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Cancer Genetics CANCER IS A GENETIC DISEASE Cancer arises through a series of somatic alterations in DNA that result in unrestrained cellular proliferation. Most of these alterations involve subtle sequence changes in DNA (i.e., mutations). The somatic mutations may originate as a consequence of random replication errors or exposure to carcinogens (e.g., radiation) and can be exacerbated by faulty DNA repair processes. While most cancers arise sporadically, clustering of can cers occurs in families that carry a germline mutation in a cancer gene. HISTORICAL PERSPECTIVE The idea that cancer progression is driven by sequential somatic muta tions in specific genes has only gained general acceptance in the past 30 years. Before the advent of the microscope, cancer was believed to be composed of aggregates of mucus or other noncellular matter. By the middle of the nineteenth century, it became clear that tumors were masses of cells and that these cells arose from the normal cells of the tissue from which the cancer originated. The molecular basis for the uncontrolled proliferation of cancer cells was to remain a mystery for another century. During that time, a number of theories for the origin of cancer were postulated. The great biochemist Otto Warburg proposed the combustion theory of cancer, which stipulated that cancer was due to abnormal oxygen metabolism. Others believed that all cancers were caused by viruses and that cancer was in fact a contagious disease. In the end, observations of cancer occurring in chimney sweeps, studies of x-rays, and the overwhelming data demonstrating cigarette smoke as a causative agent in lung cancer, together with Ames’s work on chemical mutagenesis, were consistent with the idea that cancer originated through changes in DNA. However, it was not until the somatic mutations responsible for cancer were identified at the molec ular level that the genetic basis of cancer was definitively established. Although the viral theory of cancer did not prove to be generally accurate (with exceptions such as human papillomaviruses, which can cause cervical and other cancers), the study of retroviruses led to the discovery of the first human oncogenes in the late 1970s. Oncogenes are one of the two major classes of cancer driver genes. The study of families with genetic predisposition to cancer was instrumental to the discovery of the other major class of cancer driver genes, called

tumorsuppressor genes. Current technologies permit the sequence analysis of entire cancer genomes and provide a comprehensive view of the genetic changes that cause tumors to arise and become malignant. The field that studies the various types of mutations, as well as the consequences of these mutations in tumor cells, is now known as cancer genetics.

THE CLONAL ORIGIN AND MULTISTEP NATURE OF CANCER Nearly all cancers originate from a single cell; this clonal origin is a critical discriminating feature between neoplasia and hyperplasia. Multiple cumulative mutational events are invariably required for the progression of a tumor from normal to fully malignant phenotype. The process can be seen as Darwinian microevolution in which, at each successive step, the mutated cells gain a growth advantage resulting in the expansion of a neoplastic clone (Fig. 76-1). Based on observa tions of cancer frequency increases during aging, the epidemiologists Armitage and Doll and Nordling independently proposed that cancer is a result of three discrete cellular changes. Remarkably, this early model has been validated by extensive sequencing of cancer genomes. These studies revealed that just three causal mutations are required for the development of several of the most common cancers. Overall, it is currently believed that most common solid tumors require a minimum of three mutated cancer driver genes (either oncogenes or tumorsuppressor genes) for their development. One or two mutations are sufficient for benign tumorigenesis, but not for the invasive capacity that distinguishes cancers from benign tumors. Less common tumors, such as liquid tumors (leukemias or lymphomas), sarcomas, and childhood tumors, appear to require only two driver gene alterations for malignancy. Note that a cancer driver gene is best defined as one containing a mutation that increases the selective growth advantage of the cell containing it. Normally, cell birth and cell death are in perfect equilibrium; every time a cell is born, another in the same lineage dies. Cancer driver gene mutations alter this equilibrium, so that more cells are born than die. The imbalance is often slight, so that the difference between cell birth and cell death can be less than 1%. This explains, in combination with the low rate of mutation, why tumorigenesis—the journey from a normal cell to a typical malignant, solid tumor—often takes decades.

CHAPTER 76 Cancer Genetics We now know the precise nature of the genetic alterations respon sible for nearly all malignancies and are beginning to understand how these alterations promote the distinct stages of tumor growth. The prototypical example is colon cancer, in which analyses of genomes from the entire spectrum of neoplastic growths—from normal colon Initiation Expansion Invasion FIGURE 76-1 Multistep clonal development of malignancy. In this diagram, a series of three cumulative mutations, each with a modest growth advantage acting alone, eventually results in a malignant tumor. Note that not all such alterations result in progression. The actual number of cumulative mutations necessary to transform from the normal to the malignant state has been estimated to be three for several of the most common types of cancer. (Adapted and modified from PC Nowell: The clonal evolution of tumor cell populations. Science 194:23, 1976.)

Microsatellite Instability (MIN) or Chromosomal Instability (CIN) SMAD4 or TGFb II inactivation TP53 inactivation APC inactivation or b-catenin activation KRAS or BRAF activation Early adenoma Late adenoma Carcinoma Metastasis Normal epithelium Initiation Expansion Invasion FIGURE 76-2 Progressive somatic mutational steps in the development of colon carcinoma. The accumulation of alterations in a number of different genes results in the progression from normal epithelium through adenoma to full-blown carcinoma. Genetic instability (microsatellite or chromosomal) accelerates the progression by increasing the likelihood of mutation at each step. Patients with

