymerase errors remaining after DNA replication and other forms of incompletely repaired DNA, damage resulting from exposure to exogenous genotoxins (ionizing radiation, chemical mutagens, and others), and damage related to endogenous genotoxic insult (such as reactive oxygen species). A number of genes have been implicated in the control of this checkpoint, including *p53* [92], *ATM* [123], *BRCA1* and *BRCA2* [124], and some others [2]. Functional inactivation of one or more of these genes through genetic or epigenetic mechanisms could result in a genomic instability related to the loss of the DNA damage checkpoint. Loss of this checkpoint might lead to aneuploidy directly resulting from abnormal segregation of damaged chromosomes [2].

Instability of chromosome structure

The majority of human cancers exhibit chromosomal abnormalities, including marker chromosomes with altered structure. It is generally accepted that many of the alterations of chromosome structure occurring in cancer cells confer some selective advantage to the evolving tumor. Thus, accumulation of a critical number of chromosomal aberrations or development of specific chromosomal abnormalities may represent essential steps in the process of neoplastic transformation. Three general forms of chromosomal alteration are observed in cancer cells: (i) gene amplifications, (ii) rearrangements and translocations, and (iii) large-scale deletions.

Gene amplification

The amplification of specific chromosomal segments or genes have been documented in some cancers and in many cancer cell lines [48, 125], some of which involve cellular proto-oncogenes, resulting in abnormal expression levels of the proto-oncogene products [126]. In general, gene amplification occurs late in tumorigenesis associated with tumor progression and is the recognized mechanism through which many tumors acquire resistance to chemotherapeutic agents. Thus, gene amplifications can profoundly affect tumor behavior, and can have prognostic significance for some cancers, but

may not be involved with early genetic alterations in pre-neoplastic lesions. The mechanisms governing gene amplification have not been determined with any certainty. However, several studies suggest that gene amplification occurs at much higher rates in neoplastic cells than in normal cells [48]. A role for the *p53* tumor suppressor in gene amplification has been suggested by some investigators. Evidence supporting this suggestion includes the observation that gene amplification occurs more readily in cells following inactivation of *p53* function [127, 128]. However, gene amplification can also occur in cells with normal *p53* [127]. One possibility for the role of *p53* in this process is that amplification of a chromosomal segment in a normal cell may trigger apoptosis in response to perceived DNA damage [129], whereas in the absence of normal *p53* function cells would not undergo apoptosis, but would continue to accumulate amplicons in subsequent rounds of replication [2]. Thus, this form of chromosomal instability may involve a mechanism (or a mechanistic component) that increases the ability of an affected cell to survive the genetic alteration.

Chromosomal rearrangements and translocations

Chromosomal rearrangements can take on several different forms, the most common of which are translocations. Patterns of chromosomal translocation in human cancer can be classified as complex or simple [2]. In some human cancers no consistent pattern of chromosomal abnormality can be discerned (complex translocations). These tumors exhibit complex type translocations, which may appear to be random. Among individual tumors of one type, or individual cells of a single tumor, different chromosomal aberrations may be found. Very often, these rearrangements are accompanied by large-scale loss of chromosomal segments. While it is possible that some of these chromosomal alterations are not essential to tumorigenesis, it is unlikely that any chromosomal alteration that does not confer a proliferative or adaptive advantage would be preserved in an evolving tumor. In some human cancers specific chromosomal anomalies are consistently found in a high percentage of tumors (simple translocations). These recurrent chromosomal abnormalities may reflect molecular alterations that are essential and necessary to the molecular pathogenesis of the specific tumor type. The discovery of the Philadelphia chromosome [trans(9;22)(q34;q11)] in the cancer cells of patients with chronic myelogenous leukemia was the first report suggesting the involvement of nonrandom chromosomal changes in the molecular pathogenesis of the disease [130]. Subsequent studies suggest that the neoplastic cells of 80–90% of leukemia and lymphoma patients contain some sort of demonstrable karyotypic abnormality, and many of these are uniquely associated with morphologically or clinically defined subsets of these cancers [67, 89]. Similar relationships between chromosomal alterations and definable stages of tumor development and progression have been established for some human solid tumors [8, 26],

and proposed for others [131, 132]. The role of chromosomal translocation in cancer pathogenesis is suggested to involve proto-oncogene activation by repositioning of the gene adjacent to a heterologous genetic control element. Evidence for this type of proto-oncogene activation includes studies of chromosome translocations in Burkitt's lymphoma [133]. In this cancer, the c-*myc* proto-oncogene is translocated from chromosome 8 to chromosome 14, proximal to the immunoglobulin enhancer sequences, resulting in abnormal constitutive expression of c-*myc* [58].

Large-scale chromosomal deletions

Large-scale deletions of whole chromosomes or chromosomal arms have been documented in many cancers. These deletions contribute to the abnormal allelotype of tumors, and may accompany chromosomal rearrangements and/or translocations. In most cases, such deletions are thought to be related to the presence of a tumor suppressor locus on the affected chromosomal arm. Large-scale deletions affecting several chromosomes have been documented in sporadic CRC, including deletions of 5q, 17p, and 18q [8]. Each of these chromosomal arms contains a known tumor suppressor locus; the adenomatous polyposis coli (*APC*) gene at 5q [134], the *p53* gene at 17p [56, 100], and the *DCC* (for "deleted in colorectal cancer") gene at 18q [135].

Microsatellite instability in human cancer

Microsatellite instability (MSI) is characterized by alterations to simple repeated sequences, including both expansions (insertions) and contractions (deletions), typically resulting in frameshift mutations. Microsatellites are repetitive sequences that consist of variable numbers of repeated units of one to four (or more) nucleotides. Such sequences are numerous and randomly distributed throughout the human genome. Mutational alterations of numerous adenine mononucleotide repeat motifs (polyA tracts) was the first characteristic used to define MSI in human tumors [136, 137]. These early studies of sporadic CRC suggested that 12% of all tumors harbor these mutations, with as many as 1×10^5 mutated polyA tracts per tumor [137]. Subsequent studies demonstrated frequent microsatellite alterations in hereditary CRC [61] and sporadic tumors [138] when higher order repeated units were examined.

Determination of microsatellite instability in human tumors

Tumors with MSI contain numerous altered microsatellite sequences, but not all microsatellite sequences are altered in tumors with MSI [59, 139]. In fact some studies have shown dramatic differences in susceptibility to mutation of

