

Cancer Genetics: A Primer for Surgeons

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Contemporary ideas of carcinogenesis envisage a series of stochastic genetic changes that confer a selective growth advantage over healthy cells. These changes collectively lead to the disruption of coordinated networks of intercellular communication and cause a fundamental change in cellular behavior, which affects processes, such as proliferation, differentiation, and apoptosis. This progressive dysregulation of cellular function implies that cancer is not a morphologic entity, but a process in which the malignant phenotype is gradually acquired. Rates of proliferation and differentiation are stringently regulated within normal tissues of a multicellular organism such that organs do not exceed a specific size and tissue renewal is proportionate and confined to replacement of damaged or effete cells only. An important mechanism for growth control in multicellular organisms is density-dependent growth inhibition, which ensures that no single cell has unrestrained growth and competition for space and nutrients is “fair.” This mechanism may be mediated by an increase in cellular requirements for macromolecular growth factors. As confluency is reached with crowding of cells, their innate sensitivity to these growth factors decreases, perhaps as a result of a reduction in the density of cell surface receptors [1]. Polypeptide growth factors are a group of regulatory molecules that have been well characterized from serum and cell tissue extracts. There seems to be a close relationship between growth factor production and growth of many types of tumor. They are functionally divided into positive and negative growth

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factors depending on whether epithelial proliferation is stimulated (mitogenic) or inhibited, respectively. Control of the cell cycle and hence rate of tumor growth is determined by the balance of growth factors acting on a cell. Although cells have an inherent program that influences rates of proliferation, differentiation, and cell death, this sea of soluble growth factors represents a principle mechanism for modulation and regulation of cellular activity by exogenous stimuli. Aberrant function of autocrine and paracrine growth factor loops leads to excessive proliferation and promotes neoplastic development [2].

Cancer genes

The existence of multiple mitogenic growth factors may guarantee a rapid growth phase during the early stages of embryogenesis thereby maximizing the chances of sustained viability. A consequence of this collective mitogenic potential of cells may be a lower threshold for development of hyperproliferative states that presage cancer. The sequence of events leading to formation of a tumor is ultimately attributable to genetic mutations and changes in gene expression, although the latter can be modified by host factors. Concepts of carcinogenesis over the past 2 decades have been dominated by the paradigm of oncogenes and more recently tumor suppressor genes [3]. The malignant phenotype is considered to arise from an accumulation, either randomly or sequentially, of alterations within these two operational classes of genes at the somatic cell level. Oncogenes are derived from normal cellular counterparts termed proto-oncogenes, which have some sequence homology with tumor-producing viruses. Activated oncogenes represent a positive or "gain-of-function" change resulting in a growth advantage over normal cells in possession of the inactivated proto-oncogene. The latter code for various proteins, including polypeptide growth factors and their receptors, together with several key components of the signal transduction process and nuclear regulators of the cell cycle. The normal proto-oncogene product may simply be produced in excessive amounts rather than activation being associated with an abnormal gene product. In both scenarios, there are increased rates of cell proliferation and persistence of genetically aberrant cells. By contrast, tumor suppressor genes are characterized by mutations that lead to loss of function. Tumor suppressor genes are natural elements of a cell's genetic code and products of these genes exert a negative (suppressive) influence on cellular proliferation but promote pathways leading to programmed cell death. In addition to acting as a kind of brake on the cell cycle, they have a crucial role in maintenance of genomic integrity and fidelity of DNA replication. Mutations within these tumor suppressor genes essentially produce tumors by default, whereas oncogenic events within a cell tend to have an executive influence on malignant change.

Inherited and sporadic forms of cancers

The genetic alterations within a cell that form the basis for malignant transformation can be either inherited or acquired [4]. Germline mutations are present within all cells, whereas somatic mutations affect individual cells within a particular tissue. Although most cancers arise from changes in gene expression consequent to acquired mutations within somatic cells, a minority (5%) of tumors develop within the setting of an inherited genetic predisposition. The cells of such individuals possess a pre-existing germline mutation and require fewer subsequent events for induction of carcinogenesis. Occasionally a genetic susceptibility may be manifest as a systemic effect whereby spontaneous mutations within all tissues become more frequent or carcinogens are metabolized less efficiently. Table 1 shows examples of specific tumors resulting from an inherited genetic predisposition that are associated with various familial cancer syndromes.

Epidemiologic studies of inherited and sporadic forms of certain cancers have provided much insight into the genetic initiation process. Knudson [5,6] proposed a “two-hit” hypothesis in which mutations in both alleles of a gene pair were a prerequisite for cancer development (Fig. 1). Individuals who have an inherited predisposition already possessed a mutation in one allele (present in all cells) and thus required only one further somatic mutation for tumor formation. Sporadic forms of the cancer depended on two somatic mutations, the chances of which were correspondingly smaller for any equivalent mutation rate. Knudson’s hypothesis is especially applicable to those tumors arising from loss of function in tumor suppressor genes; usually inactivation of both alleles is essential before levels of the gene product decrease sufficiently to induce malignant change. By contrast, oncogenes behave in a dominant manner and mutation within a single allele may be sufficient for tumor development. Sometimes heterozygosity at an oncogene locus (eg, 5q21) results in a premalignant phenotype, such as colonic polyps, with malignant transformation once mutation occurs in the second allele. Fearon and Vogelstein [7] proposed a model for colorectal carcinogenesis based on sequential genetic changes and progression from normal to dysplastic epithelium, polyp formation, and eventual colon cancer (see discussion of cytoplasmic tumor suppressor genes).

Carcinogenesis

Carcinogenesis is a multistage process with the sequential acquisition of mutations within the genome [8]. It remains unclear whether invasive malignancy develops once a critical number and type of mutations are present within a cell, or whether serial accumulation is mandatory, whereby mutations are acquired in a particular order. Many genetic changes are already present in premalignant and in situ forms of cancer. The incidence of

Table 1
Genes involved in familial cancer syndromes

Syndrome	Gene	Tumors
Ataxia telangiectasia	ATM	Lymphoma Breast cancer in heterozygotes
Bloom syndrome	BLM	Solid tumors
Cowden syndrome	PTEN1	Breast cancer Hamartoma
Familial adenomatous polyposis	APC	Colorectal cancer Desmoids Osteomas Duodenal cancer
Familial breast ovarian cancer	BRCA1, BRCA2	Breast cancer Ovarian cancer Male breast cancer (BRCA2)
Familial retinoblastoma	RB1	Retinoblastoma Osteosarcoma
Fanconi anemia	FACC, FACA	AML
Gorlin syndrome (basal cell nevus syndrome)	PATCHED	Basal cell cancer Medulloblastoma Ovarian fibroma
Hereditary nonpolyposis colon cancer (HNPCC or Lynch syndrome)	hMLH1, hMSH2, hPMS2, hMSH6	Colorectal cancer Endometrial cancer Gastric cancer Ovarian cancer Uroepithelial cancer
Hereditary papillary renal cancer	MET	Papillary renal cancer Other cancers
Juvenile polyposis	SMAD4	Hamartomatous polyps Colorectal cancer
Li Fraumeni syndrome	p53, hCHK2	Soft tissue sarcoma Breast cancer Brain tumors Leukemia
Multiple endocrine neoplasia 1 (MEN1)	MEN1	Parathyroid hyperplasia Endocrine pancreatic tumors Pituitary tumors
Multiple endocrine neoplasia 2 (MEN2)	RET	Medullary thyroid cancer Pheochromocytoma
Neurofibromatosis	NF1, NF2	Neurofibroma Acoustic neuroma Meningioma Schwannoma
Peutz-Jeghers syndrome	LKB1	Hamartomatous polyps Breast cancer Other tumors
Tuberous sclerosis	TSC1, TSC2	Renal angiomyolipomas Rhabdomyoma
von Hippel-Lindau syndrome	VHL	Renal cell cancer Hemangioblastoma Retinal angioma Pheochromocytoma
Wilms tumor	WT1	Wilms tumor
Xeroderma pigmentosum	XPB, XPD, XPA	Skin cancer