familial polyposis are already one step into this pathway because they inherit a germline alteration of the APC gene. TGF, transforming growth factor. epithelium through adenoma to carcinoma—have identified mutations that are highly characteristic of each type of lesion (Fig. 76-2). PART 4 Oncology and Hematology TWO TYPES OF CANCER GENES: ONCOGENES AND TUMOR-SUPPRESSOR GENES Oncogenes and tumor-suppressor genes exert their effects on tumor growth through their ability to determine cell fates, influence cell sur vival, and contribute to genome maintenance. The underlying molecu lar mechanisms can be extremely complex. While tightly regulated in normal cells, oncogenes acquire mutations that typically relieve this control and lead to increased activity of the gene products. This activat ing mutational event occurs in a single allele. In contrast, the normal function of tumor-suppressor genes is usually to restrain cell growth, and this function is lost in cancer. Because of the diploid nature of mammalian cells, both alleles must be inactivated for a cell to com pletely lose the function of a tumor-suppressor gene. Thus, two genetic events are required to inactivate a tumor-suppressor gene, while only one genetic event is required to activate an oncogene. A subset of tumor-suppressor genes controls the ability of the cell to maintain the integrity of its genome. Cells with a deficiency in these genes acquire an increased number of mutations throughout their genomes, including those in oncogenes and tumor-suppressor genes. This “mutator” phenotype was first hypothesized by Loeb to explain how the multiple rare mutational events required for tumorigenesis can occur in the lifetime of an individual. A mutator phenotype under lies several forms of cancer, such as those associated with deficiencies in DNA mis match repair. The great majority of cancers do not harbor repair deficiencies, and their rate of mutation is similar to that observed in normal cells. Many of these cancers, however, appear to harbor a different kind of genetic instability, affecting the loss or gains of whole chromosomes or large parts thereof (as explained in more detail below). TABLE 76-1 Oncogenes Commonly Altered in Human Cancers ONCOGENE FUNCTION ALTERATION IN CANCER NEOPLASM AKT1 Serine/threonine kinase Point mutation Skin BRAF Serine/threonine kinase Point mutation Melanoma, thyroid, colorectal CCND1 Cell cycle progression Amplification Esophageal, head and neck CTNNB1 Signal transduction Point mutation Colon, liver, uterine, melanoma EGFR Signal transduction Point mutation Lung FLT3 Signal transduction Point mutation AML IDH1 Chromatin modification Point mutation Glioma MDM2 Inhibitor of p53 Amplification Sarcoma, glioma MDM4 Inhibitor of p53 Amplification Breast MYC Transcription factor Amplification Prostate, ovarian, breast, liver, pancreatic ONCOGENES IN HUMAN CANCER Work by Peyton Rous in the early 1900s revealed that a chicken sarcoma could be transmitted from animal to animal in cellfree extracts, suggesting that cancer could be induced by an agent acting positively to MYCL1 Transcription factor Amplification Ovarian, bladder MYCN Transcription factor Amplification Neuroblastoma PIK3CA Phosphoinositol-3-kinase Point mutation Multiple cancers KRAS GTPase Point mutation Pancreatic, colorectal, lung NRAS GTPase Point mutation Melanoma Abbreviation: AML, acute myeloid leukemia.

promote tumor formation. The agent responsible for the transmission of the cancer was a retrovirus (Rous sarcoma virus [RSV]), and the oncogene responsible was identified 75 years later as V-SRC. Other oncogenes were also discovered through their presence in the genomes of retroviruses that are capable of causing cancers in chickens, mice, and rats. The nonmutated cellular homologues of these viral genes are called proto-oncogenes and are often targets of mutation or aberrant regula tion in human cancer. Whereas many oncogenes were discovered on the basis of their presence in retroviruses, other oncogenes, particularly those involved in translocations characteristic of par ticular leukemias and lymphomas, were identi fied through

genomic approaches. Investigators cloned the sequences surrounding the chromo somal translocations observed cytogenetically and identified the genes activated at the breakpoints (see below). Some of these were oncogenes previ ously found in retroviruses (like ABL, involved in chronic myeloid leukemia [CML]), whereas others were new (like BCL2, involved in B-cell lymphoma). In the normal cel lular environment, proto-oncogenes have crucial roles in cell prolifera tion and differentiation. Table 76-1 is a partial list of oncogenes known to be involved in human cancer. The normal growth and differentiation of cells is controlled by growth factors that bind to receptors on the surface of the cell. The signals generated by the membrane receptors are transmitted inside the cells through signaling cascades involving kinases, G proteins, and other regulatory proteins. Ultimately, these signals affect the activity of transcription factors in the nucleus, which regulate the expression of genes crucial in cell proliferation, cell differentiation, and cell death. Oncogene products function at critical steps in these signaling path ways (Chap. 77). Inappropriate activation of these pathways can lead to tumorigenesis. MECHANISMS OF ONCOGENE ACTIVATION ■ ■POINT MUTATION Point mutation (alternatively known as single nucleotide substitution) is a common mechanism of oncogene activation. For example, point mutations in KRAS are present in >95% of pancreatic cancers and 40% of colon cancers. Activating KRAS mutations are less common in other cancer types, although they can occur at significant frequencies in leukemia, lung, and thyroid cancers. Remarkably—and in contrast

to the diversity of mutations found in tumor-suppressor genes—most of the activated KRAS alleles contain point mutations in codons 12, 13, or 61. These mutations lead to constitutive activation of the mutant RAS protein. The restricted pattern of mutations observed in onco genes compared to that of tumor-suppressor genes reflects the fact that gain-of-function mutations must occur at specific sites, while a broad variety of mutations can lead to loss of activity. Indeed, inactivation of a gene can in theory be accomplished through the introduction of a stop codon anywhere in the coding sequence, whereas activations require precise substitutions at residues that can somehow lead to an increase in the activity of the encoded protein under particular circumstances within the cell. ■ ■DNA AMPLIFICATION The second mechanism for activation of oncogenes is DNA sequence amplification, leading to overexpression of the gene product. This increase in DNA copy number may cause cytologically recognizable chromosome alterations referred to as homogeneous staining regions (HSRs) if integrated within chromosomes, or double minutes (dmins) if extrachromosomal. Numerous genes have been reported to be amplified in cancer. Several of these genes, including NMYC and LMYC, were identified through their presence within the amplified DNA sequences of a tumor and their homology to known oncogenes. Because amplified regions often include hundreds of thousands of base pairs, multiple oncogenes may be amplified in a single amplicon in some cancers. For example, MDM2, GLI1, CDK4, and TPSPAN31 at chromosomal location 12q1315 have been shown to be co-amplified in several types of sarcomas and other tumors; which of these genes play the causal role in the neoplastic process is still an active area of research. Amplification of a cellular gene is often a predictor of poor prognosis; for example, ERBB2/HER2 and NMYC are often amplified in aggressive breast cancers and neuro blastoma, respectively. ■ ■CHROMOSOMAL REARRANGEMENT Chromosomal alterations provide important clues to the genetic changes in cancer. The chromosomal alterations in human solid tumors such as carcinomas are heterogeneous and complex and occur as a result of the frequent chromosomal instability observed in these tumors (see below). In contrast, the chromosome alterations in myeloid and lymphoid tumors are often simple translocations, that is, reciprocal transfers of chromosome arms from one chromosome to another. The breakpoints of recurring chromosome abnormalities usually