individual microsatellite loci [140]. In addition, two distinct patterns of microsatellite alteration have been described in human cancers that display MSI, and specific microsatellite markers tend to be altered in a characteristic pattern [141, 142]. The pattern of alteration observed at a specific microsatellite locus may reflect the nature of the genomic instability displayed by a tumor. Several factors influence the probability of mutation at a specific microsatellite locus: (i) the type of repeated sequence (mononucleotide, dinucleotide, etc.), (ii) the length of the microsatellite sequence (number of repeated units), (iii) the location of the microsatellite sequence within the genome, and (iv) the underlying molecular lesion. Thus, no single type of microsatellite will be diagnostic for MSI in all tumors. This is supported by the observation that numerous polyA repeats are altered in various human cancers [143, 144], but not all neoplasms that exhibit MSI demonstrate alterations in polyA sequences, and may only show alterations in higher order repeats [145]. A direct relationship has been observed between the length of polyA tracts and their mutation frequency among genetically unstable tumors [146], consistent with the suggestion that the probability of sustaining a mutation in an individual microsatellite sequence is proportional to the length of its sequence [147]. Extensive comparison of the mutation of dinucleotide *versus* higher order repeat units (trinucleotide or tetranucleotide) in human tumors suggests that larger alleles are more susceptible to mutation in genetically unstable tumors [148]. Studies with cancer cell lines that harbor MMR gene mutations demonstrate instability of specific classes of microsatellites. Cells possessing a defect in *hPMS2* exhibit instability of trinucleotide repeats [149], while cells deficient for *hMSH3* or *hMSH6* demonstrate an inability to correct mismatches in dinucleotide (or higher order) repeats [150]. Furthermore, cells lacking *hMSH* demonstrate minimal levels of dinucleotide instability, while cell lines lacking *hMSH2* or *hMLH1* demonstrate profound dinucleotide instability [151]. In addition, specific MMR gene mutations can affect the extent of hypermutability at microsatellite sequences [140]. The microsatellite mutation rate in cells lacking *hMLH1* and *hMSH3* is tenfold greater than that of cells lacking *hPMS2* and *hMSH6* [140]. These observations suggest that individual MMR complexes exhibit specificity for certain types of mismatches, and that the MSI displayed by cancer cells may be directly related to the number [152] and nature [140] of MMR gene mutations.

Frequency of microsatellite instability in human cancer

A large number of studies have documented the occurrence of MSI in human cancer [59]. Tumors from patients diagnosed with HNPCC frequently exhibit MSI (141/159 tumors, 89%), while only 15% (887/5724 tumors) sporadic colorectal cancers demonstrate MSI [59]. However, sporadic CRC occurring in young patients (<35 years of age) and in patients with multiple primary tumors exhibit MSI at higher frequency (64% and 71%, respectively) than sporadic

CRC in general [153]. Sporadic gastric cancers exhibit MSI in 19% (276/1485) of tumors [59], while gastric carcinomas from patients with multiple primary tumors or familial predisposition exhibit an elevated frequency of MSI (61% and 32%, respectively) compared to sporadic tumors [154, 155]. Several other cancers exhibit MSI in 15–35% of tumors examined. Sporadic breast cancers demonstrate MSI in 17% (64/372 tumors) of cases, but this percentage varies widely between studies [59]. The combined results of six studies failed to detect MSI at even one locus among 522 tumors examined [59], suggesting that the actual frequency of occurrence of MSI among breast cancers is very low. MSI has been documented in 24% (168/713) of tumors of the endometrium, 13% (16/123) of ovarian cancers, 27% (25/92) of esophageal tumors, 28% (25/88) of liver tumors, 29% (78/272) of non-small cell lung cancers, and 32% (79/247) of prostate cancers [59]. Hodgkin's disease and some forms of leukemia exhibit MSI in a high percentage of cases. However, additional studies will be needed to determine more precisely the prevalence of this genetic abnormality in these neoplasms, particularly among the various forms of leukemia. MSI is rare (<10% tumors) among gliomas, neuroblastomas, and cancers of the testicles, thyroid, and uterine-cervix. Evidence for the involvement of MSI in some other tumors has been produced, although the numbers of tumors examined and the numbers of studies conducted are limited.

Mismatch repair defects lead to nicrosatellite instability

The molecular defects responsible for the MSI in human tumors involve the genes that encode proteins required for normal MMR [156]. These include *hMSH2* [157, 158], *hMSH3* [159], and *hMSH6/GTBP* [160, 161], which are human homologs of the bacterial *MutS* gene, and *hMLH1* [162, 163], *hPMS1* [164], *hPMS2* [164], and *hMLH3* [165], which are human homologs of the bacterial *MutL* gene. One or more of these genes are mutated in the germline of the majority of individuals with HNPCC [166], and somatic mutations have been identified in sporadic CRC that display MSI [167]. MMR gene defects have also been identified in other cancers that exhibit MSI [52, 59, 168, 169].

The proteins involved with MMR operate in concert to recognize mispaired or unpaired nucleotides, and facilitate their removal and repair [170]. This mechanism differs from nucleotide-excision repair, which recognizes and repairs abnormal (adducts) nucleotides [168]. The observation that microsatellite mutations consist of expansion or contraction of the repeated sequence through insertion or deletion of variable numbers of repeat units suggests that such mutations arise through a slippage mechanism during replication of these simple repeat sequences [171]. Strand slippage of the primer at a repetitive sequence during replication generates a misaligned intermediate that is stabilized by correct base pairing between discrete repeat units on the misaligned strand. Such a misaligned intermediate is normally repaired through the proofreading function of the polymerase complex, or by post-replication repair

mechanisms [172]. If the intermediate is not repaired, subsequent rounds of replication will generate insertion or deletion mutations in the newly synthesized DNA strands. The relative location of the unpaired repeat sequence in the replication intermediate determines whether an insertion or deletion will result (contraction or expansion of the microsatellite).

Microsatellite mutation rates in DNA repair-proficient and -deficient cells

The spontaneous mutation rate of a dinucleotide microsatellite repeat sequence in normal human fibroblasts has been estimated to be 12.7×10^{-8} mutations/cell/generation [173], suggesting that dinucleotide repeat sequences are remarkably stable in normal human cells. MMR-proficient cancer cells exhibit a microsatellite mutation rate of 9.8×10^{-6} mutations/cell/generation, whereas MMR-deficient cells exhibited mutation rates of 1.6×10^{-4} and 3.3×10^{-3} mutations/cell/generation, respectively [174]. The estimated mutation rates at the dinucleotide repeat in MMR-deficient tumor cells were 16-fold and 337-fold higher than that of the MMR-proficient tumor cells, and 1260-fold and 25984-fold higher than that of normal fibroblasts [173]. These results highlight the propensity for spontaneous mutation at microsatellite repeat sequences of tumor cells that are deficient for MMR, and support the notion that cells displaying MSI harbor sustained lesions in MMR.

Specific mismatch repair genes are responsible for specific forms of microsatellite instability

Genetic complementation studies have produced direct evidence for the involvement of specific chromosomal loci or specific genes in MMR-deficient tumor cells that exhibit MSI. Transfer of human chromosome 2, which contains the *hMSH2* and *hMSH6* genes, restores genetic stability and MMR-proficiency to *hMSH2*-mutant cancer cells [175]. Furthermore, cells containing chromosome 2 demonstrate microsatellite stability at a trinucleotide repeat (D7S1794) and a dinucleotide repeat (D14S73), whereas cells containing other transferred chromosomes (such as chromosome 17) continue to exhibit instability at these loci [175]. Furthermore, transfer of chromosome 2 restores genetic stability to cancer cells that carry mutations of both *hMSH6* and DNA polymerase δ [161, 176], suggesting that the DNA polymerase δ defect is not the primary determinant of genetic instability in these cells [175]. However, other studies question whether *hMSH6* plays a major role in MSI [177]. Transfer of chromosome 3, which contains *hMLH1*, into tumor cells that are homozygous for *hMLH1* mutation, restores MMR and stability to the D5S107 dinucleotide microsatellite repeat [178]. In similar studies, transfer of human chromosome 5 (containing *hMSH3*) or human chromosome 2, into tumor cells resulted in partial correction of the MMR defect [159]. More recent studies

have utilized single gene transfer to correct MMR deficiency. Cancer cells that harbor an *hPMS2* mutation and display MMR deficiency [149] show increased microsatellite stability and reduced mutation rate at the *HPRT* locus, and cell extracts can perform strand-specific MMR following transfection with a wild-type *hPMS2* gene [179]. Likewise, transfection of tumor cells with *hMSH6* resulted in restoration of MMR, increased stability of the BAT26 polyA tract, and reduction in the mutation rate at the *HPRT* locus [180].

