Tumor-Suppressor Genes

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A genetic basis for the development of cancer has been hypothesized for roughly a century, and support for this proposal has been provided by familial, epidemiologic, and cytogenetic studies. Nevertheless, only in the past 25 years has there been definitive evidence that cancer is a genetic disease. A current view is that cancers arise through a multistage process in which inherited and somatic mutations of cellular genes lead to clonal selection of variant progeny with the most robust and aggressive growth properties. Two classes of genes, protooncogenes and tumor-suppressor genes, are targets for the mutations. The vast majority of the mutations that contribute to the development and behavior of cancer cells are somatic (ie, arising during tumor development) and present only in the neoplastic cells of the patient. Although only a small fraction of all mutations in cancer cells are constitutional and thus present in all somatic cells of affected individuals, such mutations not only predispose to cancer, but can also be passed on to future generations.

The identification and function of protooncogenes and their oncogenic variants are reviewed in other chapters in this book. However, we briefly mention their general properties in an effort to compare them with tumor-suppressor genes. More than 50 different protooncogenes have been identified through various experimental strategies. In general, protooncogenes have critical roles in a variety of growth regulatory pathways, and their protein products are distributed throughout many subcellular compartments. The oncogenic variant alleles present in cancers have sustained gain-of-function alterations resulting from point mutations, chromosomal rearrangements, or gene amplifications of the protooncogene sequences. In the overwhelming majority of cancers, mutations in protooncogenes arise somatically in the tumor cells, although germ line mutations activating the function of the *RET* gene have been identified in those with multiple endocrine neoplasia type 2 and in familial medullary thyroid cancer. In analogous fashion, germ line mutations in the *MET* gene have been found in affected members of families with hereditary papillary renal cell carcinoma.

Whereas oncogenic alleles harbor activating mutations, tumor-suppressor genes are defined by their inactivation in human cancer. As is reviewed below, a large number of tumor-suppressor genes have been hypothesized to exist. Thus far, approximately 20 tumor-suppressor genes have been identified and definitively implicated in cancer development. Like protooncogenes, the cellular functions of the tumor-suppressor genes appear to be diverse. A subgroup of tumor-suppressor genes deserves further mention here, namely, the deoxyribonucleic acid (DNA) repair-pathway genes. DNA repair pathway gene defects recently have been implicated in a fairly broad spectrum of human cancers. Like other tumor-suppressor genes, the DNA repair genes are inactivated in human cancers. However, because of their cellular function, it might be argued that they constitute a unique subset of the tumor-suppressor genes. Specifically, whereas protein products of many tumor-suppressor genes are likely to be directly involved in growth inhibition or differentiation, many DNA repair pathway proteins, such as those involved in recognizing DNA mismatches, have a more passive role in cell growth regulation. As such, their inactivation in tumor cells results in an increased rate of mutations in other cellular genes, including protooncogenes and other tumor-suppressor genes.

Enormous progress has been made in the identification of inherited and somatic mutations in tumor-suppressor genes in human cancer, as well as in defining the means by which loss-of-function mutations in these genes contribute to the development of cancer. It is not possible to summarize all of these findings here. Rather, the principal aims of this chapter are to review the somatic cell genetic and epidemiologic studies that established the existence of tumor-suppressor genes; the identification and cloning of a few tumor-suppressor genes, such as the retinoblastoma and *p53* genes and a few other genes; selected studies of the function of tumor-suppressor genes in growth regulation and differentiation; and the role of DNA mismatch repair gene mutations in common human cancers.

GENETIC BASIS FOR TUMOR DEVELOPMENT

That cancer in man and other animals might be inherited has been appreciated for more than a century. In 1866, Broca described a family in which many members developed breast or liver cancer, and he proposed that an inherited abnormality within the affected tissue allowed tumor development.[1] Following the rediscovery of Mendel's work, studies of the rates of spontaneous mammary tumor formation among various inbred strains of mice led Haaland to argue that tumorigenesis could behave in a formal sense as a mendelian genetic trait.[2] Similarly, Warthin's analysis of the pedigrees of cancer patients at the University of Michigan Hospital between 1895 and 1913 identified four multigenerational families with susceptibilities to specific cancer types that appeared to be transmitted as autosomal dominant mendelian traits (Figure 7-1).[3] Although these and other studies suggested the existence of an inherited genetic basis for some cancers, other explanations for familial clustering were possible (eg, shared exposure to a carcinogenic agent in the environment or diet). Furthermore, it was argued that most cancers in humans appeared to arise as sporadic, isolated cases.

A role for somatic mutations in the development of cancer was first proposed by Boveri, who noted that in sea urchin eggs fertilized by two sperm, abnormal mitotic divisions leading to the loss of chromosomes occurred in daughter cells, and atypical tissue masses could be seen in the resulting gastrula.[4] He believed these abnormal tissues appeared physically similar to the poorly differentiated tissue masses seen in tumors, and hypothesized that cancer arose from a cellular aberration producing abnormal mitotic figures. Boveri's hypothesis apparently did not gain favor at the time, initially because of the lack of direct experimental support from studies of the karyotypes of animal and human tumors and later because of uncertainty about whether the changes in chromosome number in tumors were a cause or an effect of the neoplastic phenotype.

A landmark observation in the search to identify a genetic basis for cancer was reported by Rous in 1911, when he showed that sarcomas could be reproducibly induced in chickens by cell-free filtrates of a sarcoma that had previously arisen in another chicken.[5] Although this observation provided strong evidence that neoplasms could be virally induced, the observation also provided support for the view that cancer could be attributed to discrete genetic elements. Sixty years after Rous' initial report, the oncogenic region of the Rous sarcoma virus was identified. Further characterization and cloning of the transforming sequences demonstrated that the oncogenicity of the virus was dependent on v-*src*, a transduced and mutated copy of the c-*src*

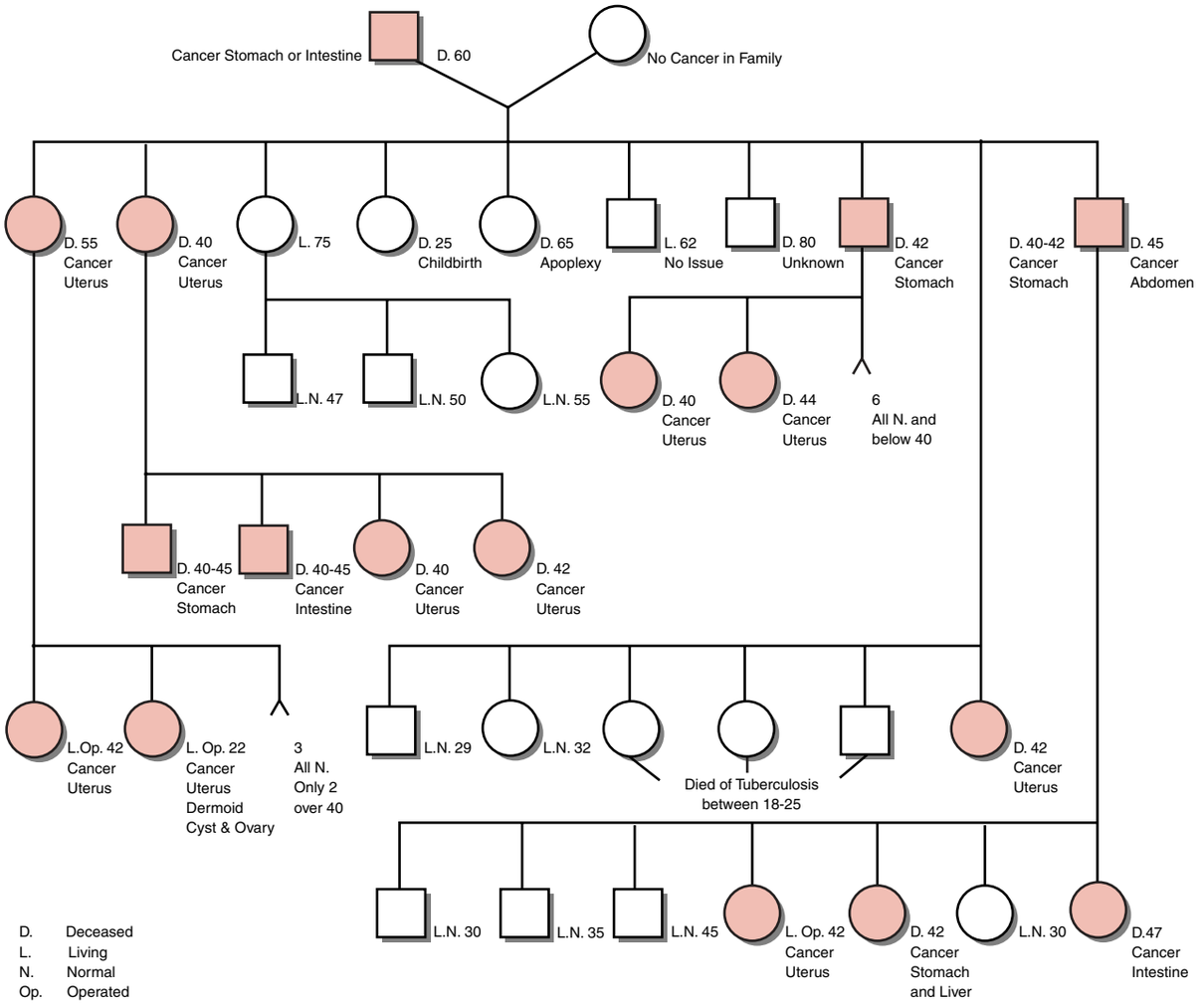

Figure 7-1 The inheritance of cancer in a family (family G). The affected members with cancer are indicated by shaded figures and by the type of cancer in each case. The family demonstrates a dominant inheritance pattern for the development of cancer, of either the colon, stomach, or uterus, a syndrome now referred to as hereditary nonpolyposis colorectal cancer (HNPCC). Recent studies demonstrate that cancer predisposition in families with HNPCC results from germ line mutation of a DNA repair gene allele (see text) (kindred described by AS Warthin, 1913; the mutation in this kindred was recently described in ref. 270). (Reproduced with permission from Fearon ER, Vogelstein B. Tumor suppressor and DNA repair gene defects in human cancer. In: Holland JF, Frei E, Bast RC, et al, editors. Cancer medicine, 4th ed. Baltimore: Williams & Wilkins; 1997. p. 97–117.

cellular protooncogene. Subsequently, all oncogenes of acutely transforming ribonucleic acid (RNA) tumor viruses have been found to be transduced cellular genes (the protooncogenes). Although the biochemical mechanisms by which most viral oncogenes cause neoplastic transformation are still not fully defined at present in general terms, the viral oncogenes appear to cause transformation because they are mutated versions of cellular protooncogenes and/or are expressed aberrantly. In human cancers, somatic mutations generate oncogenic alleles from protooncogenes.