occur at the site of cellular oncogenes. Table 76-2 lists representative examples of recurring chromosome alterations in malignancy and the associated gene(s) rearranged or deregulated by the chromosomal rearrangement. Translocations are often observed in liquid tumors in general and are particularly common in lymphoid tumors, probably because these cell TABLE 76-2 Representative Oncogenes at Chromosomal Translocations GENE (CHROMOSOME) TRANSLOCATION MALIGNANCY BCR-ABL (9;22)(q34;q11) Chronic myeloid leukemia BCL1 (11q13.3)–IgH (14q32) (11;14)(q13;q32) Mantle cell lymphoma BCL2 (18q21.3)–IgH (14q32) (14;18)(q32;q21) Follicular lymphoma FLI-EWSR1 (11;22)(q24;q12) Ewing's sarcoma LCK-TCRB (1;7)(p34;q35) T-cell acute lymphocytic leukemia PAX3-FOXO1 (2;13)(q35;q14) Rhabdomyosarcoma PAX8-PPARG (2;3)(q13;p25) Thyroid IL21R-BCL6 (3;16)(q27;p11) Non-Hodgkin's lymphoma TAL1-TCTA (1;3)(p34;p21) Acute T-cell leukemia TMPRSS2-ERG Rearrangement on Chr21q22 Prostate

types have the capability to rearrange their DNA to generate antigen receptors. Indeed, antigen receptor genes are commonly involved in the translocations, implying that an imperfect regulation of receptor gene rearrangement may be involved in their pathogenesis. In addition to transcription factors and signal transduction molecules, translocation may result in the overexpression of cell cycle regulatory proteins or proteins such as cyclins and of proteins that regulate cell death. Recur rent translocations have more recently been identified in solid tumors such as prostate cancers. For example, fusions between TMPRSS2 and ERG, which are normally located in tandem on chromosome 21, con tribute to more than one-third of all prostate cancers.

The first reproducible chromosome abnormality detected in human malignancy was the Philadelphia chromosome detected in CML. This cytogenetic abnormality is generated by reciprocal translocation involving the ABL oncogene on chromosome 9, encoding a tyrosine kinase, being placed in proximity to the breakpoint cluster region (BCR) gene on chromosome 22. Figure 76-3 illustrates the genera tion of the translocation and its protein product. The consequence of expression of the BCR-ABL gene product is the activation of signal transduction pathways leading to cell growth independent of normal external signals. Imatinib, a drug that specifically blocks the activity of Abl tyrosine kinase, has shown remarkable efficacy with little tox icity in patients with CML. The successful targeting of BCR-ABL by imatinib is the paradigm for molecularly targeted anticancer therapies. CHAPTER 76 CHROMOSOMAL INSTABILITY IN SOLID TUMORS Solid tumors generally contain an abnormal number of chromosomes, a state known as aneuploidy. Chromosomes from aneuploid tumors also exhibit structural alterations such as translocations, deletions, and amplifications. These abnormalities reflect an underlying defect in cancer cells known as chromosomal instability. While aneuploidy is a striking cellular phenotype, chromosomal instability is manifest as only a small increase in the tendency of cells to gain, lose, or rearrange chromosomes during any given cell cycle. This intrinsically low rate of chromosome aberration implies that cancer cells become aneuploid only after many generations of clonal expansion. The molecular basis of aneuploidy remains incompletely understood. It is widely believed that defects in checkpoints, the quality-control mechanisms that halt the cell cycle if chromosomes are damaged or misaligned, contribute to chromosomal instability. This hypothesis emerged from experimental observations that the tumor suppressor p53 controls checkpoints that regulate the initiation of DNA replication and the onset of mitosis. These processes are therefore defective in many cancer cells. The mitotic spindle checkpoint, which ensures proper chromosome attach ment to the mitotic spindle before allowing the sister chromatids to separate, is also altered in some

cancers, irrespective of p53 status. The precise relationship between checkpoint deficiency, p53, and chromosomal instability remains unclear, but it is believed that even a subtle perturbation of the highly orchestrated process of cell divi sion can impact the ability of a cell to faithfully replicate and segregate its complement of chromosomes. From a therapeutic standpoint, the checkpoint defects that are prevalent in cancers have been proposed as vulnerabilities that may be exploited by novel agents and combinatorial strategies. Cancer Genetics In contrast to the genome-wide cytogenetic changes that are typical indications of an underlying chromosomal instability, more focal pat terns of chromosomal rearrangement have been recurrently detected in many cancer types. A curious phenomenon known as chromothripsis causes dozens of distinct breakpoints that are localized on one or sev eral chromosomes. These striking structural alterations are thought to reflect a single event in which a chromosome is fragmented and then imprecisely reassembled. In some cancer types, chromothripsis contributes to oncogene amplification and tumor suppressor gene inactivation in a substantial proportion of tumors. While the exact pro cess that underlies chromothripsis remains obscure, a transient period of extreme instability stands in contrast to the gradual loss, gain, and rearrangement of chromosomes that are typically observed in serially cultured cancer cells.