Epigenetic silencing of mismatch repair genes leads to microsatellite instability

Mutational inactivation of MMR genes has been documented in numerous human tumors that display MSI. However, in many cases the underlying molecular defect in MMR cannot be identified, suggesting that additional MMR genes exist, or that alternative mechanisms for microsatellite mutation are operational in these tumors. Several studies have produced strong evidence that epigenetic regulation of MMR gene expression may be responsible for loss of MMR function in tumors that display MSI. Initially, a strong correlation between general methylation status and MMR proficiency in CRC cell lines was noted [181]. Cell lines that were deficient for MMR and showed MSI demonstrated hypermethylation of endogenous and exogenous DNA sequences [181]. Subsequently, several laboratories examined expression of *hMLH1*, methylation of the *hMLH1* promoter, and MSI status among sporadic CRC [182–184]. Tumors exhibiting high level MSI, no detectable expression of *hMLH1*, and no *hMLH1* point mutation, also showed hypermethylation of the *hMLH1* promoter region [182–184]. In cell lines that exhibit loss of *hMLH1* and hypermethylation of the *hMLH1* promoter, treatment with 5-aza-2'-deoxycytidine resulted in re-expression of *hMLH1* and restoration of MMR capacity [183]. These results suggest that inactivation of *hMLH1* through hypermethylation of its promoter may represent the principle mechanism of gene inactivation in sporadic CRC characterized by widespread MSI. Consistent with this suggestion, the *hMLH1* promoter has been shown to be hypermethylated in 122/167 (73%) CRC with MSI, but in only 20/138 (14%) of microsatellite stable CRC [59]. Similar relationships between *hMLH1* promoter hypermethylation and MSI have been observed in gastric and endometrial cancers [185–187]. In contrast to the relationship observed in sporadic cancers with MSI, tumors from HNPCC patients that harbor mutations in MMR genes do not show *hMLH1* promoter hypermethylation.

Oxidative stress and loss of mismatch repair function

Chronic inflammation is known to contribute to DNA damage related to excess levels of free radicals. MMR-proficient cells are protected from mutational

alteration of microsatellite sequences after exposure to low levels of hydrogen peroxide [66]. However, MMR-deficient cells demonstrate numerous microsatellite alterations in response to oxidative stress, and oxygen radical scavengers diminish the damaging effects of free radicals in these cells [188]. These observations suggest that DNA damage related to free radical exposure can contribute to MSI. Many forms of cancer are closely associated with chronic inflammation, leading to the suggestion that oxidative stress may significantly contribute to DNA damage elevating the risk for neoplastic transformation in affected tissues. Ulcerative colitis is an inflammatory bowel disease associated with increased risk for colorectal cancer [189]. MSI is one of the features of ulcerative colitis and related pre-neoplastic lesions [190], suggesting a role for MMR defects in this condition. The accumulation of microsatellite mutations in ulcerative colitis could be related to a failure of the MMR pathways to correct the excess damage resulting from elevated levels of free radicals [191]. Alternatively, MMR function may be disabled through DNA methylation [192] or directly by oxidative stress [193]. The inactivation of MMR function in response to oxidative stress is mediated by oxidative damage to MMR complexes, possibly involving hMutSα, hMutSβ, and hMutLα [193]. This type of mechanism could account for MSI in chronically inflamed non-neoplastic tissues [194], as well as cancers associated with inflammatory processes [195, 196].

Molecular targets of microsatellite instability

Numerous simple repeat sequences are found in the human genome. Some of these occur within the coding regions of structural genes. These genes may be targets for mutation in cells that display MSI [197, 198]. The *TGFβRII* gene contains two simple repeat sequences: (i) a 10-bp adenine mononucleotide tract and (ii) a 6-bp GT repeat [199]. This gene represents the first recognized target for inactivation due to microsatellite mutations in human tumors and cell lines, and both simple repeat sequences are subject to mutation [200]. Mutation of the $(GT)_3$ repeat region in one tumor by insertion of an additional GT repeat unit resulted in a frameshift, which was predicted to significantly alter the C terminus of the receptor protein [200]. Additional mutations were documented in the $(A)_{10}$ repeat region of the *TGFβRII* gene (deletion of one or two bases), resulting in frameshifts that were predicted to give rise to truncated receptor proteins [200]. Inactivating *TGFβRII* mutations involving these simple repeat regions have now been identified in a significant number of human tumors that exhibit MSI, including sporadic and hereditary CRC, as well as cancers of the stomach, endometrium, and acute lymphoblastic leukemia [59]. However, cancers of the esophagus [201] and gliomas [202] display no microsatellite mutations involving the *TGFβRII* gene.

A number of other genes that function in various aspects of normal cellular homeostasis (growth control and DNA repair) exhibit frameshift mutations at

microsatellite loci, including *APC*, *BAX*, *E2F-4*, *IGFIIR*, *hMSH3*, *hMSH6*, *TCF-4*, *BLM*, and others [59, 198, 203, 204]. Mutation in these genes have been identified in a significant percentage (as high as 50–55%) of gastrointestinal cancers (HNPCC, sporadic CRC, stomach) that exhibit MSI [59]. However, some other cancers that display MSI do not contain these mutations [205], suggesting that these genes may be preferential targets in tumors of the gastrointestinal tract. Other tumors with MSI may mutate different genes from those that have been identified to be susceptible to this form of genetic event [198, 204].

Conclusions

A large amount of evidence has now accumulated suggesting a genetic basis for the development of neoplastic disease in humans. However, the genetic damage documented in human cancers includes both large-scale alterations (chromosomal aberrations and ploidy changes) and DNA sequence alterations (single nucleotide changes or alterations in short segments of DNA). In addition, the patterns of genetic damage within a single tumor can vary from a few molecular alterations at specific loci to genome-wide mutations involving a large number of loci. Several distinct forms of genomic instability may provide the molecular basis for neoplastic transformation in humans. Cells undergoing neoplastic transformation may accumulate genetic damage related to progressive genomic instability, or due to episodic genomic instability. Transforming mutations could arise through either of these mechanisms, involving chromosomal alterations or sequence alterations (point mutations and/or MSI). Although the significance of mutations to the etiological mechanisms of tumor development has been debated, a causal role for genetic lesions in the genesis of cancer is commonly accepted. Thus, genetic lesions represent an integral part of the processes of neoplastic transformation, tumorigenesis, and tumor progression, and as such represent potentially valuable markers for cancer detection, diagnosis, staging, and prediction of clinical outcome [3, 4].