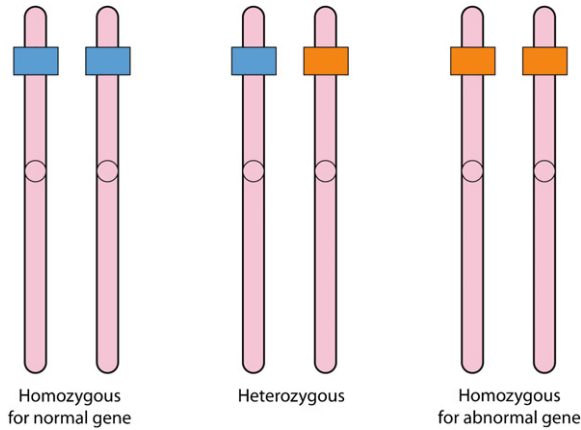


Fig. 1. Knudson proposed that the genetic changes within a somatic cell that underlie development of cancer are related to germline mutations, which are inherited in a Mendelian pattern. Individuals who have a familial predisposition already possess a mutation in one of a pair of alleles and require only one further mutation for malignancy to develop. By contrast, those individuals who do not have a genetic predisposition must acquire a mutation in each allele of a pair. Identification of the genes involved in hereditary susceptibility would provide insight into somatic cell mutations within sporadic tumors.

many common cancers (such as those of the breast, prostate, colon, or skin) increases with age with kinetics dependent on the fourth or fifth power of elapsed time. This observation suggests that a minimum of four or five events must occur before tumor development [9]. Moreover, the association of cancer with increasing age suggests that continuous exposure to low levels of environmental or endogenous carcinogens may have a cumulative effect and perhaps act on tissues more susceptible to neoplastic change.

Among those cancers that are attributable to inheritable forms of the disease, the development of malignancy is almost inevitable. In such cases, either the homozygous (tumor suppressor genes) or heterozygous state (oncogenes) confers a high level of genetic susceptibility and no further genetic mutation may be necessary for tumor initiation (eg, retinoblastoma). In other circumstances, the chance of developing cancer depends on a balance of genetic predisposition and acquired mutations. For most human cancers there is no inherited risk and these are termed sporadic tumors. They depend exclusively on somatic mutations. These latter genetic alterations result from one of two interrelated processes.

There is an intrinsic error rate for DNA synthesis and repair within normal tissues, which results in acquired mutations that are passed on to the cell progeny during replication. This phenomenon leads to a background rate of spontaneous mutation that has been estimated to be a 1 in 1 million chance for any particular gene each time a cell divides. This figure represents a very low baseline somatic mutation rate and the

chance of a key cancer gene being mutated spontaneously must be extremely low. A powerful positive selection pressure operates from the outset for cancer-promoting mutations, however, and this effectively magnifies the impact of low-frequency events. Once a mutation has occurred in a stem cell, a malignant clone of cells arises that replicates the initial mutation thousands of times. As the clone size approaches about 100 cells, the chance of a second mutation within this primary clone increases significantly, which in turn enhances any selective growth advantage and this new clone outgrows the first one. This process is continued and leads to acquisition of a collection of cellular features typical of the malignant phenotype.

This baseline rate of spontaneous somatic mutation is augmented by environmental factors interacting with cellular DNA either directly or indirectly. These include not only exposure to agents, such as radiation and chemical carcinogens, but also the genotoxic effects of endogenous agents, such as free radicals, which induce oxidative stress. Various chemicals are known to be carcinogenic and some of the mechanisms for induction of tumors have been elucidated. Although many of the well-documented industrial cancer risks have been minimized in recent years, several potential sources of carcinogens exist within the environment of contemporary western society. Most of these involve low levels of exposure, including low-density ionizing radiation and ultraviolet irradiation. These contribute to the background rate of somatic mutation and in some cases can greatly increase rates of malignancy.

The origin of cancer cells

One of the most challenging aspects in the research and treatment of cancer is tumor heterogeneity. Not only is there variation between individuals with respect to a designated tumor type, but cells composing a single tumor are far from homogeneous. Any theory for the origin of cancer cells must provide an explanation for cellular heterogeneity and address the questions of whether cancer is monoclonal or polyclonal in origin and whether cancers originate from stem cells or differentiated somatic cells.

Stem cells

Growth and development of normal tissues proceeds to a point at which the rate of cell proliferation is balanced by cell loss. Some tissues undergo hypertrophy or hyperplasia in response to normal physiologic processes (eg, breast and uterine tissues), but regress spontaneously on withdrawal of an external stimulus. Furthermore, during the process of normal development and tissue renewal, the progeny of stem cells differentiate into mature cells that have characteristic biochemical and functional properties. Stem

cells themselves originate from multipotential precursor cells that give rise to stem cells with a degree of genetic restriction and reduced potential. All stem cells have the capacity for self-renewal and can proliferate indefinitely. They undergo asymmetric cell divisions to produce a pool of identical progenitor cells or transit amplifying cells that differentiate into the cellular type appropriate for a particular location (Fig. 2). Stem cells for one particular lineage cannot differentiate into cells of another lineage and are “determined” once formed. The phenotype of these stem cells is influenced by environmental factors that control replacement of senescent cells from undifferentiated stem cells [10].