Despite the significance of oncogenes in the genesis of many different human tumor types, many of the altered properties of cancer cells appear to be attributable to the inactivation of normal cellular genes. These cellular genes, hypothesized to regulate cellular proliferation and growth in a negative fashion, have been termed tumor-suppressor genes.

SOMATIC CELL GENETIC STUDIES OF TUMORIGENESIS

Several oncogenes, particularly those in RNA tumor viruses, were identified and molecularly cloned through their ability to induce neoplastic growth properties upon their introduction into appropriate recipient cells. In contrast, essentially all of the initial evidence supporting the existence of tumor-suppressor genes was derived indirectly prior to the identification and molecular cloning of any tumor-suppressor genes. A difficulty in using functional approaches to identify tumor-suppressor genes is that the genes would be expected to suppress key traits of cancer cells, such as their uncontrolled proliferation, unlimited life span, and tumorigenicity in animals. As might have been predicted, selection methods for directly identifying suppressed cells in a background of fully transformed cells have proven elusive. Despite the theoretical and practical difficulties inherent in functional approaches to define tumor-suppressor genes, such studies do provide strong, albeit indirect, support for the existence of these genes.

The studies of Ephrussi et al[6] and Harris[7] provided compelling evidence that the ability of cells to form a tumor behaves as a recessive trait at the cellular level. They observed that the growth of murine tumor cells in syngeneic animals could be suppressed when the malignant cells were fused to nonmalignant cells, although reversion to tumorigenicity often occurred when the hybrids were propagated for extended periods in culture. The reappearance of malignancy was found to be associated with specific chromosome losses. Their interpretation, that malignancy can be suppressed in somatic cell hybrids, was subsequently supported by additional studies of mouse, rat, and hamster intraspecies somatic cell hybrids, as well as interspecies hybrids between rodent tumor cells and normal human cells.[8,9] The karyotypic instability of the rodent–human hybrids, however, complicated the analysis of the human chromosomes involved in the suppression process. Stanbridge and his colleagues overcame this problem by studying hybrids made by fusing human tumor cell lines to normal, diploid human fibroblasts.[10,11] Their analysis confirmed that hybrids retaining both sets of parental chromosomes were suppressed, with tumorigenic variants arising only rarely after chromosome losses in the hybrids. Moreover, it was demonstrated that the loss of specific chromosomes, and not simply chromosome loss in general, correlated with the reversion to tumorigenicity. Tumorigenicity could be suppressed even if activated onco-

genes, such as mutant *RAS* genes, were expressed in the hybrids.[11,12]

The observation that the loss of specific chromosomes was associated with the reversion to malignancy suggested that a single chromosome (and perhaps even a single gene) might be sufficient to suppress tumorigenicity. To directly test this hypothesis, single chromosomes were transferred from normal cells to tumor cells, by using the technique of microcell-mediated chromosome transfer. It was found that the transfer of a single chromosome 11 into the HeLa cervical carcinoma cell line suppressed the tumorigenic phenotype of the cells.[13] Similarly, transfer of chromosome 11 into a Wilms tumor cell line was found to suppress tumorigenicity, whereas the transfer of several other chromosomes had no effect.[14] Many studies have demonstrated that transfer of even very small chromosome fragments will specifically suppress the tumorigenic properties of certain cancer cell lines.

Although tumorigenic growth in immunocompromised animals can often be suppressed in hybrids resulting from fusion between malignant and normal cells or by transfer of unique chromosome fragments, other traits characteristic of the parental tumor cells, such as immortality and anchorage-independent growth in vitro, may be retained. This observation is consistent with the notion that most malignant tumors arise as a result of multiple genetic alterations. Suppression of tumorigenicity following cell fusion or microcell chromosome transfer might thus represent correction of only one of many alterations. Furthermore, the data suggest that some of the genes that influence the lifespan of normal cells may be distinct from the genes that suppress the tumorigenic phenotype. However, because each of these classes of genes can suppress at least some phenotypic properties of tumor cells (eg, tumorigenicity or immortality), the two classes of genes are not usually distinguished one from another, and both types are referred to as tumor-suppressor genes.

In summary, although somatic cell genetic approaches did not lead to the identification of specific tumor-suppressor genes in human cancer, the approaches provided early and persuasive evidence for the existence of critical growth-regulating genes in normal cells that can suppress phenotypic traits of immortal or even fully cancerous cells.

RETINOBLASTOMA—A PARADIGM FOR TUMOR-SUPPRESSOR GENE FUNCTION

Essentially concurrent with the initial cell fusion experiments of Harris and colleagues, Knudson's analysis of the age-specific incidence of retinoblastoma led him to propose that two "hits" or mutagenic events were necessary for retinoblastoma development.[15] Retinoblastoma occurs sporadically in most cases, but in some families, it displays autosomal dominant inheritance. In an individual with the inherited form of the disease, Knudson proposed that the first hit is present in the germ line, and thus in all cells of the body. However, the presence of a mutation at the susceptibility locus was argued to be insufficient for tumor formation, and a second somatic mutation was hypothesized to be necessary for promoting tumor formation. Given the high likelihood of a somatic mutation occurring in at least one retinal cell during development, the dominant inheritance pattern of retinoblastoma in some families could be explained. In the nonhereditary form of retinoblastoma, both mutations were proposed to arise somatically within the same cell. Although each of the two hits could theoretically have been in different genes, subsequent studies (see below) led to the conclusion that both hits were at the same genetic locus, ultimately inactivating both alleles of the retinoblastoma (*RB1*) susceptibility gene. Knudson's hypothesis served not only to illustrate mechanisms through which inherited and somatic genetic changes might collaborate in tumorigenesis, but it also linked the notion of recessive genetic determinants for human cancer to somatic cell genetic findings on the recessive nature of tumorigenesis.

The first clue to the location of a putative gene responsible for inherited retinoblastoma was obtained from karyotypic analyses of patients with retinoblastoma. Constitutional deletions of chromosome 13 were observed in some cases.[16] Subsequent cytogenetic studies of patients with retinoblastoma identified detectable germ line deletions of chromosome 13 in only about 5% of all patients. However, in cases where deletions were observed, the common region of deletion was centered around chromosome band 13q14.[17] Levels of esterase D, an enzyme of unknown physiologic function, were found to be reduced in patients with deletions of 13q14, when compared with karyotypically normal family members.[18] This finding implied that the *esterase D* gene might be contained within chromosome band 13q14. Indeed, analysis of the segregation patterns of esterase D isozymes and retinoblastoma development in families with inherited retinoblastoma established that the *esterase D* and *RB1* loci were genetically linked.[19]

Subsequently, a child with inherited retinoblastoma was found to have esterase D levels approximately one-half of normal, although no deletion of chromosome 13 was seen in karyotype studies of his blood cells and skin fibroblasts.[20] Interestingly, tumor cells from this patient had a complete absence of esterase D activity, despite harboring one apparently intact copy of chromosome 13. Based on these findings, it was proposed that the copy of chromosome 13 retained in the tumor cells had a submicroscopic deletion of both the *esterase D* and *RB1* loci. Moreover, it was concluded that the initial *RB1* mutation in the child was recessive at the cellular level (ie, cells with inactivation of one *RB1* allele had a normal phenotype). The effect of the predisposing mutation, however, could be unmasked in the tumor cells by a second event, such as the loss of the chromosome 13 carrying the wild-type *RB1* allele. This proposal was entirely consistent with Knudson's two-hit hypothesis.[15,21]

To establish the generality of these observations, Cavenee, White, and their colleagues undertook studies of retinoblastomas, both inherited and sporadic types, by using DNA probes from chromosome 13. Probes detecting DNA polymorphisms were used, so that the two parental copies of chromosome 13 in the cells of the patient's normal and tumor tissues could be distinguished from one another. By using such markers to compare paired normal and tumor samples from each patient, they were able to demonstrate that loss of heterozygosity (ie, the loss of one parental set of markers) for chromosome 13 alleles had occurred during tumorigenesis in more than 60% of the cases studied.[22] Loss of heterozygosity (LOH) for chromosome 13, and specifically for the region of chromosome 13 containing the *RB1* gene, occurred via a number of different mechanisms (Figure 7-2). In addition, through study of inherited cases, it was shown that the copy of chromosome 13 retained in the tumor cells was derived from the affected parent and that the chromosome carrying the wild-type *RB1* allele had been lost.[22,23] These data established that the unmasking of a predisposing mutation at the *RB1* gene, whether the initial mutation had been inherited or had arisen somatically in a single developing retinoblast, occurred by the same chromosomal mechanisms.