References

1 Bishop JM (1991) Molecular themes in oncogenesis. *Cell* 64: 235–248
2 Lengauer C, Kinzler KW, Vogelstein B (1998) Genetic instabilities in human cancers. *Nature* 396: 643–649
3 Mao L, Sidransky D (1994) Cancer screening based on genetic alterations in human tumors. *Cancer Res* 54: 1939s–1940s
4 Sidransky D (1995) Molecular markers in cancer: can we make better predictions? *Int J Cancer* 64: 1–2
5 Renan MJ (1993) How many mutations are required for tumorigenesis? Implications from human cancer data. *Mol Carcinog* 7: 139–146
6 Loeb KR, Loeb LA (2000) Significance of multiple mutations in cancer. *Carcinogenesis* 21: 379–385

7 Cohen AM, Minsky BD, Schilsky RL. (1997) Cancer of the colon. In: VT DeVita, S Hellman, SA Rosenberg (eds): *Cancer: Principles and Practice of Oncology*, *5th ed*. Lippincott-Raven, Philadelphia, 1144–1197
8 Kinzler KW, Vogelstein B (2001) Colorectal tumors. In: CR Scriver, AL Beaudet, WS Sly, D Valle (eds): *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. McGraw-Hill. New York 1033–1062
9 Crow JF (1997) The high spontaneous mutation rate: is it a health risk? *Proc Natl Acad Sci USA* 94: 8380–8386
10 Cairns J (1998) Mutation and cancer: the antecedents to our studies of adaptive mutation. *Genetics* 148: 1433–1440
11 Ruddon RW (1995) *Cancer Biology, 3rd ed*. Oxford University Press, New York
12 Albertson DG, Collins C, McCormick F, Gray JW (2003) Chromosome aberrations in solid tumors. *Nat Genet* 34: 369–376
13 Hussain SP, Harris CC (1998) Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes. *Cancer Res* 58: 4023–4037
14 Hedenfalk I, Ringner M, Ben-Dor A, Yakhini Z, Chen Y, Chebil G, Ach R, Loman N, Olsson H, Meltzer P et al. (2003) Molecular classification of familial non-BRCA1/BRCA2 breast cancer. *Proc Natl Acad Sci USA* 100: 2532–2537
15 Chung CH, Parker JS, Karaca G, Wu J, Funkhouser WK, Moore D, Butterfoss D, Xiang D, Zanation A, Yin X et al. (2004) Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell* 5: 489–500
16 Meyerson M, Franklin WA, Kelley MJ (2004) Molecular classification and molecular genetics of human lung cancers. *Semin Oncol* 31: 4–19
17 Hoang CD, D'Cunha J, Tawfic SH, Gruessner AC, Kratzke RA, Maddaus MA (2004) Expression profiling of non-small cell lung carcinoma identifies metastatic genotypes based on lymph node tumor burden. *J Thorac Cardiovasc Surg* 127: 1332–1341; discussion 1342
18 Warner GC, Reis PP, Jurisica I, Sultan M, Arora S, Macmillan C, Makitie AA, Grenman R, Reid N, Sukhai M et al. (2004) Molecular classification of oral cancer by cDNA microarrays identifies overexpressed genes correlated with nodal metastasis. *Int J Cancer* 110: 857–868
19 Cleator S, Ashworth A (2004) Molecular profiling of breast cancer: clinical implications. *Br J Cancer* 90: 1120–1124
20 Troester MA, Hoadley KA, Sorlie T, Herbert BS, Borresen-Dale AL, Lonning PE, Shay JW, Kaufmann WK, Perou CM (2004) Cell-type-specific responses to chemotherapeutics in breast cancer. *Cancer Res* 64: 4218–4226
21 Armitage P, Doll R (1957) A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. *Br J Cancer* 11: 161–169
22 Knudson AG Jr, (1971) Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68: 820–823
23 Baylin SB (2002) Mechanisms underlying epigenetically mediated gene silencing in cancer. *Semin Cancer Biol* 12: 331–337
24 Herman JG, Baylin SB (2003) Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 349: 2042–2054
25 Fazzari MJ, Greally JM (2004) Epigenomics: beyond CpG islands. *Nat Rev Genet* 5: 446–455
26 Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. *Cell* 61: 759–767
27 Jackson AL, Loeb LA (1998) The mutation rate and cancer. *Genetics* 148: 1483–1490
28 Loeb LA (2001) A mutator phenotype in cancer. *Cancer Res* 61: 3230–3239
29 Loeb LA, Christians FC (1996) Multiple mutations in human cancers. *Mutat Res* 350: 279–286
30 Perucho M (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem* 377: 675–684
31 Orr-Weaver TL, Weinberg RA (1998) A checkpoint on the road to cancer. *Nature* 392: 223–224
32 Goyette MC, Cho K, Fasching CL, Levy DB, Kinzler KW, Paraskeva C, Vogelstein B, Stanbridge EJ (1992) Progression of colorectal cancer is associated with multiple tumor suppressor gene defects but inhibition of tumorigenicity is accomplished by correction of any single defect via chromosome transfer. *Mol Cell Biol* 12: 1387–1395
33 Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. *N Engl J Med* 319: 525–532
34 Duesberg P, Rausch C, Rasnick D, Hehlmann R (1998) Genetic instability of cancer cells is proportional to their degree of aneuploidy. *Proc Natl Acad Sci USA* 95: 13692–13697

35 Strauss BS (1998) Hypermutability in carcinogenesis. *Genetics* 148: 1619–1626
36 Sieber OM, Heinimann K, Tomlinson IP (2003) Genomic instability—the engine of tumorigenesis? *Nat Rev Cancer* 3: 701–708
37 Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R, Harris CC (1996) Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. *Nucleic Acids Res* 24: 141–146
38 Moolgavkar SH, Luebeck EG (2003) Multistage carcinogenesis and the incidence of human cancer. *Genes Chromosomes Cancer* 38: 302–306
39 Boesen JJ, Niericker MJ, Dieteren N, Simons JW (1994) How variable is a spontaneous mutation rate in cultured mammalian cells? *Mutat Res* 307: 121–129
40 Eldridge SR, Gould MN (1992) Comparison of spontaneous mutagenesis in early-passage human mammary cells from normal and malignant tissues. *Int J Cancer* 50: 321–324
41 Wittenkeller JL, Storer B, Bittner G, Schiller JH (1997) Comparison of spontaneous and induced mutation rates in an immortalized human bronchial epithelial cell line and its tumorigenic derivative. *Oncology* 54: 335–341
42 Loeb LA (1991) Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res* 51: 3075–3079
43 Loeb LA (1997) Transient expression of a mutator phenotype in cancer cells. *Science* 277: 1449–1450
44 Richards B, Zhang H, Phear G, Meuth M (1997) Conditional mutator phenotypes in hMSH2-deficient tumor cell lines. *Science* 277: 1523–1526
45 Bhattacharyya NP, Skandalis A, Ganesh A, Groden J, Meuth M (1994) Mutator phenotypes in human colorectal carcinoma cell lines. *Proc Natl Acad Sci USA* 91: 6319–6323
46 Eshleman JR, Lang EZ, Bowerfind GK, Parsons R, Vogelstein B, Willson JK, Veigl ML, Sedwick WD, Markowitz SD (1995) Increased mutation rate at the hprt locus accompanies microsatellite instability in colon cancer. *Oncogene* 10: 33–37
47 Glaab WE, Tindall KR (1997) Mutation rate at the hprt locus in human cancer cell lines with specific mismatch repair-gene defects. *Carcinogenesis* 18: 1–8
48 Tlsty TD, Margolin BH, Lum K (1989) 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
49 Cairns J (1975) Mutation selection and the natural history of cancer. *Nature* 255: 197–200
50 Foulds L (1958) The natural history of cancer. *J Chronic Dis* 8: 2–37
51 Nowell PC (1976) The clonal evolution of tumor cell populations. *Science* 194: 23–28
52 Eshleman JR, Markowitz SD (1996) Mismatch repair defects in human carcinogenesis. *Hum Mol Genet* 5 Spec No: 1489–1494
53 Lengauer C, Kinzler KW, Vogelstein B (1997) Genetic instability in colorectal cancers. *Nature* 386: 623–627
54 Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B (1998) Mutations of mitotic checkpoint genes in human cancers. *Nature* 392: 300–303
55 Greenblatt MS, Bennett WP, Hollstein M, Harris CC (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 54: 4855–4878
56 Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, vanTuinen P, Ledbetter DH, Barker DF, Nakamura Y et al. (1989) Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 244: 217–221
57 Bos JL (1989) ras oncogenes in human cancer: a review. *Cancer Res* 49: 4682–4689
58 Dalla-Favera R, Martinotti S, Gallo RC, Erikson J, Croce CM (1983) Translocation and rearrangements of the c-myc oncogene locus in human undifferentiated B-cell lymphomas. *Science* 219: 963–967
59 Coleman WB, Tsongalis GJ (2002) The role of genomic instability in the development of human cancer. In: WB Coleman, GJ Tsongalis (eds): *The Molecular Basis of Human Cancer*. Humana Press, Totowa, 115–142
60 Rosenberg SM, Thulin C, Harris RS (1998) Transient and heritable mutators in adaptive evolution in the lab and in nature. *Genetics* 148: 1559–1566
61 Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR et al. (1993) Clues to the pathogenesis of familial colorectal cancer. *Science* 260: 812–816
62 Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, Lynch HT, Watson P,