Chr 9 Changed Chr 9 Chr 22 BCR BCR Chromosome translocation 9q34 ABL 22q11 FIGURE 76-3 Specific translocation seen in chronic myeloid leukemia (CML). The Philadelphia chromosome (Ph) is derived from a reciprocal translocation between chromosomes 9 and 22 with the breakpoint joining the sequences of the ABL oncogene with the BCR gene. The fusion of these DNA sequences allows the generation of an entirely novel fusion protein with modified function. PART 4 Oncology and Hematology TUMOR-SUPPRESSOR GENE INACTIVATION IN CANCER The normal role of tumor-suppressor genes is to restrain cell growth, and the function of these genes is inactivated in cancer. The three major types of somatic lesions observed in tumor-suppressor genes during tumor development are point mutations, small insertions and/or deletions known as indels, and large deletions. Point mutations or indels in the coding region of tumor-suppressor genes will frequently lead to truncated protein products or allele-specific loss of RNA expression by the process of nonsense-mediated decay. Unlike the highly recurrent point mutations that are found in critical positions of activated onco genes, known as mutational hotspots, the point mutations that cause tumor-suppressor gene inactivation tend to be distributed throughout the open reading frame. Large deletions lead to the loss of a functional product and sometimes encompass the entire gene or even the entire chromosome arm, leading to loss of heterozygosity (LOH) in the tumor DNA compared to the corresponding normal tissue DNA (Fig. 76-4). Mapping regions of LOH was a useful approach in the positional clon ing of many tumor-suppressor genes. The rate of LOH is increased in the presence of chromosomal instability, a relationship that would explain the selective forces leading to the high prevalence of aneuploidy in late-stage cancers. Gene silencing, an epigenetic change that leads to the loss of gene expression, occurs in conjunction with hypermethylation of the promoter and histone deacetylation, and is another mechanism of tumor-suppressor gene inactivation. An epigenetic modification refers to a covalent modification of chromatin, heritable by cell progeny that may involve DNA but does not involve a change in the DNA sequence. FAMILIAL CANCER SYNDROMES A small fraction of cancers occurs in patients with a genetic predispo sition. Based on studies of inherited and sporadic forms of retinoblas toma, Knudson and others formulated a hypothesis that explains the differences between sporadic and inherited forms of the same tumor type. In inherited forms of cancer, called cancer predisposition syn dromes, one allele of a particular tumor-suppressor gene is inherited in mutant form. This germline mutation is not

sufficient to initiate a tumor, however; the other allele, inherited from the unaffected parent, must become somatically inactivated in a normal stem cell for tumori genesis to be initiated. In sporadic (noninherited) forms of the same disease, all cells in the body start out with two normal copies of the tumor-suppressor gene. A single cell must then sequentially acquire

Ph Chr Chimeric gene ABL BCR ABL BCR-ABL fusion protein mutations in both alleles of the tumor-suppressor gene to initiate a tumor. Thus, biallelic mutations of the same tumor-suppressor gene are required for both inherited and noninherited forms of the disease; the only difference is that individuals with the inherited form have a “head start”: they already have one allele mutated, from conception, and only need one additional mutation to initiate the process (Fig. 76-4). This distinction explains why those with inherited forms of the dis ease develop more cancers, at an earlier age, than the general popula tion. It also explains why, even though every cell in an individual with a cancer predisposition syndrome has a mutant gene, only a relatively small number of tumors arise during their lifetime. The reason is that the vast majority of cells within such individuals are functionally normal because one of the two alleles of the tumor-suppressor gene is normal. Mutations are uncommon events, and only the rare cells that develop a mutation in the remaining normal allele will exhibit uncontrolled proliferation. The same principle applies to virtually all types of cancer predisposition syndromes, though the particular genes differ. For example, inherited mutations in RB1, WT1, VHL, APC, and BRCA1 lead to predispositions to retinoblastomas, Wilms’ tumors, renal cell carcinomas, colorectal carcinomas, and breast carcinomas, respectively (Table 76-3). Also note that the biallelic inactivation of any of these genes is not sufficient to develop cancer; it requires other, additional somatic alterations in other genes for the initiating cells to evolve to malignancy, as noted above. Roughly 100 familial cancer syndromes have been reported; the great majority are very rare. Most of these syndromes exhibit an autosomal dominant pattern of inheritance, although some of those associated with DNA repair abnormalities (xeroderma pigmentosum, Fanconi’s anemia, ataxia telangiectasia) are inherited in an autosomal recessive fashion. Table 76-3 shows a number of cancer predisposition syndromes and the responsible genes. Familial adenomatous polyposis (FAP) is a dominantly inherited colon cancer syndrome caused by germline mutations in the adeno matous polyposis coli (APC) tumor-suppressor gene on chromosome 5. Affected individuals develop hundreds to thousands of adenomas in the colon. In each of these adenomas, the APC allele inherited from the nonaffected parent has been inactivated by virtue of a somatic mutation (Fig. 76-2). This inactivation usually occurs through a gross chromosomal event resulting in loss of all or a large part of the long arm of chromosome 5, where APC resides. In other cases, the remain ing allele is inactivated by a subtle intragenic mutation of APC, which is typically a single base substitution resulting in a nonsense codon.

A1 + + B1 A2 A1 + Rb B2 Markers A and B B2 Tumor formation A1 + Rb B1 A3 B3 A1 Rb Rb B1 FIGURE 76-4 Diagram of possible mechanisms for tumor formation in an individual with hereditary (familial) retinoblastoma. On the left is shown the pedigree of an affected individual who has inherited the abnormal (Rb) allele from her affected mother. The normal allele is shown as a (+). The four chromosomes of her two parents are drawn to indicate their origin. Flanking the retinoblastoma locus are genetic markers (A and B) also analyzed in this family. Markers A3 and B3 are on the chromosome carrying the retinoblastoma disease gene. Tumor formation results when the normal allele, which this patient inherited from her father, is inactivated. On the right are shown four possible ways in which this could occur. In each case, the resulting chromosome 13