Patients with the inherited form of retinoblastoma were known to be at an increased risk for the development of a few other cancer types, particularly osteosarcomas. LOH for the chromosome 13q region containing the *RB1* locus was seen in osteosarcomas arising in patients with the inherited form of retinoblastoma, suggesting that inactivation of both *RB1* alleles was critical to the development of osteosarcomas in those with inherited retinoblastoma.[24,25] Chromosome 13q LOH was also frequently observed in sporadic osteosarcomas. These molecular studies of retinoblastomas and osteosarcomas provided strong support for Knudson's two-hit hypothesis, and suggested that a variety of tumors might arise from the unmasking of recessive mutations at different tumor suppressor loci.[11,21,23] In addition, the studies demonstrated that both the inherited and sporadic forms of a tumor appeared to arise as a result of similar genetic alterations. Moreover, osteosarcoma, a common second primary neoplasm in patients with inherited retinoblastoma, was found to have pathologic genetic mechanisms in common with retinoblastoma.

Cloning and Analysis of the *RB1* Gene

The molecular cloning of the *RB1* gene was facilitated by the identification of an anonymous DNA marker from the chromosome 13q14 region that detected DNA rearrangements in retinoblastomas.[26] Through the analysis of the DNA sequences flanking this DNA marker, a

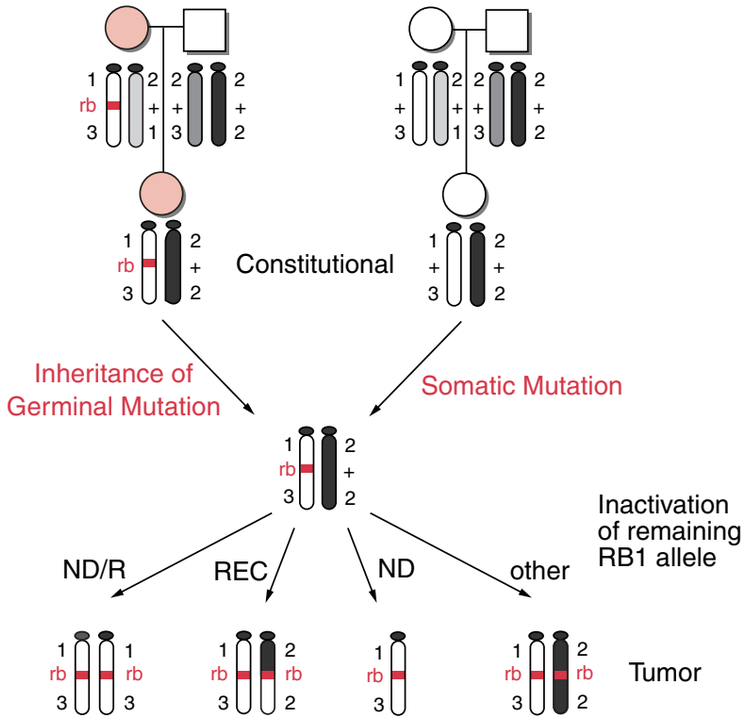

Figure 7-2 Chromosomal mechanisms that result in loss of heterozygosity for alleles at the retinoblastoma predisposition (*RB1*) locus at chromosomal band 13q14. In the inherited form of the disease (*top left*), the affected daughter inherits a mutant *RB1* allele (*rb*) from her affected mother and a normal *RB1* allele (+) from her father. Thus, she has one wild-type and one mutant *RB1* allele in all her cells (ie, constitutional genotype for RB1 is *rb*/+). The two copies of chromosome 13 in her normal cells (one from each parent) can be distinguished by using polymorphic DNA markers flanking the *RB1* locus (the polymorphic alleles are designated by number). A retinoblastoma can arise after inactivation of the remaining wild-type *RB1* allele. Among the genetic mechanisms found to inactivate the remaining wild-type *RB1* allele during tumor development are chromosome nondisjunction and reduplication of the remaining copy of chromosome 13 (ND/R); mitotic recombination (REC); nondisjunction (ND); and other mutations that inactivate the remaining *RB1* allele (Other). Shown at the *top right* is the situation in the noninherited (sporadic) form of the disease. A somatic mutation arises in a developing retinal cell and inactivates one of the *RB1* alleles. A retinoblastoma will develop if the remaining *RB1* allele is inactivated by one of the mechanisms shown. (Modified, corrected, and reproduced with permission from Fearon ER, Vogelstein B. Tumor suppressor and DNA repair gene defects in human cancer. In: Holland JF, Frei E, Bast RC, et al, editors. Cancer medicine, 4th ed. Baltimore: Williams & Wilkins; 1997. p. 97–117.)

gene with the properties expected of *RB1* was identified.[27–29] The *RB1* gene has a complex organization with 27 exons, spanning greater than 200 kilobases (kb) of DNA, and an RNA transcript of about 4.7 kb.[30] The *RB1* gene appears to be expressed ubiquitously rather than being restricted to retinoblasts and osteoblasts.

Cloning of *RB1* allowed study of mutations that inactivate the gene. Although gross deletions of *RB1* sequences have been observed in a small subset of retinoblastomas and osteosarcomas, most tumors appear to express full-length *RB1* transcripts and do not have detectable gene rearrangements when analyzed by Southern blotting.[31–35] Hence, the detection of inherited and somatic mutations in the *RB1* gene in most cases has required detailed characterization of its sequence. Mutant *RB1* alleles from both constitutional cells of individuals with the inherited form of the disease and from retinoblastomas of both inherited and sporadic types have now been quite extensively analyzed.[35,36] This analysis has provided definitive molecular evidence supporting Knudson's two-hit model. As predicted, patients with inherited retinoblastoma have been found to have one mutated and one normal allele in their constitutional (blood) cells. In retinoblastomas of such individuals, the remaining *RB1* allele has been found to be inactivated by somatic mutation, usually by loss of the normal allele through a gross chromosomal event (see Figure 7-2), but in some cases by point mutation. Multiple tumors arising in an individual patient with inherited retinoblastoma all were found to contain the same germ line mutation but had different somatic mutations affecting the remaining *RB1* allele. The vast majority of patients with a single retinoblastoma and no family history of the disease have two somatic mutations in their tumors and two normal alleles in their constitutional cells.

Although the identification of mutations in both alleles of the *RB1* gene in retinoblastomas and osteosarcomas provides strong support for the proposal that the cloned gene is, indeed, the gene whose inactivation is a crucial and likely rate-determining step in tumor formation, additional support for the critical growth regulatory function of the gene was provided by the demonstration that restoration of *RB1* function could suppress some aspects of retinoblastoma tumorigenesis. The transfer of a cloned copy of wild-type *RB1* to retinoblastoma and other tumor cells in culture affects a number of cellular properties, including morphology and differentiated phenotype, growth rate in culture, and the ability of the cells to form colonies in soft agar and progressive tumors in nude mice.[37–39] However, such studies generally involve expression of an exogenous gene at nonphysiologic levels, and the significance of the phenotypes produced is questionable. Indeed, many genes that have little or no role in tumorigenesis can inhibit the growth of transfected cells when expressed at high levels.

The observation that *RB1* is ubiquitously expressed is rather puzzling, given the spectrum of tumors that develop in patients with germ line *RB1* mutations. Patients with germ line mutations of *RB1* are at elevated risk for the development of only a rather limited number of tumor types, including retinoblastomas in childhood, osteosarcomas, soft-tissue sarcomas, and melanomas later in life. *RB1* germ line mutations fail to provide a strong predisposition to most common cancers, despite the fact that somatic *RB1* mutations have been observed in a wide variety of other cancer types, including breast, small cell lung, bladder, pancreas, and prostate cancers.[40] It is possible that retinoblastoma functions slightly differently in retinal epithelial cells than in other cell types, so that the *RB1* gene acts as a "gatekeeper" in retinal cells but not in other cell types.

Function of the Retinoblastoma Protein (p105-RB) The protein product of the *RB1* gene is a nuclear phosphoprotein with a molecular weight of about 105,000 Daltons known as p105-Rb or, more commonly, as pRb.[39] Harlow and colleagues' studies provided the first critical insights into pRb function. They demonstrated that pRb formed a complex with the E1A oncoprotein encoded by the murine DNA tumor virus adenovirus type 5.[41] Prior studies of E1A had established that it had many effects on cell growth, including cell immortalization and cooperation with other oncogenes (eg, mutated Ras oncogene alleles) in neoplastic transformation. It was thus hypothesized that functional inactivation of pRb through its interaction with E1A might contribute to some of E1A's transforming functions. Additional support for this proposal was provided by data establishing that mutations inactivating the ability of E1A to bind to pRb also inactivated E1A's transforming function.[42,43]

The significance of physical interaction between pRb and a DNA tumor virus oncoprotein was further supported by the subsequent demonstration that other DNA tumor virus oncoproteins also formed complexes with pRb, including SV40 T antigen and the E7 proteins of human papillomavirus (HPV) types 16 and 18 (Figure

7-3).[44,45] Many of the mutations inactivating the transforming activities of these oncoproteins also inactivated their ability to interact with pRb. Furthermore, E7 proteins from "high-risk" HPVs (ie, those linked to cancer development), such as HPV 16 and 18, formed complexes more tightly with pRb than did E7 proteins of "low-risk" viruses (eg, HPV types 6 and 11). These studies of pRb provided compelling evidence that DNA tumor viruses might transform cells, at least in part, by inactivating tumor-suppressor gene products. In addition, given the critical dependence of DNA tumor viruses on harnessing the cell's machinery for replication of the viral genome, the studies also provided support for the hypothesis that pRb might normally control cell growth by interacting with cellular proteins that regulated the cell's decision to enter into the DNA synthesis (S) phase of the cell cycle.