Monoclonal theory

Much evidence has accrued supporting a monoclonal origin for most human cancers. This theory implies that a single cell undergoes malignant transformation and forms a primary clone from which further subclones are derived. This process of clonal evolution has been described earlier and is predicated on a selective growth advantage for mutated cells that permits them to bridge bottlenecks imposed by restrictions of space, nutrients, and oxygen [11]. A polyclonal origin for cancer might be feasible for some

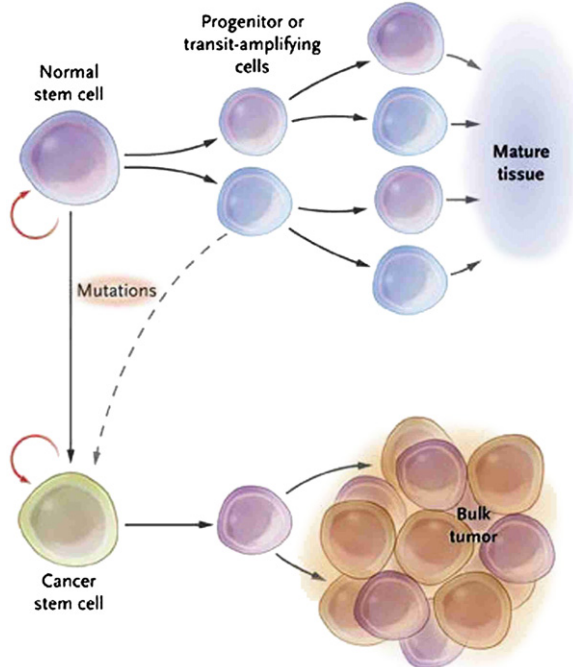


Fig. 2. All stem cells undergo asymmetric cell divisions to produce a pool of identical progenitor cells or transit amplifying cells that differentiate into the cellular type appropriate for a particular location.

inherited forms of cancer in which there are germline mutations in both alleles and genetic susceptibility operates as a field effect. For most sporadic cancers and inherited forms that depend on a further somatic mutation, however, polyclonality is highly improbable; multiple cells in close proximity would have to be transformed concurrently or within a relatively short time frame to form a single tumor.

The sequential acquisition of mutations during clonal evolution not only provides a positive selection pressure but also generates a degree of instability within the genome. This genetic instability favors further mutational change among individual cells of a clone. These additional and random mutations result in phenotypic differences between cells that are manifest as variations in rates of proliferation, cell motility, and metastatic potential, and sensitivity/resistance to therapeutic interventions. The mature tumor thus contains cells of monoclonal origin but phenotypic and genetic diversity. This process generates subclones of cells with different functional properties, some of which have the capacity to metastasize [12].

Cancer stem cell hypothesis

Many tumors are grossly similar to their tissue of origin, which is most evident for well-differentiated lesions. It was previously believed that cancers arose from dedifferentiation of the mature phenotype with reversion to a more primitive state to a greater (poorly differentiated) or lesser (well differentiated) degree. It is unlikely that a mature somatic cell would exist within tissues for a sufficiently long period to accumulate a mandatory number of mutations for malignant transformation. By contrast, stem cells have greater longevity and have the capacity for self-renewal. The cancer stem cell hypothesis proposes that the stem cell is the target for carcinogenesis and not mature somatic cells [13]. A tumor would arise from differentiation of rapidly proliferating, undifferentiated stem cells and contain varying proportions of these two stem cell types. Furthermore, the proportion of malignant stem cells that have undergone differentiation (and apoptosis) relative to those that continue to proliferate determines histologic grade. A cell may thus possess the typical features of a malignant phenotype yet still have gone through a sequential process of differentiation to a comparable point as the normal cell lineage. Cancer stem cells have now been identified in tumors of the breast and nervous system and are the focus for novel and more targeted forms of treatment. Cancer stem cells thus form a functional group of cells that initiate tumor formation and can differentiate into heterogeneous progeny that sustain tumor growth. A relatively small population of quiescent or slowly dividing cancer stem cells is responsible for the continued expansion of a tumor by spawning more differentiated cells. These latter cells constitute the bulk of the tumor (epithelial component) and have short-term proliferative capacity. The development of cancer therefore parallels normal tissue development with origin from a hierarchical lineage of cells [10].

Genetic alterations

The process of mitosis with division of a cell to produce progeny with identical genetic content is extremely complex. Although cell division is well orchestrated with a high degree of fidelity, there is an innate fallibility that leads to errors in DNA replication. The cell possesses multiple mechanisms for ongoing repair of inappropriate alterations in base-pair sequences, and can activate programmed cell death when there is overwhelming DNA damage or gross chromosomal changes. Some of the enzymes involved in these repair processes can be susceptible to mutational events and indirectly cause malignant transformation of cells by allowing persistence and propagation of gene alterations. Individuals who have Bloom syndrome's have a deficiency of DNA ligase I and are highly susceptible to developing cancer (see Table 1).

Types of mutation

In recent years there has been major progress in understanding and unraveling the genetic alterations that lead to disruption and dislocation of molecular and biochemical pathways. Although no single genetic change has been identified that causes cancer, it is now appreciated that cancer cells display a finite number of aberrant pathways and the defective portion of the DNA is relatively small in proportion to overall genome size. Exogenous agents, such as chemicals and irradiation, induce direct DNA damage, but a background mutation rate occurs from the hydrolytic interaction of water itself with DNA. This interaction results in the cleavage of glycosidic bonds which can depurinate or depyrimidate nucleotide bases or cause strand breaks. Genetic alterations associated with malignant change can be broadly divided into the following five categories:

Changes in nucleotide sequence resulting from base pair substitutions, deletions, or insertions. Deletions and insertions can lead to major problems with gene transcription and are sometimes referred to as missense mutations. These often result in a truncated protein because the altered DNA sequence cannot be read. Up to 90% of pancreatic adenocarcinomas contain missense mutations. Tautomeric changes within individual nucleotides may have minimal impact and cause minor changes in protein structure and function. Indeed, a codon with a single base alteration could be "silent" and not effect any change in amino acid sequence.

Changes in the normal diploid number of chromosomes are common in cancer. Aneuploid cells usually have a reduced complement of chromosomal material (up to 50%) and result from inappropriate segregation of chromosomes during mitosis.

Chromosomal translocations are the most common structural rearrangement in cancer cells and involve fusion of different chromosomes or

segments of a single chromosome that are noncontiguous. This interchange of chromosomal material can result in an oncogene being positioned next to transcription regulatory sequences leading to overexpression. Alternatively, a fusion gene may be formed from combination of coding sequences on either side of the breakpoint. A classic example of the latter is formation of the Philadelphia chromosome in chronic myelogenous leukemia (Fig. 3) [14]. This chromosome results from fusion of the carboxy terminus of the c-abl gene on chromosome 9 and the amino terminus of the bcr gene on chromosome 22 (bcr-abl gene product).

Gene amplifications result from multiple copies of an amplicon containing 0.5 to 10 megabases of DNA. This phenomenon occurs relatively late in the pathogenesis of cancer and probably reflects acquired genetic instability. When an amplicon encodes an oncoprotein, this is overexpressed and promotes tumorigenesis. Many oncogenes derive enhanced expression from this mechanism and are associated with particularly aggressive phenotypes. For example, the n-myc gene is frequently amplified in neuroblastomas, which are highly lethal tumors [15].

Epigenetic changes represent a nonmutational pathway for modulation of gene expression. Instead of changes in nucleotide sequence, DNA methylation and histone modification are used to maintain a gene in a closed conformation such that it cannot be accessed by DNA polymerase. Hypermethylation is a method for gene silencing and can prevent expression of tumor suppressor genes (eg, BRCA-1) even when the gene sequence is intact [16].