arrangement is shown. Note that in the first three situations, the normal allele (B1) has been lost in the tumor tissue, which is referred to as loss of heterozygosity (LOH) at this locus. Gross chromosomal losses occur more commonly than point muta tions in normal cells, explaining why chromosomal loss rather than point mutation is the predominant mechanism underlying the inacti vation of the normal allele of APC. The same is true for other cancer predisposition syndromes caused by other inherited tumor suppressor gene mutations; gross chromosomal events are generally responsible for inactivation of the tumor-suppressor gene allele inherited from the nonaffected parent. Several thousand adenomas form in FAP patients, and a small subset of the millions of cells within an adenoma will acquire a second mutation, leading to tumor progression, that is, a larger adenoma. A third mutation in such a larger adenoma may convert it to a carcinoma. If untreated (by colectomy), at least one of the adenomas will progress to cancer by the time patients are in their mid-40s. APC is a gatekeeper for colon tumorigenesis in the sense that in the absence of mutation in APC (or a gene acting within the same pathway), a colorectal tumor simply cannot be initiated. Figure 76-5 shows the germline and somatic mutations found in the APC gene. A negative regulator of a signaling pathway that determines cell fate during development, the APC protein provides differentiation and apoptotic cues to colonic epithelial cells as they migrate up the crypt. Defects in this process can lead to abnormal accumulation of cells that would otherwise differentiate and eventually undergo apoptosis. In contrast to patients with FAP, patients with hereditary nonpol yposis colon cancer (HNPCC, or Lynch syndrome) do not develop polyposis, but instead develop only one or a small number of adenomas that rapidly progress to cancer. HNPCC is due to inherited mutations in one of four DNA mismatch repair genes (Table 76-3) that are com ponents of a repair system responsible for correcting errors in newly replicated DNA. Germline mutations in MSH2 and MLH1 together account for more than 90% of HNPCC cases, and mutations in MSH6 and PMS2 account for the remainder. When a somatic mutation inac tivates the remaining wild-type allele of a mismatch repair gene, the

Chromosome arrangement in the tumor Loss of normal chr 13 Rb A3 B3 Loss and reduplication A3 A3 Rb Rb B3 A3 B3 B3 Mitotic crossing over A1 Rb Rb B3 A3 B3 CHAPTER 76 Independent mutation or small deletion A3 Cancer Genetics B3 cell develops a hypermutable phenotype characterized by profound genomic instability that is most readily apparent in short repeated sequences called microsatellites and is sometimes called microsatellite instability (MSI). The high rate of mutation in such cells impacts all genes, including oncogenes and tumor-suppressor genes, and thereby accelerates the activation of the former and the inactivation of the latter (Fig. 76-2). HNPCC can be considered a disease of tumor progression; once tumors are initiated (by an inactivating mutation of APC or by some other gene in the APC pathway), tumors rapidly progress because of the accelerated mutation rate. Progression from a tiny adenoma to carcinoma takes only a few years in HNPCC patients instead of the two or three decades this progression takes in patients with FAP (or in patients with sporadic colorectal tumors). Approximately half of HNPCC patients develop colorectal cancers by the time they are in their mid-40s—similar to that of FAP patients. This coincidence in age of onset emphasizes that both tumor initiation (abnormal in FAP patients) and tumor progression (abnormal in HNPCC patients) are the two pillars of cancer development and are equally important for cancer development. Another general principle is apparent from the comparison between FAP and HNPCC patients. The tumors in FAP patients, like those in patients without hereditary predisposition to cancers, exhibit chro mosomal instability rather than MSI. Indeed, MSI and chromosomal instability tend to be mutually exclusive in colon cancers, suggest ing that they represent alternative mechanisms for the generation of genomic instability (Fig. 76-

2). Other cancer types rarely exhibit MSI. Chromosomal instability is far more prevalent than MSI among all cancer types, perhaps explaining why nearly all cancers are aneuploid. Although most autosomal dominant inherited cancer syndromes are due to mutations in tumor-suppressor genes (Table 76-3), there are a few interesting exceptions. Multiple endocrine neoplasia type 2, a dominant disorder characterized by pituitary adenomas, medullary

TABLE 76-3 Cancer Predisposition Syndromes and Associated Genes SYNDROME GENE CHROMOSOME INHERITANCE TUMORS Ataxia telangiectasia ATM 11q22-q23 AR Breast Autoimmune lymphoproliferative syndrome FAS FASL Birt-Hogg-Dubé syndrome FLCN 17p11.2 AD Kidney (hybrid oncocytic, chromophobe) Bloom syndrome BLM 15q26.1 AR Various Cowden syndrome PTEN 10q23 AD Breast, thyroid Familial adenomatous polyposis APC MUTYH Familial melanoma CDKN2A 9p21 AD Melanoma, pancreatic Familial Wilms' tumor WT1 11p13 AD Kidney (pediatric) Hereditary breast/ovarian cancer BRCA1 BRCA2 Hereditary diffuse gastric cancer CDH1 16q22 AD Stomach Hereditary multiple exostoses EXT1 EXT2 Hereditary retinoblastoma RB1 13q14.2 AD Retinoblastoma, osteosarcoma Hereditary nonpolyposis colon cancer (HNPCC) MSH2 MLH1 MSH6 PMS2 PART 4 Oncology and Hematology Hereditary papillary renal carcinoma MET 7q31 AD Papillary kidney Juvenile polyposis syndrome SMAD4 BMPR1A Li-Fraumeni syndrome TP53 17p13.1 AD Sarcoma, breast Multiple endocrine neoplasia type 1 MEN1 11q13 AD Parathyroid, endocrine, pancreas, and pituitary Multiple endocrine neoplasia type 2a RET 10q11.2 AD Medullary thyroid carcinoma, pheochromocytoma Neurofibromatosis type 1 NF1 17q11.2 AD Neurofibroma, neurofibrosarcoma, brain Neurofibromatosis type 2 NF2 22q12.2 AD Vestibular schwannoma, meningioma, spine Nevoid basal cell carcinoma syndrome (Gorlin's syndrome) PTCH1 9q22.3 AD Basal cell carcinoma, medulloblastoma, jaw cysts Peutz-Jeghers syndrome STK11/LKB1 19p13.3 AD Gastrointestinal, breast Tuberous sclerosis TSC1 TSC2 von Hippel–Lindau disease VHL 3p25-26 AD Kidney, cerebellum, pheochromocytoma Abbreviations: AD, autosomal dominant; AR, autosomal recessive. Number of mutations Number of mutations Somatic

O ARM 15 aa 20 aa Basic E/D APC

Germline

1000 1200 1400 FIGURE 76-5 Germline and somatic mutations in the tumor-suppressor gene adenomatous polyposis coli (APC). APC encodes a 2843-amino-acid protein with six major domains: an oligomerization region (O), armadillo repeats (ARM), 15-amino-acid repeats (15 aa), 20-amino-acid repeats (20 aa), a basic region, and a domain involved in binding EB1 and the Drosophila discs large homologue (E/D). Shown are 650 somatic and 826 germline mutations representative of the mutations that occur within the APC gene (from the APC database at www.umd.be/APC). All known pathogenic mutations of APC result in the truncation of the APC protein. Germline mutations are found to be relatively evenly distributed up to codon 1600 except for two mutation hotspots surrounding amino acids 1061 and 1309, which together account for one-third of the mutations found in familial adenomatous polyposis (FAP) families.