The functional activity of pRb is regulated by phosphorylation during normal progression through the cell cycle.[39,46–48] Accordingly, pRb appears to be predominantly unphosphorylated or hypophosphorylated in the G1 phase of the cell cycle and maximally phosphorylated in G2 (Figure 7-4). The critical phosphorylation events regulating the function of pRb are likely to be mediated at the boundary between the G1 and S phases of the cell cycle by cyclin and cyclin-dependent kinase (cdk) protein complexes.[39,40] Presumably, phosphorylation of pRb, particularly at the G1-S boundary, inactivates its ability to interact with cellular proteins that regulate entry into S phase. For example, when it is not phosphorylated, pRb forms complexes with proteins in the E2F family and inhibits transcription by recruiting proteins involved in transcriptional repression.[39] When phosphorylated, pRb can no longer efficiently form complexes with E2Fs (see Figure 7-4). The E2F proteins, when dimerized with their DP (differentiation-regulated transcription factor) partner proteins, are then capable of activating the expression of a number of genes that are likely to regulate/promote entry into S phase, including DNA polymerase α, thymidylate synthase, ribonucleotide reductase, cyclin E, and dihydrofolate reductase.[39] That E2F family members directly affect cellular proliferation was recently shown in conditional mouse knockout models.[49] Several other cellular proteins that bind to pRb have been identified, but their functions and the significance of their interactions with pRb remain less-well characterized than pRb's interactions with E2Fs.

The retinoblastoma protein shares significant similarity with two proteins known as p107 and p130. Like Rb, these proteins have been found to

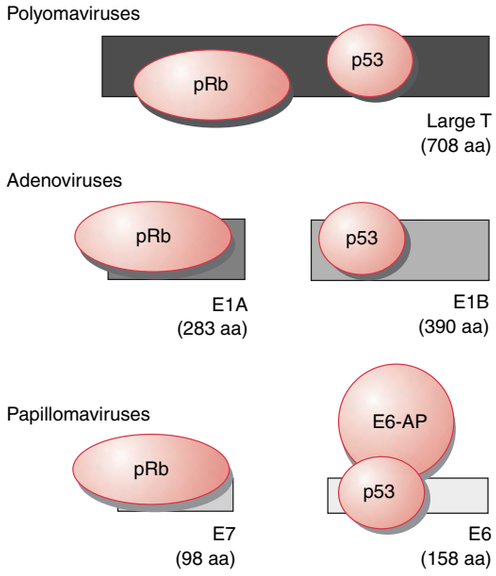

Figure 7-3 Representation of interactions between tumor-suppressor gene products and proteins encoded by DNA tumor viruses. Large T antigen from polyomaviruses (such as simian virus 40 [SV40]) binds both the retinoblastoma (pRb) and p53 proteins. For the adenoviruses and the high-risk human papillomaviruses (HPV types 16 and 18), different viral protein products complex with pRb and p53. A cellular protein known as E6-associated protein (E6-AP) cooperates with the HPV E6 protein to complex and degrade p53. (Modified with permission from Werness BA, Levine AJ, Howley PM: Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1990;248:76. Modified, corrected, and reproduced with permission from Fearon ER, Vogelstein B. Tumor suppressor and DNA repair gene defects in human cancer. In: Holland JF, Frei E, Bast RC, et al, editors. Cancer medicine, 4th ed. Baltimore: Williams & Wilkins; 1997. p. 97–117.)

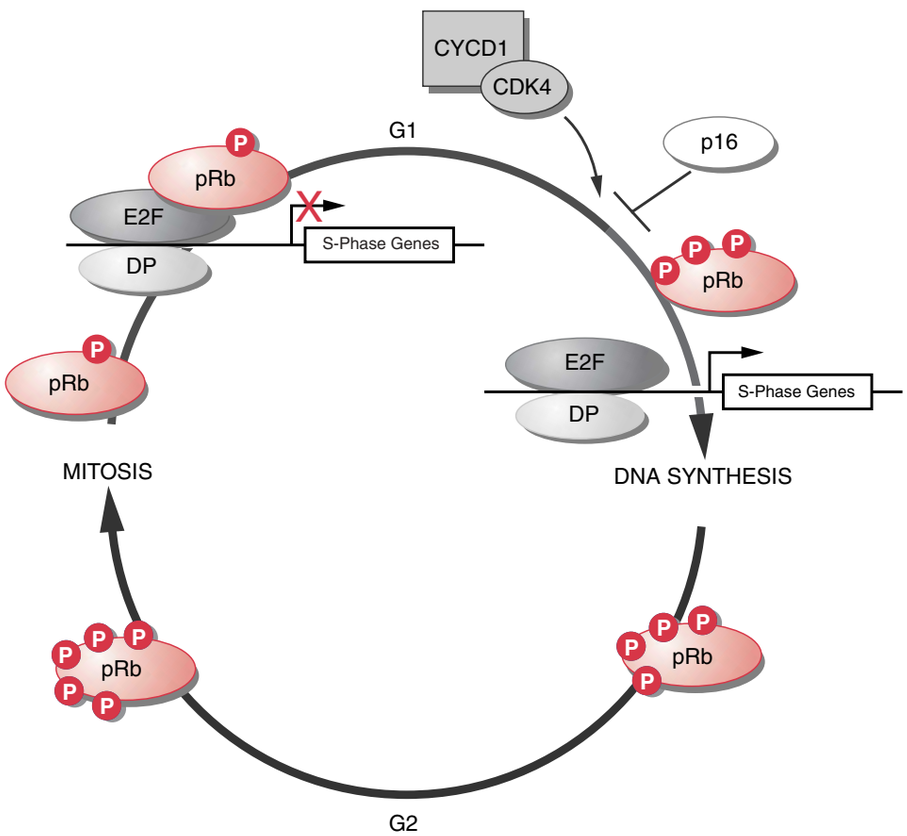

Figure 7-4 Phosphorylation regulates the function of pRb during the cell cycle. The pRb protein is hypophosphorylated in the G1 phase of the cell cycle, and phosphorylation (P) of specific sites appears to increase during progression through the cell cycle. A protein complex that appears to phosphorylate pRb prior to DNA synthesis (S-phase) includes a cyclin (CYC) and a cyclin-dependent kinase (CDK) (probably, cyclin D1 and CDK4). The CYCD1/CDK4 complex is regulated by the p16 inhibitor protein, which is itself the product of a tumor-suppressor gene on chromosome 9p known as *INK4a* (see text). In its hypophosphorylated state, pRb binds to E2F transcriptional regulatory proteins. E2F proteins dimerize with DP proteins and activate the transcription of genes, including those involved in DNA synthesis. However, when pRb is brought to the promoter regions of genes via its interaction with E2F proteins, pRb represses the expression of the E2F target genes. Phosphorylation of pRb releases it from the E2F/DP protein complex and results in gene activation. The figure also indicates that pRb phosphorylation increases in G2 with pRb dephosphorylated at or near anaphase. (Modified and reproduced with permission from Fearon ER, Vogelstein B. Tumor suppressor and DNA repair gene defects in human cancer. In: Holland JF, Frei E, Bast RC, et al, editors. Cancer medicine, 4th ed. Baltimore: Williams & Wilkins; 1997. p. 97–117.)

form complexes with certain DNA tumor virus proteins.[50–52] Because of their similarity to pRb, the p107 and p130 proteins have been termed pRb "cousins." Although all three proteins may have related cellular functions, there is only rather limited evidence indicating that mutations in the *p107* or *p130* genes contribute to cancer development. Germ line mutations in *p107* and *p130* have not been reported in humans, somatic mutations in the *p130* gene have been seen in only a small fraction of small cell lung and nasopharyngeal cancers,[53,54] and somatic mutations in *p107* appear to be even rarer or absent in cancer. Furthermore, whereas germ line inactivation of the mouse *pRb* gene predisposes the animals to pituitary adenomas and carcinomas as well as thyroid tumors, germ line inactivation of the murine homologs of the *p130* and *p107* genes appears to have no effect on tumor predisposition.[55,56] Future studies will undoubtedly shed further light on the means by which loss of pRb function, but not that of p107 or p130, contributes to cancer development. Nevertheless, a reasonable hypothesis is that pRb, via its apparently selective interactions with certain E2F family members, such as E2F1, may regulate expression of cellular genes distinct from those regulated by p107 and p130.