Genetic instability

The process of clonal selection amplifies the rate of spontaneous mutation in somatic cells to permit emergence of tumors. The frequency of mutational events is further enhanced by the existence of genetic instability which occurs pari-passu with clonal evolution. As cells progressively acquire cancer-related mutations, the genome becomes more unstable and prone to

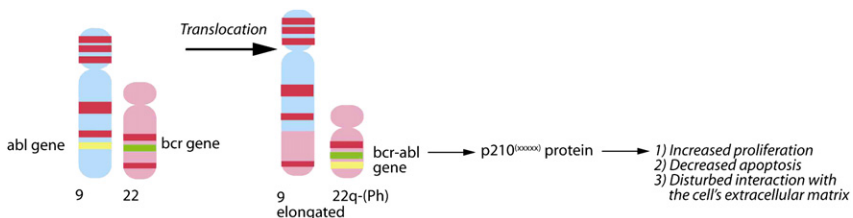


Fig. 3. Formation of the Philadelphia chromosome in chronic myelogenous leukemia resulting from fusion of the carboxy-terminus of the c-abl gene on chromosome 9 and the amino-terminus of the bcr gene on chromosome 22 (bcr-abl gene product).

genetic alteration, which is a general property of the cellular DNA and not attributable to specific mutations. Genetic instability seems to be inherent in a large proportion of cancers and greatly increases the probability of further spontaneous mutations that contribute to neoplastic development. Although cells are vulnerable to genetic damage, there are specific mechanisms that serve to maintain the integrity of the genome [17].

Caretaker genes: These genes are involved in recognition and repair of nucleotide base pair abnormalities or DNA strand breaks. Base excision and nucleotide excision repair are two general mechanisms that may be involved and have been implicated in BRCA-1 and BRCA-2 defects and their interaction with the repair protein Rad51 [18].

Gatekeeper genes: These genes control entry of cells into the replicative cycle and activate cell cycle arrest in the presence of damaged DNA. This set of gatekeeper genes works in collaboration with caretaker genes so that sufficient time and opportunity exist for repair of damaged DNA before onset of cell division. If this gatekeeper function fails, there is a risk that damaged portions of DNA will be passed on to daughter cells as potential cancer-promoting mutations. Gatekeepers operate around the cell cycle control or checkpoints (G1/S and G2/M) and interact with signal transduction pathways that are rapidly integrating stimulatory and inhibitory signals from within and outside the cell during the gap periods of the cell cycle (G1 and G2). The cell assesses the final polarity of the net signaling and together with an analysis of DNA integrity directs gatekeeper activities. Activation of gatekeeper pathways influences one or another of these checkpoints and prevents either the onset of DNA synthesis or entry into mitosis. Specific defects in caretaker and gatekeeper genes can lead to dramatic rates of genetic instability and rapid progression to a lethal phenotype.

Genetic instability can be manifest at one of two levels. Most instability is observed at the level of the chromosome, with large-scale deletions, duplication, or interchange of whole or large segments of chromosomes. Less commonly, nucleotide instability results from substitution, deletion, or insertion of nucleotides. Interestingly, there is an inverse relationship between instability at the chromosomal and nucleotide levels suggesting that these pathways may be mutually exclusive.

Chromosomal instability

Karyotypic analyses indicate that most cancers of epithelial origin display aneuploidy [19]. This finding suggests that several genes, when mutated, lead to this form of instability, which is at least 10-fold higher in aneuploid compared with diploid tumors. The molecular basis for this chromosomal instability is heterogeneous, but defects in specific checkpoints discussed earlier promote this form of genetic instability [20]. “Spindle checkpoint” genes

ensure that segregation of chromosomes on the mitotic spindle proceeds without error, but mutations in these genes are commonly detected in human cancer [21].

Defects in a second checkpoint, referred to as a “DNA damage checkpoint,” are probably a more frequent cause of chromosomal instability. This checkpoint prevents cells with damaged DNA from entering mitosis; replication of damaged DNA results in abnormalities of chromosomal segregation and mitotic recombination. Gross structural alterations in chromosomes occur if DNA replication goes ahead in the presence of either a single or double strand break. Some inherited forms of cancer predisposition are linked to these DNA strand break pathways and genes such as ATM (ataxia telangiectasia mutated), ATR (ATM and Rad-3 related), BRCA-1, BRCA-2, and p53 are DNA damage checkpoint genes that have been implicated in human malignancy [22]. Within normal cells, functional p53 prompts cell cycle arrest in G1 in the presence of inappropriate chromosomal segregation. By contrast, a defective p53 protein allows cells to progress through the G1/S transition and eventually aneuploidy occurs in daughter cells. A further mechanism for chromosomal instability is abnormal number and function of centrosomes. Centrosomes act to nucleate the ends of the mitotic microtubule spindle along which sister chromosomes separate during mitosis [23]. A final mechanism for chromosomal instability is by way of dysfunctional telomeres [24]. The latter are ribonuclear protein complexes located at the ends of all functional eukaryotic chromosomes. Telomeric dysfunction promotes end-to-end fusions and fusion-bridge-breakage cycles that result in gross structural chromosomal abnormalities [25]. Several mechanisms exist, therefore, whereby cells may become aneuploid with chromosomal instability. Collectively, these are responsible for the relatively frequent occurrence of these complex lesions.

Nucleotide instability

Instability at the nucleotide level is relatively uncommon in cancers and probably reflects the impact of environmental carcinogens or the background rate of somatic mutation. Defects in two main cellular DNA repair systems can lead to significant levels of genetic instability, however.

Nucleotide excision repair: This process is responsible for detection and repair of bulky DNA lesions induced by exogenous mutagens [26] and was first recognized in individuals who had xeroderma pigmentosum. The latter possess an inherited defect in this DNA repair system characterized by severe UV photosensitivity and susceptibility to skin cancers [27]. The disease is autosomally recessive and heterozygotes are not at increased risk for malignancy. The disease thus poses a significant clinical risk only in the context of an inherited predisposition.

DNA mismatch repair: This system is responsible for correction of DNA replication errors, including base–base mismatches and abnormal nucleotide

loops resulting from insertions/deletions of DNA and incorporated during the replicative process. Base–base mismatches typically affect nonrepetitive DNA sequences, whereas insertional/deletion loops occur at sites of repetitive DNA sequences. These lesions lead to gains or losses of short mono- or dinucleotide repeat units (eg, poly(A) or poly(CA) repeats) within sections of the genome called microsatellite regions. The microsatellite sequences are characterized by identical nucleotide repeats and are frequently observed in the coding regions of genes. This type of nucleotide replication error is known as microsatellite instability and has been identified in most tumors developing in patients who have hereditary nonpolyposis coli (HNPCC), otherwise known as Lynch syndrome [28,29]. This disease is caused directly by mutations in genes required for DNA mismatch repair that were initially investigated in yeast; human homologs were later identified [30]. Mismatch repair defects accelerate the mutation rate for hereditary and sporadic forms of colon cancer, however. Furthermore, mismatch repair defects can be detected in more than 10% of all colorectal, stomach, and endometrial cancers. It is now recognized that at least 95% of HNPCC cases are attributable to mutations in the human homologs of two mismatch repair genes (MSH2 and MLH1) [31]. A correspondingly lower proportion (15%) of sporadic colorectal cancers exhibit microsatellite instability and this often results from a nonmutational event (epigenetic inactivation of the MLH1 gene) [29].

Chromosomal translocations

Two principle forms of chromosomal translocations occur in human cancers:

Complex translocations: These are the more common type of translocation and probably represent a stochastic event with no predictable pattern of repetition within tumors of the same histopathologic subtype.