Tallqvist G, Juhola M et al. (1994) Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 54: 1645–1648
63 Feig DI, Reid TM, Loeb LA (1994) Reactive oxygen species in tumorigenesis. *Cancer Res* 54: 1890s–1894s
64 Jackson AL, Chen R, Loeb LA (1998) Induction of microsatellite instability by oxidative DNA damage. *Proc Natl Acad Sci USA* 95: 12468–12473
65 Baba S (1997) Recent advances in molecular genetics of colorectal cancer. *World J Surg* 21: 678–687
66 Yamada NA, Parker JM, Farber RA (2003) Mutation frequency analysis of mononucleotide and dinucleotide repeats after oxidative stress. *Environ Mol Mutagen* 42: 75–84
67 Mitelman F (1994) *Catalog of Chromosome Aberrations in Cancer, 5th ed*. Wiley-Liss Publishers, New York
68 Meltzer PS, Kallioniemi A, Trent JM (2001) Chromosome alterations in human solid tumors. In: CR Scriver, AL Beaudet, WS Sly, D Valle (eds): *The Metabolic and Molecular Bases of Inhierited Disease, 8th ed*. McGraw-Hill, New York, 575–596
69 Cahill DP, Lengauer C (2001) Tumor genome instabilities. In: CR Scriver, AL Beaudet, WS Sly, D Valle (eds): *The Metabolic and Molecular Bases of Inherited Disease, 8th ed*. McGraw-Hill, New York 611–612
70 Wang Z, Cummins JM, Shen D, Cahill DP, Jallepalli PV, Wang TL, Parsons DW, Traverso G, Awad M, Silliman N et al. (2004) Three classes of genes mutated in colorectal cancers with chromosomal instability. *Cancer Res* 64: 2998–3001
71 Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R (1989) Allelotype of colorectal carcinomas. *Science* 244: 207–211
72 Tsuchiya E, Nakamura Y, Weng SY, Nakagawa K, Tsuchiya S, Sugano H, Kitagawa T (1992) Allelotype of non-small cell lung carcinoma—comparison between loss of heterozygosity in squamous cell carcinoma and adenocarcinoma. *Cancer Res* 52: 2478–2481
73 Seymour AB, Hruban RH, Redston M, Caldas C, Powell SM, Kinzler KW, Yeo CJ, Kern SE (1994) Allelotype of pancreatic adenocarcinoma. *Cancer Res* 54: 2761–2764
74 Roncalli M, Borzio M, Bianchi P, Laghi L (2000) Comprehensive allelotype study of hepatocellular carcinoma. *Hepatology* 32: 876
75 Miller BJ, Wang D, Krahe R, Wright FA (2003) Pooled analysis of loss of heterozygosity in breast cancer: a genome scan provides comparative evidence for multiple tumor suppressors and identifies novel candidate regions. *Am J Hum Genet* 73: 748–767
76 Thiagalingam S, Laken S, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B, Lengauer C (2001) Mechanisms underlying losses of heterozygosity in human colorectal cancers. *Proc Natl Acad Sci USA* 98: 2698–2702
77 El-Naggar AK, Vielh P (2004) Solid tumor DNA content analysis. *Methods Mol Biol* 263: 355–370
78 Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D (1994) Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. *Genes Chromosomes Cancer* 10: 231–243
79 Kallioniemi OP, Kallioniemi A, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D (1993) Comparative genomic hybridization: a rapid new method for detecting and mapping DNA amplification in tumors. *Semin Cancer Biol* 4: 41–46
80 Bayani JM, Squire JA (2002) Applications of SKY in cancer cytogenetics. *Cancer Invest* 20: 373–386
81 Patel AS, Hawkins AL, Griffin CA (2000) Cytogenetics and cancer. *Curr Opin Oncol* 12: 62–67
82 Radford DM, Fair KL, Phillips NJ, Ritter JH, Steinbrueck T, Holt MS, Donis-Keller H (1995) Allelotyping of ductal carcinoma *in situ* of the breast: deletion of loci on 8p, 13q, 16q, 17p and 17q. *Cancer Res* 55: 3399–3405
83 Boige V, Laurent-Puig P, Fouchet P, Flejou JF, Monges G, Bedossa P, Bioulac-Sage P, Capron F, Schmitz A, Olschwang S, Thomas G (1997) Concerted nonsyntenic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelotype. *Cancer Res* 57: 1986–1990
84 Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih Ie M, Vogelstein B, Lengauer C (2002) The role of chromosomal instability in tumor initiation. *Proc Natl Acad Sci USA* 99: 16226–16231
85 Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C (2003) The significance of unstable chromosomes in colorectal cancer. *Nat Rev Cancer* 3: 695–701