THE *P53* GENE

Studies in the late 1970s revealed that a cellular phosphoprotein with a relative molecular mass of about 53,000 Daltons formed a tight complex with SV40 T antigen; hence, the p53 protein was so named.[57–59] Further work established that p53 also formed a complex with other viral oncogene products, including the adenovirus E1B protein, and that p53 was present at low levels in normal cells and high levels in many tumors and tumor cell lines.[59–62] These initial findings suggested that increased levels of p53 might contribute to cancer. Consistent with this notion, gene transfer studies provided data demonstrating that *p53* functioned as an oncogene in in vitro experiments.[62–65] However, subsequent studies showed that p53 was in reality a tumor-suppressor gene. The first definitive evidence for this conjecture came from the studies of human tumors.[66]

The rationale for the human cancer studies was the observation that chromosome 17p LOH was common in a number of different tumor types, including colorectal, bladder, breast, and lung cancer.[67,68] Detailed mapping showed that the region of 17p that was lost in colorectal cancers included the p53 gene.[66] Analysis of the sequence of the *p53* alleles retained in those cancers with 17p LOH demonstrated the remaining *p53* allele was mutated,[66] in perfect accord with Knudson's hypothesis for the alterations expected in tumor-suppressor genes. These observations were soon extended to other cancer types[69,70] and explained many previous observations on p53 that had been confusing when p53 was believed to be an oncogene.[71–75] Additional evidence that *p53* functions as a tumor-suppressor gene in human cancer is provided by gene transfer studies, but as noted above, such overexpression studies cannot be easily interpreted because many genes with no role in neoplasia can inhibit the growth of transfected cells.[76–79] Based on the types of tumors in which *p53* mutations have been found and the prevalence of *p53* mutations in those tumor types, *p53* is believed to be the most frequently mutated genes in human cancer.[80] The vast majority of the somatic mutations in *p53* are missense mutations leading to amino acid substitutions in the central portion of the protein.[80]

Detailed characterization of the particular base substitutions in the *p53* gene revealed distinctly different spectra of p53 mutations in different types of cancer (reviewed in ref. 80). For example, most *p53* mutations in colorectal cancers appear to have arisen spontaneously as a result of deamination of methylated cytosine bases, leading to C → T transition mutations. By contrast, many of the *p53* mutations seen in lung cancers are transversion mutations (eg, G → T) that may have arisen as result of direct interactions of *p53* gene sequences with carcinogens present in tobacco smoke. Furthermore, some of the most compelling data to link mutagenic and carcinogenic agents with cancer induction come from study of the *p53* mutations seen in squamous cell cancers of the skin and hepatocellular cancers. In squamous cell cancers arising in ultraviolet light-exposed skin areas, a sizable fraction of the p53 mutations presumably arose from the generation of pyrimidine dimer premutagenic lesions. Similar studies of the *p53* gene in hepatocellular cancers arising in individuals from geographic areas with very high exposures to aflatoxin identified mutations that are similar to those generated by aflatoxin in in vitro studies.[80]

Germ line mutations in the *p53* gene have been seen in those affected by the Li–Fraumeni syndrome (LFS), as well as in a small subset of pediatric patients with sarcomas or osteosarcomas who do not meet the more strict criteria for diagnosis of LFS.[81–83] Those with LFS are at risk for the development of a number of tumors, including soft-tissue sarcomas, osteosarcomas, brain tumors, breast cancers, and leukemias. Between one-half and two-thirds of patients with LFS have germ line mutations in the central core domain of the *p53* coding sequences that resemble the somatic mutations frequently seen in the *p53* gene in various sporadic cancers.[84] Some LFS patients and families with phenotypic features of LFS have germ line mutations in a gene termed hCHK2 that phosphorylates p53 and controls the cell's response to DNA-damaging events.[85]

In addition to somatic and inherited mutations in the gene, *p53* function can be inactivated by other mechanisms.[86] As noted above, the majority of cervical cancers contain high-risk or cancer-associated HPV genomes (ie, HPV type 16 or 18). The E6 gene product of high-risk, but not low-risk, HPV types binds to a cellular protein known as E6AP (for E6-associated protein) and stimulates p53 degradation.[87–91] A cellular p53-binding protein known as MDM2 is overexpressed in a subset of soft-tissue sarcomas as a result of gene amplification involving chromosome 12q sequences.[92] DNA transfection studies have shown that the *MDM2* gene can function as an oncogene when overexpressed. The oncogenic function of MDM2 is presumably mediated through its binding to and inactivation of p53. MDM2 masks p53's transcriptional activation domain and promotes p53's ubiquitination and subsequent degradation by the proteasome.[93–95] Consistent with the notion that MDM2 is a critical inhibitor of p53 function, sarcomas with *MDM2* amplification and overexpression rarely harbor somatic mutations in *p53*.[96] Disruption of the MDM2 gene in the germ line of mice is lethal, probably because such disruption allows unregulated activity of p53. Accordingly, disruption of the murine p53 gene rescues MDM2-deficient mice from embryonic lethality.[97]

p53 Function Although it may yet be found to have other functions, the p53 protein has been shown to function as a transcriptional regulatory protein.[98,99] In its wild-type state, the p53 protein is capable of binding to specific DNA sequences with its central core domain (Figure 7-5). The aminoterminal sequences of p53 function as a transcriptional activation domain, and the carboxy terminal sequences appear to be required for p53 to form dimers and tetramers with itself. p53 activates transcription of a number of genes with roles in the control of the cell cycle, including *WAF1/CIP1/p21* (which encodes a regulator of Cdk activity),[100] *GADD45* (a growth-arrest DNA damage-inducible gene),[101] MDM2 (as noted above, encoding a protein that is a known negative regulator of p53), and 14–3–3σ (a regulator of G2/M progression),[102] as well as various genes that likely function in apoptosis, including BAX, NOXA, and PUMA, and a number of genes encoding proteins involved in the generation of reactive oxygen species.[98,99,103–105] Other studies suggest that p53 may also function to repress the transcription of certain genes.[98] While the specific mechanisms of p53 repression are not well understood and its importance to neoplasia is unclear, several candidate targets of p53 repression have been suggested, including the gene for the microtubule-associated protein MAP4,[106] the multidrug-resistance–associated protein 1 (MRP1),[107] and the gene for FKBP25, an FK506/rapamycin-binding protein.[108]

The vast majority of *p53* mutations in common human cancers are missense mutations.[80] These missense mutations are scattered through the central domain of the *p53* coding region (exons 5 to 9). Based on the structure of the p53 protein, the missense mutations all appear to have marked effects on p53 protein's capability to bind to its cognate DNA recognition sequence through either of two mechanisms.[109] Some mutations (eg, mutations at codons 248 or 273) alter p53 sequences that are directly responsible for sequence-specific DNA binding. Other

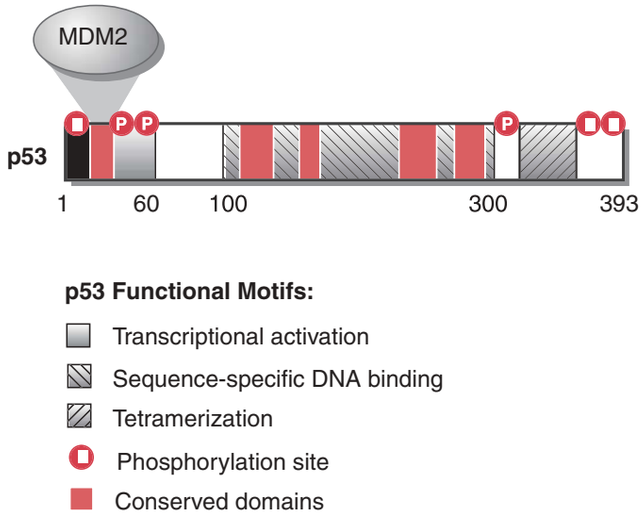

Figure 7-5 p53 functional motifs. Sequences of p53 involved in transcriptional activation, sequence-specific DNA binding, tetramerization, and binding by the MDM2 protein are indicated. The five distinct regions of p53 sequence that are highly conserved between p53 proteins of diverse species are indicated. In addition, the locations of several sites in the protein that are phosphorylated (P) and that regulate p53 function are indicated. (Modified and reproduced with permission from Fearon ER, Vogelstein B. Tumor suppressor and DNA repair gene defects in human cancer. In: Holland JF, Frei E, Bast RC, et al, editors. Cancer medicine, 4th ed. Baltimore: Williams & Wilkins; 1997. p. 97–117.)

mutations (eg, codon 175) appear to affect the folding of p53 and thus indirectly affect its ability to bind to DNA.

The cellular function(s) of p53 remain a subject of intense interest, although a number of insights have emerged.[98] Under some circumstances, p53 acts at the G1/S checkpoint to regulate the cell's decision to synthesize DNA, although p53 also appears to have a critical function at G2/M.[110,111] In perhaps other settings, p53 appears to exert control over the cell's decision to undergo apoptosis or programmed cell death. Of interest with regard to the possible role of p53 in cancer pathogenesis is that loss of p53 function affects the ability of cells to arrest cell growth at the G1/S checkpoint in response to stressful stimuli.[101,112] Furthermore, and of particular interest with regard to cancer treatment, are data from models suggesting that some tumor cells lacking p53 function are less sensitive to γ-irradiation and some chemotherapeutic agents, such as cisplatin.[113,114] Recently, "knock in" mouse cells were generated that contained dominant negative *p53* missense mutations.[115] In this system, these missense variants of p53 also conferred resistance to radiation and chemotherapy. Nevertheless, studies of other tumor cells suggest that p53 status shows a very different relationship to chemotherapeutic response, with cells that lack functional p53 being markedly sensitive to DNA-damaging agents but resistant to 5-fluorouracil.[116] Thus far, studies of primary human cancers have emphasized that there is likely to be a rather complex relationship between *p53* mutational status and the responsiveness of cancer cells to chemotherapy and/or radiation therapy. Finally, some studies provide data indicating that p53 inactivation may protect cells from arrest of growth in response to a relatively broad array of cellular stresses, including hypoxia and nucleotide deprivation.[117,118] Hopefully, further work on p53 function will clarify and enrich our understanding of the normal functions of p53, the basis for p53's frequent inactivation in many different cancers, and the consequences of p53 inactivation on tumor growth and response to therapy.