10q24 1q23 AD Lymphomas 5q21 1p34.1 AD AR Colorectal (early onset) 17q21 13q12.3 AD Breast, ovarian, prostate 8q24 11p11-12 AD Exostoses, chondrosarcoma 2p16 3p21.3 2p16 7p22 AD Colon, endometrial, ovarian, stomach, small bowel, ureter carcinoma 18q21 AD Gastrointestinal, pancreatic 9q34 16p13.3 AD Angiofibroma, renal angiomyolipoma MCR

1600 1800 2000 2200 2400 2600 2800 Amino acid number

carcinoma of the thyroid, and (in some pedigrees) pheochromocy toma, is due to gain-of-function mutations in the proto-oncogene RET on chromosome 10. Similarly, gain-of-function mutations in the tyrosine kinase domain of the MET oncogene lead to hereditary papil lary renal carcinoma. Interestingly, loss-of-function mutations in the RET gene cause a completely different disease, Hirschsprung's disease (aganglionic megacolon [Chaps. 339 and 400]). Although the heritable forms of cancer have taught us much about the mechanisms of growth control, most forms of cancer do not follow simple Mendelian patterns of inheritance. The majority of human can cers arise in a sporadic fashion, solely as a result of somatic mutation, and in the absence of any mutations in cancer-predisposing genes in their germlines. GENETIC TESTING FOR FAMILIAL CANCER The discovery of cancer susceptibility genes raises the possibility of DNA testing to predict the risk of cancer in individuals of affected families. An algorithm for cancer risk assessment and decision making in high-risk families using genetic testing is shown in Fig. 76-6. Once a mutation is discovered in a family, subsequent testing of asymptomatic family members is crucial. A negative gene test in these individuals can prevent years of anxiety, providing comfort in the knowledge that their cancer risk is no higher than that of the general population. On the other hand, a positive test may lead to alteration of clinical man agement, such as increased frequency of cancer screening and, when feasible and appropriate, prophylactic surgery. Potential negative con sequences of a positive test result include psychological distress (anxi ety, depression) and discrimination, although the Genetic Information Patients (1) from family with a known cancer syndrome, (2) from a family with a history of cancer, (3) with early onset cancer Pretest counseling Review of family history to confirm/identify possible cancer syndromes and candidate genes Informed consent Testing of cancer patient Negative test: no disease-causing mutations identified Identification of disease-causing mutation Screening of asymptomatic family members Negative test: family member has no increased risk of cancer FIGURE 76-6 Algorithm for genetic testing in a family with cancer predisposition. The key step is the identification of a disease mutation in a cancer patient, which is an indication for the testing of asymptomatic family members. Asymptomatic family members who test positive may require increased screening or surgery, whereas those who test negative are at no greater risk for cancer than the general population. It should be emphasized that no molecular assay used for this sort of testing is 100% sensitive; negative results must be interpreted with this caveat in mind.

Nondiscrimination Act (GINA) makes it illegal for predictive genetic information to be used to discriminate in health insurance or employ ment. Testing should therefore not be conducted without counseling before testing is administered and during and after disclosure of the test result.

It is now feasible to obtain high-quality sequence of all of the proteincoding DNA sequences, and even of the entire genome, in any given individual. In such studies, numerous variants in DNA sequences will inevitably be identified in every subject, but the significance of the vast majority of these DNA sequence findings will be unclear. Even muta tions in tumor-suppressor genes can be difficult to interpret unless there is an obvious functional implication, such as the truncation of the open reading frame, or that particular mutation has previously been correlated with cancer in other individuals. Germline mutations associated with cancer predisposition are uncommon in individuals without a family history of cancer, though they do occur. Much more common are variants of unknown significance (VUS). VUS that are found during genetic testing cannot be used to evaluate

the relative risk of cancer but may nonetheless cause anxiety because they represent a deviation from the reference allele that is established as “normal.” Because of the low yield of informative mutations that modify cancer risk and the frequent identification of VUS, it is generally not appropri ate to use DNA sequencing to assess cancer risk in individuals without a family history of cancer. However, there are exceptions. Testing may be appropriate in some subpopulations with a known increased risk, even without a personal family history. For example, two mutations in the breast cancer susceptibility gene BRCA1, 185delAG and 5382insC, exhibit a sufficiently high frequency in the Ashkenazi Jewish popula tion that genetic testing based on ethnicity alone may be warranted. CHAPTER 76 Cancer Genetics It is important that genetic test results be communicated to families by trained genetic counselors. To ensure that the families clearly under stand its advantages and disadvantages and the impact it may have on disease management and psyche, genetic testing should never be done before counseling. Significant expertise is needed to communicate the results of genetic testing to families. VIRUSES IN HUMAN CANCER Several human malignancies are associated with viruses. Examples include Burkitt’s lymphoma (Epstein-Barr virus; Chap. 199), hepato cellular carcinoma (hepatitis viruses), cervical cancer (human papil lomavirus [HPV]; Chap. 203), and T-cell leukemia (retroviruses; Chap. 207). There are several types of HPV, including the high-risk types 16 and 18 that are strongly associated with the development of cervical, vulvar, vaginal, penile, anal, and oropharyngeal cancer. The mechanisms of action of all these viruses involve inactivation of tumorsuppressor genes. For example, HPV proteins E6 and E7 bind to and inactivate cellular tumor suppressors p53 and pRB, respectively. This is the reason that HPV is such a potent initiator of cancer: infection with a virus is tantamount to having two of the three mutant driver genes required for cancer, that is, one viral oncogene inactivates p53 and the other inactivates Rb. Once these two inactivated gene products initiate tumorigenesis, only one additional mutant gene is required for these tumors to progress to malignancy. ■ ■CANCER GENOMES The advent of relatively inexpensive technologies for rapid and high-throughput DNA sequencing has facilitated the comprehensive analysis of numerous genomes from many types of tumors. This unprecedented view into the genetic nature of cancer has provided remarkable insights. Most cancers do not arise in the context of a mutator phenotype, and accordingly, the number of mutations in even the most advanced cancers is relatively modest. Common solid tumors harbor 30–70 subtle mutations that are nonsynonymous (i.e., result in an amino acid change in the encoded protein). Liquid tumors such as leukemias, as well as pediatric tumors, typically have fewer than 20 mutations. The vast majority of the mutations detected in tumors are not functionally significant; they simply arose by chance in a single cell that gave rise to an expanding clone. Such mutations, which pro vide no selective advantage to the cell in which they occur, are known

as passenger mutations. As noted above, only a small number of the mutations confer a selective growth advantage and thereby promote tumorigenesis. These functional mutations are known as driver muta tions, and the genes in which they occur are called driver genes.