Simple translocations: These seem to be non-stochastic events and are characterized by distinctive patterns of breakpoints and chromosomal rearrangements in specific cancers. These simple types of translocation are most likely not due to any underlying genetic instability but instead may reflect low-frequency aberrations in normal physiologic recombination events. Translocations provide the opportunity for an oncogene to come under the influence of a strong promoter, either from repositioning next to regenerating sequences or fusion of two disparate coding regions.

Gene amplification

Gene amplification occurs toward the later stages of the neoplastic continuum and is a further manifestation of genetic instability. Gene

amplification results in exaggerated expression of otherwise normal genes, although the term oncogene encompasses overexpression of a normal gene and an intrinsic gene abnormality that leads to enhanced functional activity of the gene. Defects in the apoptotic pathway may permit cells with amplified chromosomal segments to survive (eg, p53 abnormalities).

Cell cycle checkpoints and cancer

The progression of cells through the normal cell cycle is closely regulated as part of the complex process of cell division. The aforementioned checkpoints exert a restraining influence on cell cycle progression and help ensure fidelity of DNA replication and that DNA repair processes are not compromised by time limitation. These control mechanisms minimize propagation of heritable mutations and reduce the risk for cancer development. Genes encoding proteins that promote cell cycling are frequently subject to activation in human cancers through gain-of-function mutation or gene amplification.

Oncogenes

It is now acknowledged that human cancer is a multifactorial process with several key steps being prerequisite for development of cancer. The original term “oncogene” implied that cancer might be caused by change in a single gene, but this is an oversimplified and outdated concept. A revolution in understanding carcinogenesis at the molecular level originated from work on RNA tumor viruses, which can rapidly induce tumors after inoculation into animal cells [32,33]. These viruses contain reverse transcriptase and can synthesize DNA with a complementary base pair sequence to viral RNA. This DNA can then be incorporated into host DNA and cause malignant transformation. Both normal and malignant cells contain DNA sequences that are homologous or identical to the oncogenic segments of these so-called “retroviruses.” These are termed cellular proto-oncogenes and correspond to viral (v-onc) oncogenes. These have probably arisen during evolution from incorporation of the cellular counterparts into viral structures. There is a remarkable level of conservation of these ancestral oncogenes.

The cellular homolog of viral oncogenes, the proto-oncogenes, are clearly not functioning in a tumorigenic capacity in most cells within animal tissues. These genetic sequences have oncogenic potential and this is expressed when the sequence is part of the viral genome—v-onc as opposed to c-onc. It may be surmised that cellular proto-oncogenes become activated either by overexpression of the normal gene product (quantitative change) or by alteration of the proto-oncogene to yield an abnormal product with oncogenic activity (qualitative change).

Not all cellular oncogenes have a viral homolog and other methods have been used to identify these other oncogenes. These include gene transfer, insertional mutagenesis, and analysis of chromosomal translocation and sites of amplification. In the former process, viruses activate cellular oncogenes (for which there is no viral counterpart, v-onc) by insertion of viral replicative sequences adjacent to the cellular DNA. These function as a promoter or enhancer and promote malignant transformation [34]. Most oncogenes code for protein products that form components of mitogenic growth signaling pathways. Stimulation of these pathways leads to increased rates of proliferation and promotes tumor formation. One of the earliest oncogene products to be characterized was from the src gene [35]. The protein product of the cellular homolog (c-src) is located mainly on the cytoplasmic side of the plasma membrane and is capable of autophosphorylation and phosphorylation of other proteins [36]. Phosphorylation occurs on tyrosine residues and these so-called “tyrosine protein kinases” can be divided into two main classes: those forms that are membrane associated but without any obvious transmembrane or extracellular domains, and those with a prominent extracellular domain that constitute a potential site for ligand binding. These are members of the growth factor receptor family and play an important role in mediation of external growth stimulatory signals (eg, erbB1, erbB2, PDGFR, IGFR1). These two types of tyrosine kinases constitute distinct ways in which mutant forms of the protein can function as an oncogene. A third category of oncogene is represented by nuclear proteins that are more proximate effectors in cell cycle control.

Receptor protein tyrosine kinases as oncogenes

Amplification of the genes controlling receptor protein tyrosine kinase is common in human cancers, resulting in overexpression and enhanced responsiveness to positive growth factor signals. This overexpression of receptors tends to promote ligand-independent dimerization with constitutive activation of the receptor, which can lead to stimulation of downstream mitogenic pathways in the absence of any external stimulus [37]. Epidermal growth factor receptor (EGFR) and HER2/neu are otherwise known as erbB1 and erbB2, respectively, and belong to a family of receptor protein tyrosine kinases (erbB 1–4), so-called because of their homology to the erythroblastoma viral gene product v-erbB [38]. Genes for both of these growth factor receptors are frequently amplified in breast, pancreas, and lung cancers. Furthermore, EGFR/erbB1 is overexpressed in up to 80% of head and neck cancers with levels of expression correlating inversely with survival [39]. Ligand binding to receptor protein tyrosine kinase usually leads to downstream activation of Ras and the mitogen-activated protein kinase cascades. The binding of EGF and similar ligands (TGF α) to the EGFR is a classic example of this [40]. Interestingly, HER2/neu (erbB2) has no natural ligand and functions as an amplifier by forming heterodimers

with other members of the *erbB* family. Overexpression results in formation of homodimers of *erbB2*, which have constitutively active tyrosine kinase activity. In contrast to *EGFR*, this activated *HER2/neu* has a much broader range of potential downstream substrates that can transduce mitogenic, growth stimulatory signals [41]. Mutations in the *c-ret* and *c-met* receptor protein tyrosine kinase oncogenes are found in some familial cancer syndromes, such as multiple endocrine neoplasia (MEN) 2A and 2B and familial forms of medullary thyroid cancer (*c-ret*) [42].

Cytoplasmic protein tyrosine kinases as oncogenes

The cytoplasmic portion of receptor protein tyrosine kinases converge on a common second messenger system called ras proteins. The primary role of these proteins is to act as shuttling molecules that couple receptor activation to downstream effector pathways involved in regulation of cellular proliferation, differentiation, and survival. Ras proteins are small GTPases that oscillate between inactive guanosine diphosphate (GDP)-bound and active guanosine triphosphate (GTP)-bound forms. This activated form of GTP-bound ras can interact with multiple downstream effectors to influence a spectrum of cellular processes varying from DNA synthesis to cell morphology and adhesion. The system is switched off by GTPase-activating proteins (GAPs) that hydrolyze GTP-bound ras to its GDP-bound form. Activating mutations of ras are found in approximately 30% of human cancers and permit cells to partially bypass receptor protein tyrosine kinase-signaling pathways [43]. Oncogenic sequences usually result from a missense point mutation and the ras protein is maintained in the activated GTP-bound state. A single amino acid substitution can render the GTP-bound form resistant to hydrolysis by GAPs. Generation of a continuous mitogenic signal provides a powerful driver for tumorigenesis. K-ras mutations are common in solid tumors: pancreatic (>90%), colorectal, endometrial, biliary tract, lung, and cervical. They occur together with H-ras mutations in about one third of leukemias and other myeloid malignancies, whereas H-ras mutations alone are found in bladder tumors. Downstream targets include Raf, which is a serine/threonine kinase that coordinates with ras to phosphorylate the kinase MEK, which in turn phosphorylates MAP kinase [44].