THE *INK4A* LOCUS AND THE $P16^{INK4A}$ AND $P19^{ARF}$ GENES

Studies of the *INK4a* locus on chromosome 9p illustrate well how observations from initially disparate lines of investigation often converge to implicate a particular locus as a critical factor in cancer development. LOH of chromosome 9p was frequently found in many different tumor types, including melanomas, gliomas, and non-small cell lung, bladder, and head and neck cancers, as well as leukemias.[119–122] Of considerable interest were observations establishing that a subset of such tumors had homozygous (complete) deletions affecting the 9p21 region,[123–125] strongly supporting the existence of a tumor suppressor gene in the region. In addition to the frequent somatic alterations of chromosome 9p sequences in cancers, linkage studies of some families with inherited melanoma indicated a melanoma predisposition gene mapped to essentially the same region of 9p.[126]

These observations stimulated great interest in the chromosome 9p region presumed to contain the tumor suppressor gene(s). One of the genes identified in the region as a result of positional cloning efforts was initially termed *MTS1*.[127] Sequence analysis of *MTS1* showed that it was identical to a previously described gene, encoding the Cdk inhibitor protein known as p16.[128] Because the p16 protein functioned by inhibiting Cdk4 and Cdk6, the protein was termed an INK4 protein. Another highly related gene, mapping immediately next to the *p16/MTS1* gene on chromosome 9p, was found to encode a second INK4 protein, known as p15 (Figure 7-6). The gene encoding the p16 protein is most often termed *INK4a* and the gene for p15 is *INK4b*.[129,130] Subsequent studies show that heterozygous mutations in *INK4a* are present in some patients with inherited melanoma, and in some families with inherited melanoma and pancreatic cancer.[131–134] Somatic mutations in *INK4a* are present in a significant fraction of many different cancer types, including but not limited to melanomas, gliomas, pancreatic and bladder cancers, and leukemias. In some tumors, deletions affecting the *INK4a* gene also involve the *INK4b* gene. In rare tumors, deletions inactivate *INK4b* but not *INK4a*.[135] The prevalence and specific nature of *INK4a* mutations vary markedly from one tumor type to another. In contrast to other tumor-suppressor genes, like *RB1* and *p53*, homozygous deletion is a fairly common mechanism of *INK4a* inactivation in cancer.[136]

Detailed studies of the *INK4a* locus led to the identification of a novel alternative transcript containing nucleotide sequences identical to those in transcripts for the $p16^{INK4a}$ protein, but with unique 5' sequences (see Figure 7-6).[129,130,137] The alternative *INK4a* locus transcript encodes a protein known as $p19^{ARF}$ with p19 denoting its apparent molecular weight and ARF denoting *a*lternative *r*eading *f*rame. The human version of the mouse $p19^{ARF}$ protein is sometimes referred to as $p14^{ARF}$ because of its smaller apparent molecular weight in gel electrophoresis studies. However, both proteins appear to have identical functions, and the discussion below uses the $p19^{ARF}$ terminology because it is found more frequently in the literature. The $p19^{ARF}$ protein contains sequences from a distinct first exon (exon 1β). Exon 1β is located upstream of exon 1α, the first exon present in transcripts for p16 (see Figure 7-6). Exon 1β is spliced to exon 2, which, along with exon 3, is present in the transcripts for both the $p19^{ARF}$ and $p16^{INK4a}$ proteins. However, the $p19^{ARF}$ protein shares no sequence similarity with the $p16^{INK4a}$ protein because $p19^{ARF}$ synthesis initiates at a unique methionine codon in exon 1β and continues through exon 2, using an alternative open reading frame with no similarity to the $p16^{INK4a}$ open reading frame. Careful studies of somatic and inherited mutations at the *INK4a* locus indicate that localized mutations inactivating the $p16^{INK4a}$ protein are common in human cancer, but that localized mutations inactivating $p19^{ARF}$ are uncommon.[129,130] However, the frequent occurrence of homozygous deletions at the *INK4a* locus implies that mutational inactivation of both proteins may be strongly selected for during tumor development (see Figure 7-6). Other findings suggest that $p16^{INK4a}$ and $p19^{ARF}$ expression may be lost in some tumor types as a result of methylation of DNA regulatory sequences at the *INK4a* locus (see Figure 7-6).[138–140] Furthermore, studies of mice with germ line inactivation of $p19^{ARF}$ and $p16^{INK4a}$ indicate that these proteins function as tumor-suppressor genes in vivo.[141–143]

The mechanism through which the $p16^{INK4a}$ protein controls tumorigenic growth is apparently through its inhibition of Cdk4 activity. As indicated above, phosphorylation of pRb impedes its ability to transcriptionally regulate E2F-target genes (see Figure 7-4). The cyclin

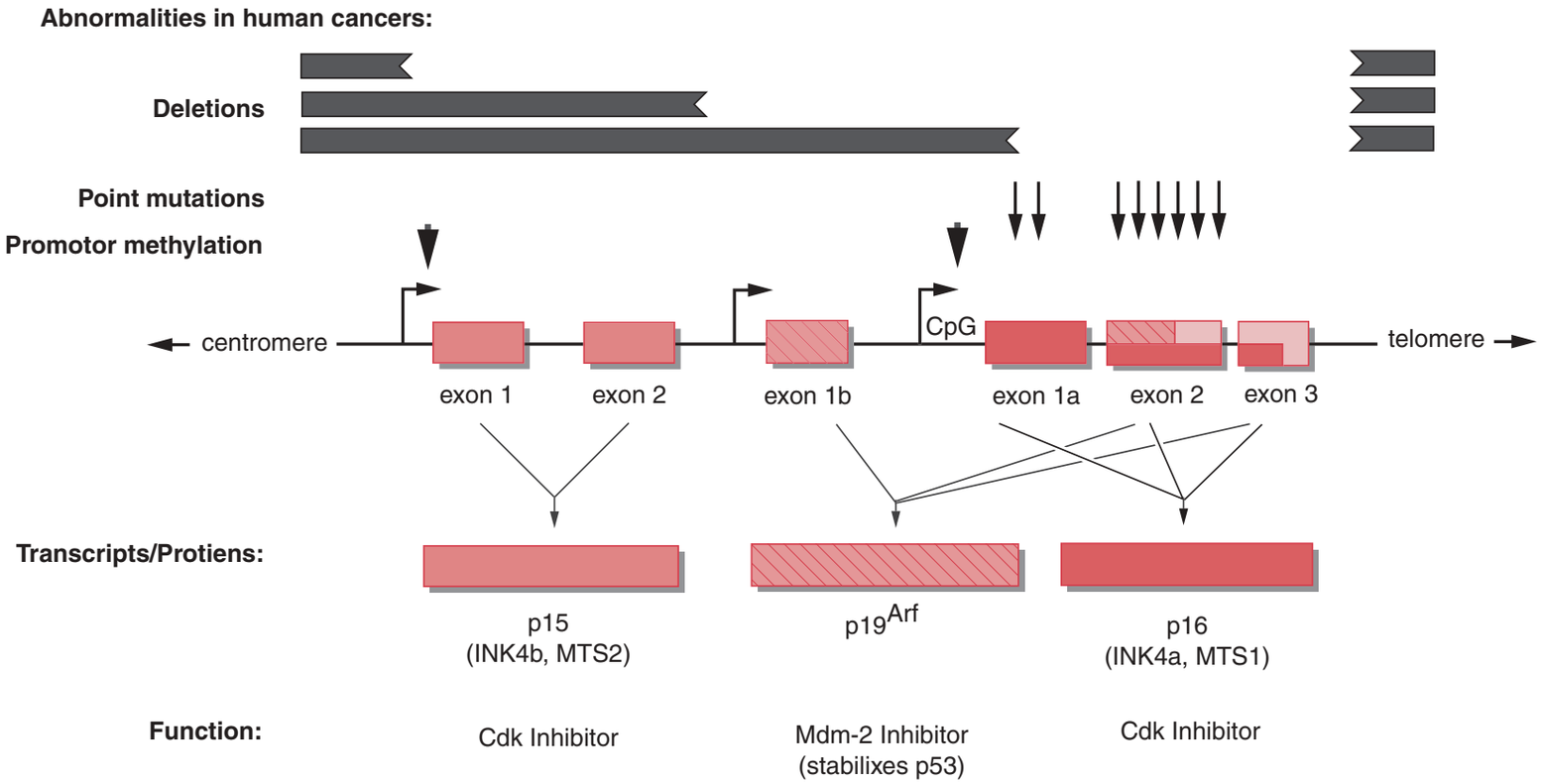

Figure 7-6 Genomic structure, mutations, and transcripts of the *INK4b* (p15) and *INK4a* (p16/p19^{ARF}) locus. The origin of the p15, p16, and p19^{ARF} transcripts is shown schematically, along with a representative depiction of genomic deletions, point mutations (arrows), and promoter methylation (arrowheads) noted in human cancers. The exons of the *INK4b* and *INK4a* loci are shown as rectangles. The transcripts/proteins and presumed functions of the transcripts/proteins are indicated. The speckled rectangles indicate the open reading frame in transcripts encoding p15; the hatched rectangles indicate the open reading frame present in transcripts encoding p19^{ARF}; and the solid rectangles indicate the open reading frame present in transcripts encoding p16. The size of the locus, exons, and transcripts are not shown to scale. (Modified and reprodcued with permission from Haber DA. Splicing into senescence: the curious case of p16 and p19^{ARF}. Cell 91:555–558, 1997.