86 Ghadimi BM, Sackett DL, Difilippantonio MJ, Schrock E, Neumann T, Jauho A, Auer G, Ried T (2000) Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. *Genes Chromosomes Cancer* 27: 183–190
87 Harwood J, Tachibana A, Davis R, Bhattacharyya NP, Meuth M (1993) High rate of multilocus deletion in a human tumor cell line. *Hum Mol Genet* 2: 165–171
88 Phear G, Bhattacharyya NP, Meuth M (1996) 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
89 Le Beau MM (1997) Molecular biology of cancer: Cytogenetics. In: VT DeVita, S Hellman, SA Rosenberg (eds): *Cancer: Principles and Practice of Oncology, 5th ed*. Lippincott-Raven, Philadelphia 103–119
90 Gollin SM (2004) Chromosomal instability. *Curr Opin Oncol* 16: 25–31
91 Kastan MB, Kuerbitz SJ (1993) Control of G1 arrest after DNA damage. *Environ Health Perspect* 101, Suppl 5: 55–58
92 Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 51: 6304–6311
93 Solomon E, Borrow J, Goddard AD (1991) Chromosome aberrations and cancer. *Science* 254: 1153–1160
94 Jong YJ, Li LH, Tsou MH, Chen YJ, Cheng SH, Wang-Wuu S, Tsai SF, Chen CM, Huang AT, Hsu MT, Lin CH (2004) Chromosomal comparative genomic hybridization abnormalities in early- and late-onset human breast cancers: correlation with disease progression and TP53 mutations. *Cancer Genet Cytogenet* 148: 55–65
95 Sugai T, Takahashi H, Habano W, Nakamura S, Sato K, Orii S, Suzuki K (2003) Analysis of genetic alterations, classified according to their DNA ploidy pattern, in the progression of colorectal adenomas and early colorectal carcinomas. *J Pathol* 200: 168–176
96 Filatov L, Golubovskaya V, Hurt JC, Byrd LL, Phillips JM, Kaufmann WK (1998) Chromosomal instability is correlated with telomere erosion and inactivation of G2 checkpoint function in human fibroblasts expressing human papillomavirus type 16 E6 oncoprotein. *Oncogene* 16: 1825–1838
97 Honma M, Momose M, Tanabe H, Sakamoto H, Yu Y, Little JB, Sofuni T, Hayashi M (2000) Requirement of wild-type p53 protein for maintenance of chromosomal integrity. *Mol Carcinog* 28: 203–214
98 Tarapore P, Fukasawa K (2000) p53 mutation and mitotic infidelity. *Cancer Invest* 18: 148–155
99 Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B (2001) Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. *Cancer Res* 61: 818–822
100 Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JK, Hamilton S, Vogelstein B (1990) p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res* 50: 7717–7722
101 Eshleman JR, Casey G, Kochera ME, Sedwick WD, Swinler SE, Veigl ML, Willson JK, Schwartz S, Markowitz SD (1998) Chromosome number and structure both are markedly stable in RER colorectal cancers and are not destabilized by mutation of p53. *Oncogene* 17: 719–725
102 Kramer A, Neben K, Ho AD (2002) Centrosome replication, genomic instability and cancer. *Leukemia* 16: 767–775
103 Lingle WL, Barrett SL, Negron VC, D'Assoro AB, Boeneman K, Liu W, Whitehead CM, Reynolds C, Salisbury JL (2002) Centrosome amplification drives chromosomal instability in breast tumor development. *Proc Natl Acad Sci USA* 99: 1978–1983
104 Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, Schryver B, Flanagan P, Clairvoyant F, Ginther C et al. (1998) A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. *Embo J* 17: 3052–3065
105 Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR, Sen S (1998) Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat Genet* 20: 189–193
106 Wolf G, Elez R, Doermer A, Holtrich U, Ackermann H, Stutte HJ, Altmannsberger HM, Rubsamen-Waigmann H, Strebhardt K (1997) Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. *Oncogene* 14: 543–549
107 Tanaka T, Kimura M, Matsunaga K, Fukada D, Mori H, Okano Y (1999) Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. *Cancer Res* 59: 2041–2044

108 Carroll PE, Okuda M, Horn HF, Biddinger P, Stambrook PJ, Gleich LL, Li YQ, Tarapore P, Fukasawa K (1999) Centrosome hyperamplification in human cancer: chromosome instability induced by p53 mutation and/or Mdm2 overexpression. *Oncogene* 18: 1935–1944
109 Tarapore P, Fukasawa K (2002) Loss of p53 and centrosome hyperamplification. *Oncogene* 21: 6234–6240
110 Tutt A, Gabriel A, Bertwistle D, Connor F, Paterson H, Peacock J, Ross G, Ashworth A (1999) Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. *Curr Biol* 9: 1107–1110
111 Deng CX (2002) Roles of BRCA1 in centrosome duplication. *Oncogene* 21: 6222–6227
112 Bharadwaj R, Yu H (2004) The spindle checkpoint, aneuploidy, and cancer. *Oncogene* 23: 2016–2027
113 Hartwell LH, Kastan MB (1994) Cell cycle control and cancer. *Science* 266: 1821–1828
114 Cahill DP, da Costa LT, Carson-Walter EB, Kinzler KW, Vogelstein B, Lengauer C (1999) Characterization of MAD2B and other mitotic spindle checkpoint genes. *Genomics* 58: 181–187
115 Li Y, Benezra R (1996) Identification of a human mitotic checkpoint gene: hsMAD2. *Science* 274: 246–248
116 Jaffrey RG, Pritchard SC, Clark C, Murray GI, Cassidy J, Kerr KM, Nicolson MC, McLeod HL (2000) Genomic instability at the BUB1 locus in colorectal cancer, but not in non-small cell lung cancer. *Cancer Res* 60: 4349–4352
117 Myrie KA, Percy MJ, Azim JN, Neeley CK, Petty EM (2000) Mutation and expression analysis of human BUB1 and BUB1B in aneuploid breast cancer cell lines. *Cancer Lett* 152: 193–199
118 Sato M, Sekido Y, Horio Y, Takahashi M, Saito H, Minna JD, Shimokata K, Hasegawa Y (2000) Infrequent mutation of the hBUB1 and hBUBR1 genes in human lung cancer. *Jpn J Cancer Res* 91: 504–509
119 Yamaguchi K, Okami K, Hibi K, Wehage SL, Jen J, Sidransky D (1999) Mutation analysis of hBUB1 in aneuploid HNSCC and lung cancer cell lines. *Cancer Lett* 139: 183–187
120 Imai Y, Shiratori Y, Kato N, Inoue T, Omata M (1999) Mutational inactivation of mitotic checkpoint genes, hsMAD2 and hBUB1, is rare in sporadic digestive tract cancers. *Jpn J Cancer Res* 90: 837–840
121 Gualberto A, Aldape K, Kozakiewicz K, Tlsty TD (1998) An oncogenic form of p53 confers a dominant, gain-of-function phenotype that disrupts spindle checkpoint control. *Proc Natl Acad Sci USA* 95: 5166–5171
122 Shigeta T, Takagi M, Delia D, Chessa L, Iwata S, Kanke Y, Asada M, Eguchi M, Mizutani S (1999) Defective control of apoptosis and mitotic spindle checkpoint in heterozygous carriers of ATM mutations. *Cancer Res* 59: 2602–2607
123 Canman CE, Lim DS (1998) The role of ATM in DNA damage responses and cancer. *Oncogene* 17: 3301–3308
124 Zhang H, Tombline G, Weber BL (1998) BRCA1, BRCA2, and DNA damage response: collision or collusion? *Cell* 92: 433–436
125 Tlsty TD, Briot A, Gualberto A, Hall I, Hess S, Hixon M, Kuppuswamy D, Romanov S, Sage M, White A (1995) Genomic instability and cancer. *Mutat Res* 337: 1–7
126 Hogarty MD, Brodeur GM (2001) Gene amplification in human cancers: Biological and clinical significance. In: CR Scriver, AL Beaudet, WS Sly, D Valle (eds): *The Metabolic and Molecular Bases of Inherited Disease*, *8th ed.* McGraw-Hill, New York, 597–610
127 Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* 70: 923–935
128 Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM (1992) Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell* 70: 937–948
129 Oren M (1994) Relationship of p53 to the control of apoptotic cell death. *Semin Cancer Biol* 5: 221–227
130 Nowell PC, Hungerford DA (1960) Chromosome studies on normal and leukemic human leukocytes. *J Natl Cancer Inst* 25: 85–109
131 Dubeau L. (2001) Ovarian cancer. In: CR Scriver, AL Beaudet, WS Sly, D Valle (eds): *The Metabolic and Molecular Bases of Inherited Disease, 8th ed.* McGraw-Hill, New York, 1091–1096
132 Hruban RH, Wilentz RE, Kern SE (2000) Genetic progression in the pancreatic ducts. *Am J Pathol* 156: 1821–1825
133 Croce CM (1986) Chromosome translocations and human cancer. *Cancer Res* 46: 6019–6023