Nuclear proteins as oncogenes

Oncogene products residing within the nucleus itself might be expected to have a more direct influence on gene expression through binding to segments of DNA that contain gene regulatory elements. The *myc* oncogene is well characterized as a nuclear oncogene with growth stimulatory properties. Distinct forms of the gene exist in neuroblastoma/retinoblastoma (N-*myc*) and small cell lung carcinomas (L-*myc*) and all three forms of

the gene have been implicated in human malignancy [45]. The *myc* gene is universally expressed in cells and participates in a highly conserved pathway that is shared by most cells. Levels of the *myc* product are generally increased in actively dividing cells and the *myc* gene encodes transcriptional factors controlling proliferation, differentiation, and apoptosis. Abnormal expression within tumor cells may result from breakdown of a negative feedback loop whereby the *myc* product fails to appropriately regulate activity of the gene. Oncogenic forms of *myc* may result from various changes, including point mutation, amplification, and translocation.

Tumor suppressor genes

Although ultimately oncogenic growth stimulatory activities may become dominant within malignant cells, defects in tumor suppressor genes may lead to excessive proliferation and neoplastic progression by default. These genes are thus sometimes referred to as anti-oncogenes; this term implies that mutations within these genes can be the primary driving force for malignant transformation, rather representing a suppressor response to an established tumorigenic phenotype.

Tumor suppressor gene products are integral components of cell cycle regulatory pathways and have both gatekeeper and caretaker functions. Some tumor suppressor genes possess functional duality and inactivation leads to major disruption of cell cycle regulation. It is thus absence of a normal gene function rather than the presence of an abnormal gene per se that characterizes tumor suppressor gene disorders. A significant advance in understanding the concept of tumor suppressor genes came from studies into the genetic basis of retinoblastomas [46]. Familial cases who had bilateral tumors were noted to have loss of part of chromosome 13 (13q14). Sporadic cases who had unilateral tumors also showed a similar chromosomal loss and Knudson [5,6,47] proposed his famous two-hit hypothesis; in familial cases of the disease, one hit is inherited as a germline mutation, whereas the second hit is acquired early in life (perhaps in utero). It is now known that these two hits each correspond to allelic loss of a tumor suppressor gene—the retinoblastoma gene *Rb-1*. This gene was mapped to the chromosomal region 13q14 and its normal product is present in all cells except those of retinoblastoma tissue. Tumor formation is thus related to absence of the retinoblastoma gene product.

The breast cancer susceptibility genes *BRCA-1* and *BRCA-2* are tumor suppressor genes that display an autosomal dominant pattern of inheritance with variable penetrance. Mutations within these two genes account for approximately three quarters of hereditary breast cancer cases and confer a lifetime risk of between 80% and 85% by age 70 years [48] (ie, a 10-fold increase). At the cellular level, the effects of *BRCA-1* and *BRCA-2* are recessive and both copies of an allele must be lost or mutated for cancer

progression. Individuals who have a germline mutation in these genes have a dominantly inherited susceptibility and the second hit occurs in the somatic copy. Tumors from genetically predisposed patients show loss of heterozygosity in the wild-type BRCA-1 allele [49] but interestingly mutations of BRCA-1 and BRCA-2 are uncommon in sporadic breast cancers. The latter do not seem to result from acquired somatic mutations in both alleles and in this respect breast cancer genes differ from p53 and RB, which behave as classic tumor suppressor genes (mutations in both inherited and sporadic forms of cancer).

Transcriptional factors as tumor suppressor genes

The Rb gene product is a universally expressed nuclear protein that has a fundamental role in controlling progression of cells through the G1 checkpoint at the transition from G1 to S-phase entry [50]. Levels of the Rb protein are critical determinants of overall functional status and when the amount of protein decreases below a threshold value, suppressor activity is lost and the cell acquires an oncogenic phenotype. Transfection of the Rb gene into tumor cells lacking Rb expression reasserts normal features and cell behavior.

Like the Rb protein, the p53 gene product is present in a wide variety of normal cells and levels of expression are increased in up to half of all cancers. Moreover, the protein product seems to be more stable with a longer half-life in transformed cells. The p53 protein is often referred to as the guardian of the genome and has an important cell cycle checkpoint function that helps protect cells from genotoxic damage. It causes cells to arrest in the G1 phase of the cell cycle and can act as a homotetrameric transcriptional factor that is activated in response to cellular insults, such as irradiation, hypoxia, and drug-induced DNA damage. Although levels of p53 are increased in some tumors, the protein product is abnormal and p53 mutations are usually inactivating and associated with loss of gene function. Moreover, the p53 gene can act in a dominant negative manner whereby the presence of any abnormal protein product can impair function of normally expressed protein. Defective p53 introduced into the germline of transgenic mice leads to augmented tumorigenesis in the offspring of these p53-deficient mice [51]. p53 therefore functions as a tumor suppressor gene at the transcriptional level rather like the Rb gene. Indeed, the two proteins form part of a signaling network that regulates progression through the cell cycle and exerts a restraint on inappropriate growth-promoting signals. Mutations of the p53 gene occur in the Li-Fraumeni syndrome, which is associated with breast cancer, sarcomas, and adrenocortical tumors [52]. Two further key components of this network include p16 ink4a and p14ARF. The former binds and inhibits the cyclin D-dependent kinases CDK4 and CDK6 and thereby induces Rb-dependent G1 arrest [53,54]. Mutations of this gene are commonly found in familial and sporadic forms of melanoma, pancreatic, lung, and

bladder cancers. p14ARF is also a potent tumor suppressor capable of activating p53 by binding directly to the p53 inhibitor MDM2. Mutations within this gene frequently occur in T-cell leukemias.

Cytoplasmic tumor suppressor genes

The development of colorectal cancers may be attributable to absence of a normal gene product. One form of colorectal cancer is associated with the hereditary condition familial adenomatous polyposis coli (FAP), which results in formation of hundreds of polyps within the colon and rectum. A proportion of these will become dysplastic and thereafter progress to carcinoma [7]. An abnormality on chromosome 5 was originally identified in one of these patients and the defective segment localized to 5q21. Furthermore, mutations at this site (the APC gene) can be found in more than three quarters of cases of sporadic colorectal cancer. By analogy with retinoblastoma, a two-hit mechanism may apply; individuals who have FAP inherit a germline mutation of APC and require one further hit for development of cancer (heterozygosity predisposes to polyp formation alone). Sporadic forms of colorectal cancer require two somatic hits for tumor formation. Loss of function of tumor suppressor genes thus seems to be an important mechanism for carcinogenesis. The APC gene and protein product has been characterized and the latter interacts with β -catenin, which is a component of the Wnt/Wingless signaling pathway [55]. Wild-type APC protein associates with β -catenin and targets it for proteasomal degradation. When APC is mutated, however, it is no longer able to negatively regulate β -catenin. Accumulated β -catenin translocates to the nucleus where it promotes cell cycle progression by interaction with transcriptional factors LEP/TCP (lymphoid enhancer factor/T-cell factor). Mutations in β -catenin have been identified in colorectal cancer and could be linked to abnormalities on chromosomes 17 and 18, which are frequently found in familial (nonpolyposis) and sporadic forms of the disease. Chromosome 17 mutations might lead directly to p53 dysfunction and impact on the (Rb/p53/p16/p14) signaling network [53].