The frequency and distribution of driver mutations within a single tumor type can be represented as a topographical landscape. The picture that emerges from cancer genome studies reveals that most genes that are mutated in tumors are actually mutated at relatively low frequencies, as would be expected of passenger genes, whereas a small number of genes (the driver genes) are mutated in a large proportion of tumors. Only ~200 driver genes contribute to the development of solid tumors of all kinds. Driver genes that play a role in ever smaller fractions of cancers are still being

discovered. The majority of the muta tions in driver genes provide a direct selective growth advantage by altering the signaling pathways that mediate cell survival or the deter mination of cell fate. The remaining driver gene mutations indirectly provide a selective growth advantage by accelerating the mutation rate of proto-oncogenes and tumor-suppressor genes. That the same driver genes play a role in multiple cancer types was unexpected before their discovery and has important implications for the development of new “tumor-agnostic” therapeutic and diagnostic approaches. Moreover, the functions of all these driver genes can be organized into a small number of signaling pathways, as shown in Table 76-4. As a consequence of the mutations they harbor, cancer cells invari ably express mutant proteins that are only rarely found in normal cells. Some of these mutant proteins are processed and displayed on the cell surface in the context of major histocompatibility complexes, a process that would normally facilitate their recognition by the adaptive immune system. Thus, all cancers have the theoretical potential to be recognized as foreign, or “nonself,” via the display of these tumor-spe cific antigens, known as mutation-associated neoantigens (MANAs). In fact, established tumors invariably prevent the activation of local T cells by inducing an intercellular suppressive mechanism known as an immune checkpoint. Therapeutic approaches to exploit this potential vulnerability by blocking immune checkpoints have elicited striking responses in patients with several types of cancer. PART 4 Oncology and Hematology It was hypothesized that the potential immunogenicity of a tumor would be related to the total number of distinctive neoantigens it can express, which in turn is directly determined by the total number of mutations in the cancer genome. This does seem to be the case. Colorectal cancers that develop as a result of mismatch repair defi ciency and smoking-related lung cancers, both of which characteristi cally harbor large numbers of mutations, exhibit more robust responses to therapeutic immune checkpoint blockade than most other tumor types. Notably, driver mutations as well as passenger mutations that result in the expression of mutant proteins can both contribute to the display of immunogenic neoantigens. Thus, the total number of coding TABLE 76-4 Signaling Pathways Altered in Cancer REPRESENTATIVE DRIVER GENES PROCESS PATHWAY Cell survival Cell cycle regulation/ apoptosis RB1, BCL2 RAS KRAS, BRAF PIK3CA PTEN, PIK3CA JAK/STAT JAK2, FLT3 MAPK MAP3K, ERK TGF-β BMPR1A, SMAD4 Cell fate Notch NOTCH1, FBWX7 Hedgehog PTCH1, SMO WNT/APC APC, CTNNB1 Chromatin modification DNMT1, IDH1 Transcriptional regulation AR, KLF4 Genome maintenance DNA damage signaling and repair ATM, BRCA1

mutations, a metric known as mutational load, is one of the determi nants of potential immunogenicity. The ability of cancer cells to evade immune-mediated cell death is an intrinsic property that is essential for their continued growth. While tumor suppressor genes and oncogenes have been intensively studied with respect to their effects on the intracellular signaling pathways that regulate cell proliferation and cell death, little is known about how these genetic alterations affect the interactions between cancer cells and neighboring immune cells. In particular, neoplastic cells contain ing certain mutations may be culled by the immune system because the mutations create neoantigens that can be recognized by T cells. ■ ■TUMOR HETEROGENEITY The mutant cells that compose a single tumor are not genetically identical. Rather, cells obtained from different sites on a tumor will harbor common mutations as well as mutations that are unique to each sample. Genetic heterogeneity results from the ongoing acquisition of mutations during tumor growth. Each time a genome is replicated, there is a small but quantifiable probability that a mutation will spon taneously arise as a result of a replication error and be passed on to the cellular progeny. This is true in normal cells or in tumor cells. Any ran domly chosen cell from the skin of

one individual will harbor hundreds of genetic alterations that distinguish it from a different randomly chosen skin cell, and the same is true for all organs of self-renewing tissues. Tumors are actually less genetically heterogeneous than normal tissues; any two randomly chosen cells from a tumor of an individual will have fewer differences than any two randomly chosen cells from that individual's normal tissues. The reason for this decrease in hetero geneity is clonal expansion, the fundamental feature of tumorigenesis. Every time a clonal expansion occurs, a genetic bottleneck wipes out heterogeneity among the cells that did not expand; these unexpanded cells either die or form only a minute proportion of the total cells in the expanding tumor. The mutations that vary between cells of a given tumor are invari ably passenger mutations that arose since the last evolutionary bottle neck, that is, those mutations that arose during the expansion of the founder cell that gave rise to the final clonal expansion. In contrast, the passenger mutations that were present in the founder cell will be uniformly present in every cell in the tumor. In that respect, these pas senger mutations are not heterogeneously distributed and are in fact uniformly present in virtually all cancer cells. These "clonal" mutations, i.e., present in all cells of the cancers, are the main source of MANAs that can be exploited through immune checkpoint inhibitors. The total number of mutations and their distribution within tumor cells repre sent a complex interplay between the age of the patient (the older the patient, the more passenger mutations will have accumulated in the founding cell of the first clonal expansion) and the evolutionary history of the cancer (its age and number of clonal expansions it experienced). Tumor heterogeneity has been recognized for decades at the cyto genetic, biochemical, and histopathologic levels. However, it is only recently, with the advent of a deep understanding of cancer genetics, that genetic heterogeneity can be interpreted in a medically relevant fashion. The first important point to recognize about tumor hetero geneity is that it is only the variation in driver gene alterations that is important; the cellular distribution of passenger gene mutations is irrelevant except for immune-related phenomena. In this discussion of heterogeneity, we can expand the definition of "driver genes" to include those that provide a selective growth advantage in the face of therapy in addition to those that provide a selective growth advantage during tumor evolution, prior to treatment. Type I heterogeneity refers to that among tumors of the same type from different patients (Fig. 76-7). Though adenocarcinomas of the lung generally harbor mutations in three or more driver genes, the genes dif fer among the patients, and the precise mutations within the same gene can vary considerably. Type I heterogeneity is the basis for precision medicine, where the goal is to treat patients with drugs that target the proteins encoded by genetic alterations within their specific tumors. Type II heterogeneity refers to the genetic heterogeneity among differ ent cells from the same primary tumor. Tumors continue to evolve as