D1/Cdk4 complex has a critical role in regulating pRb phosphorylation and function.[140] Hence, the p16^{INK4a} protein, by virtue its regulation of Cdk4 activity, is, in turn, a critical factor in regulating pRb phosphorylation. Presumably, inactivation of p16^{INK4a} results in inappropriate phosphorylation of pRb and a subsequent inability of hyperphosphorylated pRb to bind E2Fs and appropriately regulate gene expression at the G1/S transition.

Initially, insights into the means by which p19^{ARF} functioned as a growth regulator and tumor suppressor in vitro and in vivo were lacking, in part because the p19^{ARF} protein lacks significant similarity to proteins with well-established function. It is now clear that p19^{ARF} binds directly to the MDM2 protein, and its binding blocks both MDM2-induced degradation of p53 and MDM2's effects on p53-mediated transcriptional activation of genes.[130] Hence, p19^{ARF} function is important for maintaining the appropriate function of p53 in cells, much like p16^{INK4a} function is critical for appropriate pRb function. The findings on the functions of the p16^{INK4a} and p19^{ARF} proteins emphasize the concept that oncogenes and tumor-suppressor genes do not function in isolation. Rather, they function in intricately linked cascades/networks (Figure 7-7).[98]

THE *APC* GENE

Identification of the APC Gene and Germ Line Mutations Hereditary colorectal cancer syndromes are usually subdivided into polyposis and nonpolyposis types. The polyposis types are those in which dozens or even thousands of benign tumors (polyps) are often seen prior to cancer development. In the nonpolyposis types, few if any polyps are noted prior to cancer formation, in spite of the elevated risk of cancer and the fact that most colorectal cancers are believed to arise from adenomatous precursor lesions. The nonpolyposis colorectal cancer syndromes are addressed below in the context of defects in DNA repair pathway genes. One of the polyposis syndromes is known as familial adenomatous polyposis (FAP) or adenomatous polyposis coli (APC). FAP is an autosomal dominant disorder affecting about 1 in 7,000 individuals in the United States. The syndrome is characterized by the development of hundreds of adenomatous polyps in the colon and rectum of affected individuals by early adulthood. The lifetime risk of colorectal cancer in those with the classic form of FAP is extremely high, approaching nearly 100% by age 60 years.

An observation that greatly aided localization of the *APC* gene was the demonstration by Herrera and Sandberg, in 1986, of an interstitial deletion of chromosome 5q in a patient with features of FAP, but who lacked any family history of the syndrome.[144] Subsequent DNA linkage studies confirmed that, in multiple kindreds with FAP or the related condition known as Gardner syndrome, the polyposis phenotype segregated with DNA markers near 5q21.[145,146] In 1991, positional cloning efforts ultimately identified the *APC* gene as the specific gene responsible for FAP.[147–150] The *APC* gene is large, with more than 15 exons, and alternative splicing affects the 5' untranslated portion of transcripts. The predominant *APC* transcript encodes a 2,843-amino-acid protein expressed in many adult tissues.

In the great majority of individuals with FAP or the Gardner syndrome, heterozygous germ line mutations can be identified in the *APC* gene.[151–153] All of the germ line *APC* mutations in those with FAP or Gardner syndrome appear to inactivate APC protein function. The overwhelming majority of these germ line mutations are localized nonsense or frameshift mutations in the 5' half of the coding region of APC (Figure 7-8). Consistent with Knudson's two-hit hypothesis, inactivation of the remaining wild-type *APC* allele by somatic mutation in those carrying a germ line *APC* mutation is seen in the cancers that arise.[154,155] Correlations between the loca-

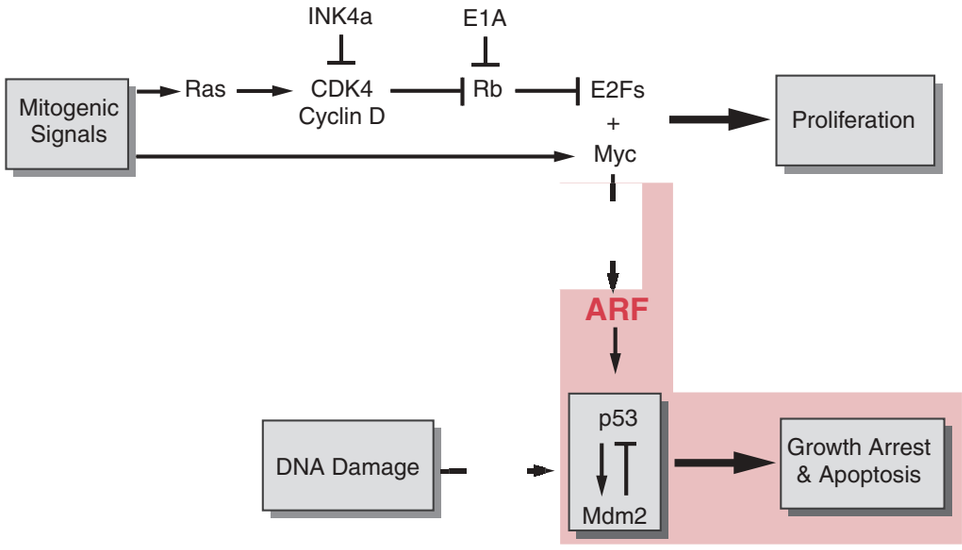

Figure 7-7 Role of the p19^{ARF} protein in checkpoint control. The p19^{ARF} protein (ARF) responds to proliferative signals normally required for cell proliferation. When these signals exceed a critical threshold, the ARF-dependent checkpoint (vertical barrel) is activated, and ARF triggers a p53-dependent response that induces growth arrest and/or apoptosis. Signals now known to induce signaling via the ARF-p53 pathway include Myc, E1A, and E2F-1. In principle, "upstream" oncoproteins, such as products of mutated Ras alleles, constitutively activated receptors, or cytoplasmic signal-transducing oncoproteins, might also trigger ARF activity via the cyclin D-cdk4-Rb-E2F or Myc-dependent pathways, both of which are normally necessary for S-phase entry. In inhibiting cyclin D-dependent kinases, p16^{INK4a} can dampen the activity of mitogenic signals. In the figure, E1A is shown to work, at least in part, by opposing Rb function. For simplicity, Myc and E2F-1 are only shown to activate p53 via the effects on ARF, although highly overexpressed levels of these proteins can activate p53 in ARF-negative cells, albeit with an attenuated efficiency. ARF activation of p53 likely depends on inactivation of Mdm2-specific function(s). DNA damage signals (eg, ionizing and UV radiation, hypoxic stress) activate p53 through multiple signaling pathways. (Reproduced with permission from Sherr CJ. Tumor surveillance via the ARF-p53 pathway. Genes Dev 1998;12:2984–91.)

tion of a particular germ line APC mutation and clinical features have been found, although clear insights into the basis for the predisposition to extracolonic tumors (eg, jaw osteomas and desmoid tumors) in those with the variant Gardner syndrome are lacking. However, some light has been shed on the variability in polyp number seen in some families with polyposis.[155,156] Mutations in the 5' region of the *APC* gene appear to be correlated with an attenuated phenotype, perhaps via mechanisms related to alternative splicing or even to reentry of the ribosome on the APC transcript downstream of the premature stop codon.[156] Mutations in 3' third of the *APC* gene are also associated with a milder polyposis phenotype than are mutations in the central third of the gene, perhaps because the mutated APC proteins retain some tumor-suppressor activity, although extracolonic features, such as desmoid tumors, may be more common in those with 3' mutations.[155] Finally, an intriguing missense mutation in the middle of the APC gene has been found in colorectal cancer prone Ashkenazi Jewish families.[157]

SOMATIC *APC* MUTATIONS IN SPORADIC COLON TUMORS Whereas germ line APC mutations are an uncommon cause of colorectal cancer in the general population and are present in only about 0.5% of all colon cancers, somatic *APC* mutations are present in the vast majority of sporadic colorectal adenomas and carcinomas.[158] The initial observation suggesting that *APC* inactivation might be common in colon tumors was the observation that the chromosome 5q region containing the *APC* gene was affected by LOH in many sporadic colorectal adenomas and carcinomas.[68,159] Since the identification of the *APC* gene, detailed analyses of the somatic mutations inactivating the *APC* gene in colorectal tumors have been carried out. The somatic *APC* mutations in sporadic tumors are similar in nature and location to the germ line *APC* mutations found in those with FAP or Gardner syndromes (see Figure 7-8). Present findings suggest that 70 to 75% of colorectal tumors, regardless of their size or particular histopathologic features, harbor a specific somatic mutation in one of their two *APC* alleles.[155]

***APC* FUNCTION** The *APC* gene encodes a large protein of roughly 300 kDa that is hypothesized to regulate cell adhesion, cell migration, or perhaps even apoptosis in the colonic crypt. The localization of the APC protein in the basolateral membrane of colonic epithelial cells, with an apparent increase in APC expression in cells near the top of the crypt implies that APC may regulate shedding or apoptosis of cells as they reach the crypt apex.[160] Perhaps consistent with this view, restoration of APC protein expression in colorectal cancer cells lacking endogenous APC expression has been reported to promote apoptosis.[161]