Receptor tumor suppressor genes

TGF β represents a family of multifunctional regulatory peptides involved in a range of processes, including development, wound healing, and carcinogenesis. The peptides are a component of the complex language of intercellular communication and potentially act as a switch that permits a biphasic functional profile. TGF β is a preeminent inhibitory growth factor and in the premalignant and early stages of cancer this tumor suppressor activity is sustained. As cells pass along the neoplastic continuum, however, functional disruption occurs and malignant epithelial cells show a reduced or absent response to the growth inhibitory effects of TGF β . Despite a dominance

of growth inhibition in the early stages of carcinogenesis, during growth of a tumor there is a shift in the balance between tumor suppressor and potential pro-oncogenic activity. In the more advanced stages of malignant disease, TGF β might promote tumor growth indirectly through the collective effects of stromal formation, angiogenesis, and immune suppression [56]. The tumor suppressor activity of TGF β has generated much interest in the potential role of this growth factor in the process of carcinogenesis and the mediation of response to some therapies. The exact function of TGF β depends on tumor stage and cellular context with relative amounts of ligand and receptor being a crucial determinant of response. TGF β receptors jointly coordinate a cellular response and mutations in the type II receptor gene lead to loss of a growth inhibitory response in colon cancer cell lines, which can be restored by transfection of the type II receptor subunit. The type II receptor is mutated in HNPCC through a mismatch repair error. Mutations within the TGF β 1 gene have been found in familial breast cancer and represent one of several low-risk genes (relative risk < 1.5) that together with BRCA-1 and BRCA-2 (and other higher-risk genes, such as PTEN and p53) contribute to approximately 25% of familial predisposition.

Mutations in genes regulating apoptosis and cell death pathways

Programmed cell death or apoptosis is an essential feature of normal development and is an ongoing process throughout the life of a complex multicellular organism. For example, selective removal of cells during the phase of tissue remodeling in organogenesis is achieved by coordinated activation of cell death programs. This process leads to generation of digits and body cavities, for example. Apoptosis is also activated when cells are subjected to an insult, such as DNA damage [57]. Cancer cells possess the ability to evade mechanisms of programmed cell death. Overexpression of the anti-apoptotic protein bcl-2 has been found in 85% of more aggressive lymphomas [58]. The bcl-2 gene is up-regulated by a chromosomal translocation (14:18) and elevated levels of bcl-2 protein bind to various proapoptotic factors (bad, bax, bid). Bcl-2 is a potent cell survival factor and prevents cytochrome c release, which in turn inhibits cell death. Inactivating mutations of p53 lead to impaired apoptotic pathways, with downstream effectors interacting with proapoptotic members of the bcl-2 family. Functioning p53 protein increases transcription of the bax gene, which promotes release of cytochrome c from mitochondria and promotes apoptosis.

Epigenetics

Most cancers display epigenetic changes that are reversible and heritable changes in gene expression without DNA sequence alterations. They act as translators between the environment and the genome and represent an

interface between genotype and phenotype. Cancer cells have an imbalance of DNA methylation; although there is widespread loss of genomic DNA methylation with neoplastic progression, there is aberrant hypermethylation of cytosine residues in CpG islands in the promoter region of genes [59]. These CpG islands are highly conserved segments of DNA with a GC content in excess of 50%. They are found in the promoter regions of almost half of mammalian genes. These CpG islands are normally protected from methylation, but aberrant methylation is widespread in human cancers and leads to selective gene silencing. Each tumor has its own pathways of methylation and hypermethylation profile. Epigenetic silencing tends to promote genetic instability with 5-methylcytosine being highly mutagenic and predisposing to C:G → A:T transitions. For example, in sporadic colon cancers there is evidence of hypermethylation and silencing of the DNA mismatch repair gene MLH1 leading to microsatellite instability [60]. Epigenetic silencing represents an important mechanism for inactivation of tumor suppressor genes. The BRCA-1 and APC genes can be inactivated by hypermethylation and in the case of the former this can act as a second hit in hereditary forms of breast cancer [61]. In sporadic cancers, there can be hypermethylation of one allele and genomic loss of the other allele. Various novel genes that can be epigenetically silenced are likely to be discovered in the future. Furthermore, it may be possible to restore normal gene expression by pharmacologic manipulation of epigenetic changes without the need for genetic engineering.

Summary

There have been great advances in our understanding of the molecular basis of carcinogenesis over the past 2 decades. Increasing understanding of the pathobiology and genetics of cancer has led to advances in treatment and risk prediction. Cancers are caricatures of normal tissues; their component cells are not foreign and genetically disparate like endogenous pathogens, but rogue cells with a finite number of genetic changes [62]. Newer forms of biologic therapies focus on blocking, bypassing, or re-regulating aberrant pathways and aim to control rather than kill cancer cells with improvement of disease-free survival and quality of life [63]. Targeting of specific growth factor pathways that drive tumor growth has become a clinical reality and this approach is consonant with the paradigm of control rather than cure.

The cellular heterogeneity of individual tumors presents a continued therapeutic challenge. There is increasing recognition that phenotypic heterogeneity for some cancers may reflect an accumulation of mutations in a large number of less highly penetrant genes rather than being attributable to simple changes in one or two dominant genes. The sophisticated methods of genetic profiling with DNA microarrays and their integration with proteomics

may ultimately allow individual tailoring of treatments and more accurate risk estimation for patients who have a hereditary predisposition. If tumors arise from transformation of stem cells (or a closely related progenitor) into malignant stem cells, then the latter must be targeted therapeutically; these cells are either quiescent or cycle relatively slowly and are resistant to conventional chemotherapy. The ability of stem cells to self-renew provides the opportunity for regeneration and clinical recurrence of cancer. Cancer stem cells retain programs for invasion and metastases together with protective mechanisms that favor survival despite exposure to potentially noxious therapies. Future research will focus on identification of biochemical pathways that are unique to cancer stem cells and thereby permit selective targeting of this important subpopulation of tumor cells.