134 Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKechnie D et al. (1991) Identification of FAP locus genes from chromosome 5q21. *Science* 253: 661–665
135 Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW et al. (1990) Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 247: 49–56
136 Peinado MA, Malkhosyan S, Velazquez A, Perucho M (1992) 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
137 Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 363: 558–561
138 Thibodeau SN, Bren G, Schaid D (1993) Microsatellite instability in cancer of the proximal colon. *Science* 260: 816–819
139 Zhang L, Yu J, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B (2001) Short mononucleotide repeat sequence variability in mismatch repair-deficient cancers. *Cancer Res* 61: 3801–3805
140 Yamada NA, Castro A, Farber RA (2003) Variation in the extent of microsatellite instability in human cell lines with defects in different mismatch repair genes. *Mutagenesis* 18: 277–282
141 Honchel R, Halling KC, Thibodeau SN (1995) Genomic instability in neoplasia. *Semin Cell Biol* 6: 45–52
142 Thibodeau SN, French AJ, Cunningham JM, Tester D, Burgart LJ, Roche PC, McDonnell SK, Schaid DJ, Vockley CW, Michels VV et al. (1998) Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. *Cancer Res* 58: 1713–1718
143 Hoang JM, Cottu PH, Thuille B, Salmon RJ, Thomas G, Hamelin R (1997) BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. *Cancer Res* 57: 300–303
144 Zhou XP, Hoang JM, Li YJ, Seruca R, Carneiro F, Sobrinho-Simoes M, Lothe RA, Gleeson CM, Russell SE, Muzeau F et al. (1998) Determination of the replication error phenotype in human tumors without the requirement for matching normal DNA by analysis of mononucleotide repeat microsatellites. *Genes Chromosomes Cancer* 21: 101–107
145 Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J (1997) Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res* 57: 4749–4756
146 Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B (1995) Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res* 55: 5548–5550
147 Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, Richards CS, Ward PA, Nelson DL (1994) Length of uninterrupted CGG repeats determines instability in the FMR1 gene. *Nat Genet* 8: 88–94
148 Mao L, Lee DJ, Tockman MS, Erozan YS, Askin F, Sidransky D (1994) Microsatellite alterations as clonal markers for the detection of human cancer. *Proc Natl Acad Sci USA* 91: 9871–9875
149 Risinger JI, Umar A, Barrett JC, Kunkel TA (1995) A hPMS2 mutant cell line is defective in strand-specific mismatch repair. *J Biol Chem* 270: 18183–18186
150 Risinger JI, Umar A, Boyd J, Berchuck A, Kunkel TA, Barrett JC (1996) Mutation of MSH3 in endometrial cancer and evidence for its functional role in heteroduplex repair. *Nat Genet* 14: 102–105
151 Oki E, Oda S, Maehara Y, Sugimachi K (1999) Mutated gene-specific phenotypes of dinucleotide repeat instability in human colorectal carcinoma cell lines deficient in DNA mismatch repair. *Oncogene* 18: 2143–2147
152 Baranovskaya S, Soto JL, Perucho M, Malkhosyan SR (2001) Functional significance of concomitant inactivation of hMLH1 and hMSH6 in tumor cells of the microsatellite mutator phenotype. *Proc Natl Acad Sci USA* 98: 15107–15112
153 Horii A, Han HJ, Shimada M, Yanagisawa A, Kato Y, Ohta H, Yasui W, Tahara E, Nakamura Y (1994) Frequent replication errors at microsatellite loci in tumors of patients with multiple primary cancers. *Cancer Res* 54: 3373–3375
154 Akiyama Y, Nakasaki H, Nihei Z, Iwama T, Nomizu T, Utsunomiya J, Yuasa Y (1996) Frequent microsatellite instabilities and analyses of the related genes in familial gastric cancers. *Jpn J*

Cancer Res 87: 595–601
155 Nakashima H, Honda M, Inoue H, Shibuta K, Arinaga S, Era S, Ueo H, Mori M, Akiyoshi T (1995) Microsatellite instability in multiple gastric cancers. *Int J Cancer* 64: 239–242
156 Jiricny J, Nystrom-Lahti M (2000) Mismatch repair defects in cancer. *Curr Opin Genet Dev* 10: 157–161
157 Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 75: 1027–1038
158 Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom-Lahti M et al. (1993) Mutations of a mutS homolog in hereditary non-polyposis colorectal cancer. *Cell* 75: 1215–1225
159 Umar A, Risinger JI, Glaab WE, Tindall KR, Barrett JC, Kunkel TA (1998) Functional overlap in mismatch repair by human MSH3 and MSH6. *Genetics* 148: 1637–1646
160 Palombo F, Gallinari P, Iaccarino I, Lettieri T, Hughes M, D'Arrigo A, Truong O, Hsuan JJ, Jiricny J (1995) GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. *Science* 268: 1912–1914
161 Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, D'Arrigo A, Markowitz S, Willson JK, Kinzler KW et al. (1995) Mutations of GTBP in genetically unstable cells. *Science* 268: 1915–1917
162 Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A et al. (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 368: 258–261
163 Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD et al. (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science* 263: 1625–1629
164 Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM et al. (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 371: 75–80
165 Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, Collins FS (2000) MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat Genet* 24: 27–35
166 Muller A, Fishel R (2002) Mismatch repair and the hereditary non-polyposis colorectal cancer syndrome (HNPCC). *Cancer Invest* 20: 102–109
167 Liu B, Nicolaides NC, Markowitz S, Willson JK, Parsons RE, Jen J, Papadopolous N, Peltomaki P, de la Chapelle A, Hamilton SR et al. (1995) Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat Genet* 9: 48–55
168 Schmutte C, Fishel R (1999) Genomic instability: first step to carcinogenesis. *Anticancer Res* 19: 4665–4696
169 Arzimanoglou II, Gilbert F, Barber HR (1998) Microsatellite instability in human solid tumors. *Cancer* 82: 1808–1820
170 Lothe RA (1997) Microsatellite instability in human solid tumors. *Mol Med Today* 3: 61–68
171 Schlotterer C, Tautz D (1992) Slippage synthesis of simple sequence DNA. *Nucleic Acids Res* 20: 211–215
172 Thomas DC, Umar A, Kunkel TA (1996) Microsatellite instability and mismatch repair defects in cancer. *Mutat Res* 350: 201–205
173 Boyer JC, Farber RA (1998) Mutation rate of a microsatellite sequence in normal human fibroblasts. *Cancer Res* 58: 3946–3949
174 Hanford MG, Rushton BC, Gowen LC, Farber RA (1998) Microsatellite mutation rates in cancer cell lines deficient or proficient in mismatch repair. *Oncogene* 16: 2389–2393
175 Umar A, Koi M, Risinger JI, Glaab WE, Tindall KR, Kolodner RD, Boland CR, Barrett JC, Kunkel TA (1997) Correction of hypermutability, N-methyl-N'-nitro-N-nitrosoguanidine resistance, and defective DNA mismatch repair by introducing chromosome 2 into human tumor cells with mutations in MSH2 and MSH6. *Cancer Res* 57: 3949–3955
176 da Costa LT, Liu B, el-Deiry W, Hamilton SR, Kinzler KW, Vogelstein B, Markowitz S, Willson JK, de la Chapelle A, Downey KM et al. (1995) Polymerase delta variants in RER colorectal tumours. *Nat Genet* 9: 10–11
177 Parc YR, Halling KC, Wang L, Christensen ER, Cunningham JM, French AJ, Burgart LJ, Price-Troska TL, Roche PC, Thibodeau SN (2000) HMSH6 alterations in patients with microsatellite