Intratumoral heterogeneity within a primary tumor Intermetastatic heterogeneity between two metastases A Clone 1 Clone 2 Liver Founder cells Founder cells Clone 4 Clone 3 Intrametastatic heterogeneity within metastatic lesions Interpatient heterogeneity D C FIGURE 76-7 Four types of tumor heterogeneity. Tumor heterogeneity is the inevitable result of cell proliferation, as new mutations are introduced during clonal expansion. In a typical tumor (upper left), founder cells that harbor a large fraction of the total mutations give rise to subclones, which continue to evolve independently. The tumors of the founding populations are shown in the middle of each circle; the distinct subclones are shown around the periphery. A. Heterogeneity among the cells of a primary tumor is known as intratumoral heterogeneity. B. Heterogeneity among the founding cells of distinct metastatic lesions (marked as 1 and 2) that arise in the same patient is known as intermetastatic heterogeneity. C. Heterogeneity among the cells of each metastatic tumor is known

as intrametastatic heterogeneity. D. Interpatient heterogeneity. The mutations in the tumors of two patients are almost completely distinct. (Reproduced with permission from B Vogelstein et al: Cancer genome landscapes. Science 339(6127):1546, 2013.) they grow, and different cells of the same cancer, in its original site (e.g., the pancreas), may acquire other driver gene mutations that are not shared among the other cells of the tumor. Such a mutation can result in a small clonal expansion that may or may not be important biologi cally. In cases in which the primary tumor can be surgically excised, such mutations are unimportant unless they give rise to type III

heterogeneity (described below). The reason they are unimportant is because all primary tumor cells, whether homogeneous or not, are removed by the surgical procedure. In primary tumors that cannot be completely excised (such as most advanced brain tumors and many pancreatic ductal adenocarcinomas), heterogeneity is biomedically important because it can give rise to drug resistance, analogously to that described for type IV heterogeneity (see below). Type III hetero geneity refers to the genetic differences among the founder cells of the metastatic lesions from the same patient. For example, a patient with melanoma may have 100 different metastases distributed throughout various organs. Only if a mutant BRAF is present in every founder cell of every metastasis, then the patient has a chance at a complete response to a BRAF inhibitor. There have been several recent detailed studies of the metastases from various tumor types. Fortunately, these studies suggest there is very little, if any, type III heterogeneity among driver genes, a necessary prerequisite for the successful implementa tion of current and future targeted therapies. Finally, type IV hetero geneity refers to that among cells of individual metastatic lesions. As the founder cell of each metastasis expands to become detectable, it acquires mutations, a small number of which can act as “drivers” when the patient is exposed to therapeutics. This type of heterogeneity is of major clinical importance, as it has been shown to be responsible for the development of resistance in virtually all targeted therapies. The development of such resistance is a fait accompli based simply on known mutation rates and genetic resistance mechanisms. The only way to circumvent acquired resistance is to treat metastatic tumors earlier (i.e., in adjuvant setting, before much tumor expansion has occurred) or to treat with combinations of drugs for which crossresistance is genetically impossible.

B Metastasis 1 Pancreas Metastasis 2 Primary tumor Patient 1 Patient 2 CHAPTER 76 Cancer Genetics PERSONALIZED CANCER DETECTION AND TREATMENT High-throughput DNA sequencing has led to an unprecedented under standing of cancer at the molecular level. A comprehensive mutation profile provides a molecular history of a given tumor and insights into how it arose. Because tumor cells and tumor DNA are shed into the blood and other bodily fluids, common driver mutations can be used as highly specific biomarkers for early detection. For diagnosed tumors, tumor-specific mutations can be used to estimate tumor burden, assess treatment responses, and detect recurrence. In some cases, information regarding specific genes and pathways that are altered provides patients and physicians with options for personalized therapy. This general approach is sometimes referred to as precision medicine. Because tumor behavior is highly variable, even within a tumor type, personalized information-based medicine can supplement and perhaps eventually supplant histology-based tumor assessment, especially in the case of tumors that are resistant to con ventional therapeutic approaches. Conversely, molecular nosology has revealed similarities in tumors of diverse histotype. The success of the precision medicine approach in any given patient depends on the pres ence of tumor-associated genetic alterations that are actionable (i.e., can be targeted with a specific drug). Examples of currently actionable changes include mutations in BRAF (targeted by the drug vemu rafenib), RET (targeted by sunitinib and

sorafenib), ALK rearrange ments (targeted by crizotinib), and mismatch repair genes (targetable by immune checkpoint inhibitors). The development of new targeted agents is at present hindered by the fact that most such agents can only target activated oncogenes, while the great majority of genetic alterations in common solid tumors are those that inactivate tumor-suppressor genes. Because all drugs, whether for use in oncology or any other purpose, can only inhibit protein actions, drugs cannot be used to directly target the proteins encoded by inactivated tumor-suppressor genes; these proteins are already inactive. More information about the pathways through which tumor-suppressor genes act may provide a way around this

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