References

- [1] Frank LM, Teich NM. Introduction to the cellular and molecular biology of cancer. New York: Oxford University Press; 1995.
- [2] Benson JR, Baum M, Colletta AA. Role of TGF beta in the anti-estrogen response/resistance of human breast cancer. *J Mammary Gland Biol Neoplasia* 1996;1(4):381–9.
- [3] Bishop JM, Weinberg RA. Molecular oncology. New York: Scientific American Inc.; 1996.
- [4] Bodmer WF. Inherited susceptibility to cancer. In: Frank LM, Teich NM, editors. Introduction to the cellular and molecular biology of cancer. 2nd edition. New York: Oxford University Press; 1995. p. 98–124.
- [5] Knudson AG Jr. Genetics of human cancer. *Annu Rev Genet* 1986;20:231–51.
- [6] Knudson AG Jr. Hereditary cancer, oncogenes, and antioncogenes. *Cancer Res* 1985;45(4):1437–43.
- [7] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61(5):759–67.
- [8] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100(1):57–70.
- [9] Stewart SA, Weinberg RA. Telomeres: cancer to human aging. *Annu Rev Cell Dev Biol* 2006;22:531–57.
- [10] Jordan CT, Guzman ML, Noble M. Cancer stem cells. *N Engl J Med* 2006;355(12):1253–61.
- [11] Greaves M. Cancer causation: the Darwinian downside of past success? *Lancet Oncol* 2002;3(4):244–51.
- [12] Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2007;58:267–84.
- [13] Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414(6859):105–11.
- [14] Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973;243(5405):290–3.
- [15] Brodeur GM. Molecular pathology of human neuroblastomas. *Semin Diagn Pathol* 1994;11(2):118–25.
- [16] Catteau A, Morris JR. BRCA1 methylation: a significant role in tumour development? *Semin Cancer Biol* 2002;12(5):359–71.
- [17] Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* 1997;386(6627):761–3.
- [18] Sharan SK, Morimatsu M, Albrecht U, et al. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* 1997;386(6627):804–10.

- [19] Weaver BA, Cleveland DW. Aneuploidy: instigator and inhibitor of tumorigenesis. *Cancer Res* 2007;67(21):10103–5.
- [20] Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004;432(7015):316–23.
- [21] Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat Rev Cancer* 2005;5(10):773–85.
- [22] Lobrich M, Jeggo PA. The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. *Nat Rev Cancer* 2007;7(11):861–9.
- [23] Mountzios G, Terpos E, Dimopoulos MA. Aurora kinases as targets for cancer therapy. *Cancer Treat Rev* 2008;34:175–82.
- [24] Hahn WC. Role of telomeres and telomerase in the pathogenesis of human cancer. *J Clin Oncol* 2003;21(10):2034–43.
- [25] Artandi SE, Attardi LD. Pathways connecting telomeres and p53 in senescence, apoptosis, and cancer. *Biochem Biophys Res Commun* 2005;331(3):881–90.
- [26] Saldivar JS, Wu X, Follen M, et al. Nucleotide excision repair pathway review I: implications in ovarian cancer and platinum sensitivity. *Gynecol Oncol* 2007;107(1 Suppl 1):S56–71.
- [27] Leibel D, Laspe P, Emmert S. Nucleotide excision repair and cancer. *J Mol Histol* 2006;37(5–7):225–38.
- [28] Soreide K, Janssen EA, Soiland H, et al. Microsatellite instability in colorectal cancer. *Br J Surg* 2006;93(4):395–406.
- [29] Vasen HF. Review article: the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *Aliment Pharmacol Ther* 2007;26(Suppl 2):113–26.
- [30] Li GM. Mechanisms and functions of DNA mismatch repair. *Cell Res* 2008;18(1):85–98.
- [31] Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993;75(5):1027–38.
- [32] Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. *Carcinogenesis* 2000;21(3):405–26.
- [33] Rous P. Viruses and tumour causation. An appraisal of present knowledge. *Nature* 1965;207(996):457–63.
- [34] Toren A, Ben-Bassat I, Rechavi G. Infectious agents and environmental factors in lymphoid malignancies. *Blood Rev* 1996;10(2):89–94.
- [35] De Larco JE, Todaro GJ. Growth factors from murine sarcoma virus-transformed cells. *Proc Natl Acad Sci U S A* 1978;75(8):4001–5.
- [36] Turner CE, Burridge K. Transmembrane molecular assemblies in cell-extracellular matrix interactions. *Curr Opin Cell Biol* 1991;3(5):849–53.
- [37] Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. *Endocr Relat Cancer* 2001;8(3):161–73.
- [38] Perona R. Cell signalling: growth factors and tyrosine kinase receptors. *Clin Transl Oncol* 2006;8(2):77–82.
- [39] Cruz JJ, Ocana A, Del Barco E, et al. Targeting receptor tyrosine kinases and their signal transduction routes in head and neck cancer. *Ann Oncol* 2007;18(3):421–30.
- [40] Lo HW, Hsu SC, Hung MC. EGFR signaling pathway in breast cancers: from traditional signal transduction to direct nuclear translocalization. *Breast Cancer Res Treat* 2006;95(3):211–8.
- [41] Baselga J, Albanell J. Mechanism of action of anti-HER2 monoclonal antibodies. *Ann Oncol* 2001;12(Suppl 1):S35–41.
- [42] Marx SJ. Molecular genetics of multiple endocrine neoplasia types 1 and 2. *Nat Rev Cancer* 2005;5(5):367–75.
- [43] Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. *Nat Rev Cancer* 2003;3(6):459–65.
- [44] Mirza AM, Gysin S, Malek N, et al. Cooperative regulation of the cell division cycle by the protein kinases RAF and AKT. *Mol Cell Biol* 2004;24(24):10868–81.
- [45] Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. *Oncogene* 1999;18(19):3004–16.

- [46] Vogel F. Genetics of retinoblastoma. *Hum Genet* 1979;52(1):1–54.
- [47] Knudson AG. Cancer genetics. *Am J Med Genet* 2002;111(1):96–102.
- [48] Ford D, Easton DF. The genetics of breast and ovarian cancer. *Br J Cancer* 1995;72(4):805–12.
- [49] Merajver SD, Pham TM, Caduff RF, et al. Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. *Nat Genet* 1995;9(4):439–43.
- [50] Harbour JW, Dean DC. Rb function in cell-cycle regulation and apoptosis. *Nat Cell Biol* 2000;2(4):E65–7.
- [51] Blackburn AC, Jerry DJ. Knockout and transgenic mice of Trp53: what have we learned about p53 in breast cancer? *Breast Cancer Res* 2002;4(3):101–11.
- [52] Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990;250(4985):1233–8.
- [53] Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell* 2002;2(2):103–12.
- [54] Sherr CJ. The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol* 2001;2(10):731–7.
- [55] Clevers H. Wnt breakers in colon cancer. *Cancer Cell* 2004;5(1):5–6.
- [56] Benson JR. Role of transforming growth factor beta in breast carcinogenesis. *Lancet Oncol* 2004;5(4):229–39.
- [57] Viktorsson K, Lewensohn R, Zhivotovsky B. Apoptotic pathways and therapy resistance in human malignancies. *Adv Cancer Res* 2005;94:143–96.
- [58] Sanchez-Beato M, Sanchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. *Blood* 2003;101(4):1220–35.
- [59] Miremadi A, Oestergaard MZ, Pharoah PD, et al. Cancer genetics of epigenetic genes. *Hum Mol Genet* 2007;16 Spec No 1:R28–49.
- [60] Esteller M. Epigenetic lesions causing genetic lesions in human cancer: promoter hypermethylation of DNA repair genes. *Eur J Cancer* 2000;36(18):2294–300.
- [61] Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. *Hum Mol Genet* 2007;16 Spec No 1:R50–9.
- [62] Pierce GB, Speers WC. Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. *Cancer Res* 1988;48(8):1996–2004.
- [63] Schipper H, Goh CR, Wang TL. Shifting the cancer paradigm: must we kill to cure? *J Clin Oncol* 1995;13(4):801–7.