instability-low colorectal cancer. *Cancer Res* 60: 2225–2231
178 Koi M, Umar A, Chauhan DP, Cherian SP, Carethers JM, Kunkel TA, Boland CR (1994) 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
179 Risinger JI, Umar A, Glaab WE, Tindall KR, Kunkel TA, Barrett JC (1998) Single gene complementation of the hPMS2 defect in HEC-1-A endometrial carcinoma cells. *Cancer Res* 58: 2978–2981
180 Lettieri T, Marra G, Aquilina G, Bignami M, Crompton NE, Palombo F, Jiricny J (1999) Effect of hMSH6 cDNA expression on the phenotype of mismatch repair-deficient colon cancer cell line HCT15. *Carcinogenesis* 20: 373–382
181 Lengauer C, Kinzler KW, Vogelstein B (1997) DNA methylation and genetic instability in colorectal cancer cells. *Proc Natl Acad Sci USA* 94: 2545–2550
182 Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burgart LJ, Thibodeau SN (1998) Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. *Cancer Res* 58: 3455–3460
183 Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Kinzler KW et al. (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 95: 6870–6875
184 Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM, Kolodner R (1997) Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 57: 808–811
185 Bevilacqua RA, Simpson AJ (2000) Methylation of the hMLH1 promoter but no hMLH1 mutations in sporadic gastric carcinomas with high-level microsatellite instability. *Int J Cancer* 87: 200–203
186 Endoh Y, Tamura G, Ajioka Y, Watanabe H, Motoyama T (2000) Frequent hypermethylation of the hMLH1 gene promoter in differentiated-type tumors of the stomach with the gastric foveolar phenotype. *Am J Pathol* 157: 717–722
187 Simpkins SB, Bocker T, Swisher EM, Mutch DG, Gersell DJ, Kovatich AJ, Palazzo JP, Fishel R, Goodfellow PJ (1999) MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. *Hum Mol Genet* 8: 661–666
188 Glaab WE, Hill RB, Skopek TR (2001) Suppression of spontaneous and hydrogen peroxide-induced mutagenesis by the antioxidant ascorbate in mismatch repair-deficient human colon cancer cells. *Carcinogenesis* 22: 1709–1713
189 Ekbom A, Helmick C, Zack M, Adami HO (1990) Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med* 323: 1228–1233
190 Willenbucher RF, Aust DE, Chang CG, Zelman SJ, Ferrell LD, Moore DH, 2nd, Waldman FM (1999) Genomic instability is an early event during the progression pathway of ulcerative-colitis-related neoplasia. *Am J Pathol* 154: 1825–1830
191 Loeb KR, Loeb LA (1999) Genetic instability and the mutator phenotype. Studies in ulcerative colitis. *Am J Pathol* 154: 1621–1626
192 Fleisher AS, Esteller M, Tamura G, Rashid A, Stine OC, Yin J, Zou TT, Abraham JM, Kong D, Nishizuka S et al. (2001) Hypermethylation of the hMLH1 gene promoter is associated with microsatellite instability in early human gastric neoplasia. *Oncogene* 20: 329–335
193 Chang CL, Marra G, Chauhan DP, Ha HT, Chang DK, Ricciardiello L, Randolph A, Carethers JM, Boland CR (2002) Oxidative stress inactivates the human DNA mismatch repair system. *Am J Physiol Cell Physiol* 283: C148–154
194 Brentnall TA, Crispin DA, Bronner MP, Cherian SP, Hueffed M, Rabinovitch PS, Rubin CE, Haggitt RC, Boland CR (1996) Microsatellite instability in nonneoplastic mucosa from patients with chronic ulcerative colitis. *Cancer Res* 56: 1237–1240
195 Iwaya T, Maesawa C, Nishizuka S, Suzuki Y, Sakata K, Sato N, Ikeda K, Koeda K, Ogasawara S, Otsuka K et al. (1998) Infrequent frameshift mutations of polynucleotide repeats in multiple primary cancers affecting the esophagus and other organs. *Genes Chromosomes Cancer* 23: 317–322
196 Suzuki H, Harpaz N, Tarmin L, Yin J, Jiang HY, Bell JD, Hontanosas M, Groisman GM, Abraham JM, Meltzer SJ (1994) Microsatellite instability in ulcerative colitis-associated colorectal dysplasias and cancers. *Cancer Res* 54: 4841–4844
197 Perucho M (2003) Tumors with microsatellite instability: many mutations, targets and paradox-

es. *Oncogene* 22: 2223–2225
198 Duval A, Hamelin R (2002) Genetic instability in human mismatch repair deficient cancers. *Ann Genet* 45: 71–75
199 Lin HY, Wang XF, Ng-Eaton E, Weinberg RA, Lodish HF (1992) Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase. *Cell* 68: 775–785
200 Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B et al. (1995) Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 268: 1336–1338
201 Tomita S, Miyazato H, Tamai O, Muto Y, Toda T (1999) Analyses of microsatellite instability and the transforming growth factor-beta receptor type II gene mutation in sporadic human gastrointestinal cancer. *Cancer Genet Cytogenet* 115: 23–27
202 Leung SY, Chan TL, Chung LP, Chan AS, Fan YW, Hung KN, Kwong WK, Ho JW, Yuen ST (1998) Microsatellite instability and mutation of DNA mismatch repair genes in gliomas. *Am J Pathol* 153: 1181–1188
203 Woerner SM, Benner A, Sutter C, Schiller M, Yuan YP, Keller G, Bork P, Doeberitz MK, Gebert JF (2003) Pathogenesis of DNA repair-deficient cancers: a statistical meta-analysis of putative Real Common Target genes. *Oncogene* 22: 2226–2235
204 Duval A, Hamelin R (2002) Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. *Cancer Res* 62: 2447–2454
205 Malkhosyan S, Rampino N, Yamamoto H, Perucho M (1996) Frameshift mutator mutations. *Nature* 382: 499–500