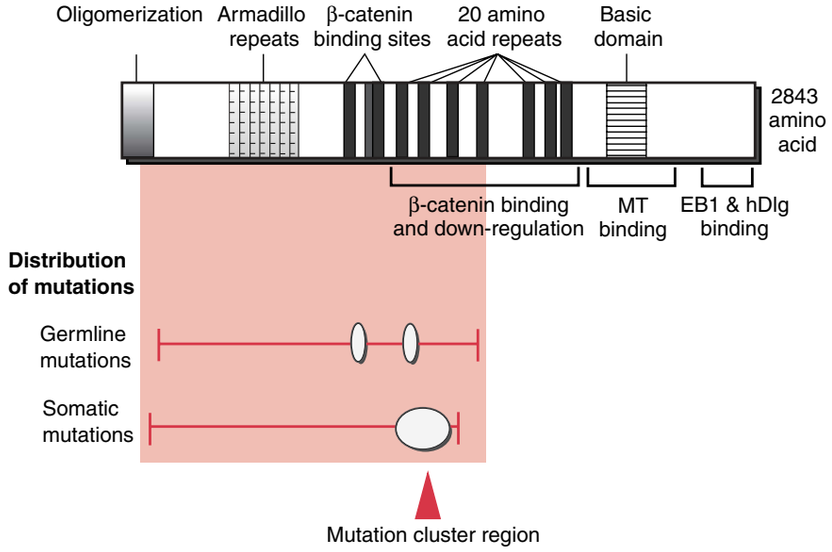

Figure 7-8 Representation of APC protein domains with respect to mutational analysis results. The relative positions of various APC domains. A putative domain involved in homo-oligomerization of APC is located at the aminoterminus. Also noted are a series of repeats of unknown function with similarity to the *Drosophila* armadillo protein, sequences known to mediate binding to β-catenin and its downregulation, a basic domain in the carboxy terminal third of the protein that appears to facilitate complexing with microtubules (MT), and sequences near the carboxy terminus of APC that are known to interact with the EB1 and human homolog of the *Drosophila* disc large (hDlg) protein. Germ line mutations in the *APC* gene (predominantly chain terminating) are dispersed throughout the 5' half of the sequence, with two apparent "hot spots" at codons 1061 and 1309. Somatic mutations in the *APC* gene in colorectal cancer appear to cluster in a region termed the "mutation cluster region," and mutations at codons 1309 and 1450 are most common. (Modified and reproduced with permission from Fearon ER. Oncogenes and tumor-suppressor genes. In: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, editors. Clinical oncology, 2nd ed. New York: Churchill Livingstone; 1999. p. 77–118.)

The APC protein binds to a number of proteins, including β-catenin, γ-catenin (also known as plakoglobin), glycogen synthase kinase 3β (GSK3β), EB1, hDLG, microtubules, and the related proteins axin and conductin.[162] With the exception of β-catenin, GSK3β, and the conductin and axin proteins, the significance and role of APC's interactions with its various binding partners is not well understood. Several lines of evidence imply that APC has a critical function in regulating β-catenin.[162,163] β-catenin is an abundant cellular protein, first identified because of its role in linking the cytoplasmic domain of the E-cadherin cell–cell adhesion molecule to the cortical actin cytoskeleton, via β-catenin's binding to α-catenin. The truncated (mutant) APC proteins present in many colorectal cancers lack some or all of the repeat motifs crucial for binding to β-catenin. APC not only binds to β-catenin, but in collaboration with an enzyme known as glycogen synthase kinase-3 (GSK3β) and other proteins, such as axin or conductin, appears to regulate the abundance of β-catenin in the cytosol. In colorectal cancers in which APC is mutated and unable to bind and/or effectively coordinate the regulation of β-catenin, β-catenin accumulates in the cell, complexes with transcription factors of the Tcf (T-cell factor) or Lef (lymphoid-enhancer factor) family, such as Tcf-4, and translocates to the nucleus (Figure 7-9). Once there, β-catenin functions as a transcriptional coactivator, activating expression of Tcf-regulated genes. Consistent with the notion that β-catenin is a critical target of APC regulation, somatic mutations in β-catenin have been found in a fraction of the colorectal cancers lacking *APC* mutations.[164–166] These mutations consistently alter GSK3β phosphorylation consensus sites near the aminoterminus of the β-catenin protein, and the mutations presumably render the defective β-catenin proteins oncogenic as a result of their resistance to degradation by APC and GSK3β. Consequently, β-catenin accumulates in the cytoplasm and nucleus and activates expression of Tcf-regulated genes (see Figure 7-9). Although somatic mutations in APC appear to be rare in cancers arising outside the colon and rectum, oncogenic mutations in β-catenin's N-terminus are also seen in a significant fraction of many different cancer types, including melanoma, hepatocellular cancer, endometrial cancer, and endometrioid-type ovarian cancer.[167]

Much work remains to define genes activated by the Tcf/β-catenin complex in cancer cells with APC defects. However, recent findings indicate that protooncogenes such as c-*MYC* and *CYCLIN D1*, extracellular proteases such as MMP-7, and nuclear receptor factors such as the peroxisome proliferator–activator receptor δ (PPARδ) may be critical targets.[168–172] Like c-MYC and cyclin D1, other Tcf/β-catenin targets with increased expression as a result of APC or β-catenin mutations presumably promote cell growth and/or inhibit cell death. Further work on APC function should offer crucial insights into the development of colon and other cancers, as well as novel strategies and targets for chemotherapy and perhaps even chemoprevention.

BRCA1 AND *BRCA2* GENES

Like several other common epithelial cancers, family history has long been hypothesized to be a major breast cancer risk factor, with greatest risk in those who have a history of breast cancer in multiple first-degree relatives. However, only in the late 1980s was evidence obtained that predisposition to breast cancer in some families could be attributed to a highly penetrant, autosomal dominant allele. Subsequently, in 1990, Hall and colleagues reported the localization of one such breast cancer predisposition gene, termed *BRCA1* (for breast cancer predisposition gene 1),

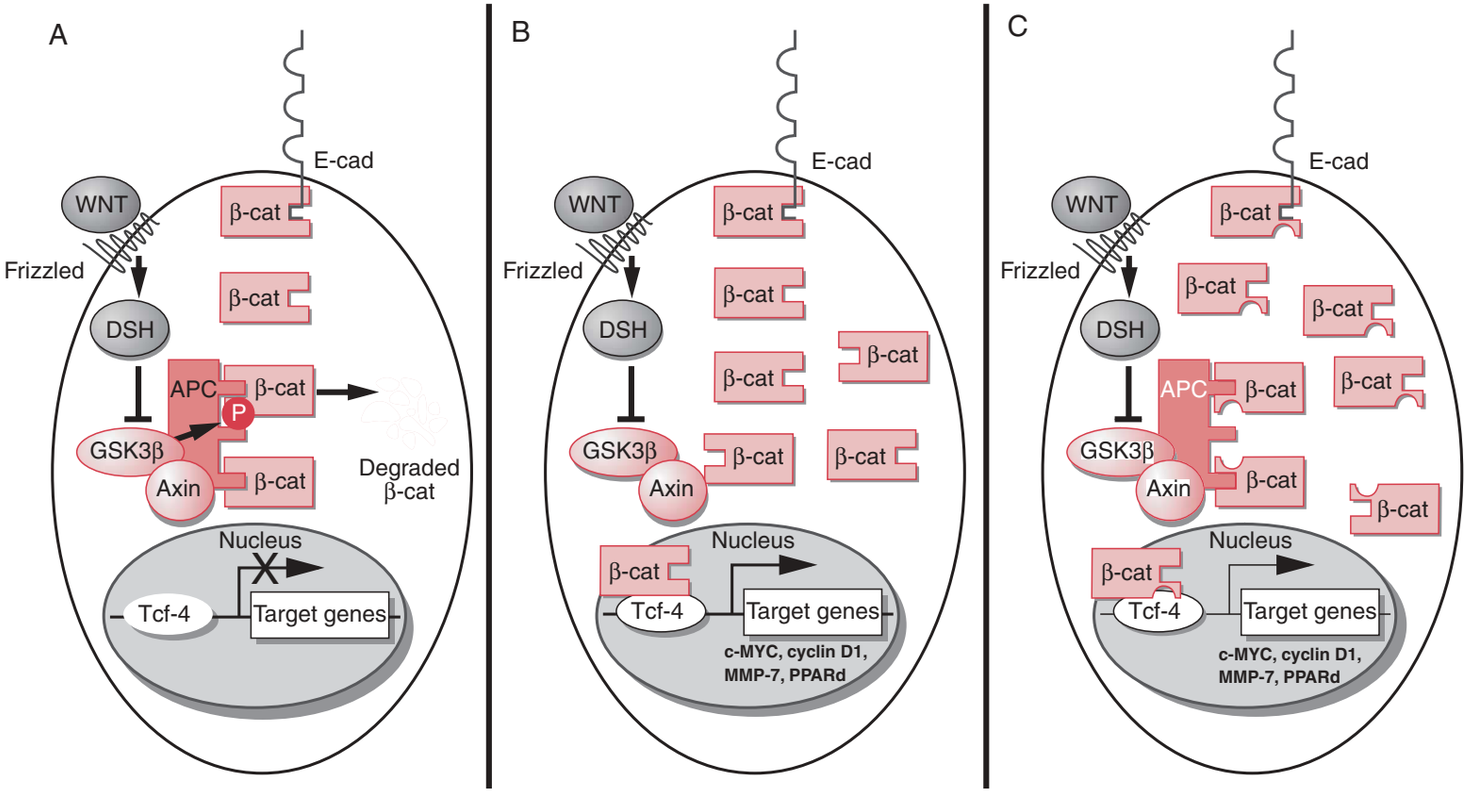

Figure 7-9 A model indicating the function of the APC, axin, and GSK3β proteins in the regulation of β-catenin (β-cat) in normal cells, and the consequence of APC or β-cat defects in cancer cells. β-cat is an abundant cellular protein, and much of it is often bound to the cytoplasmic domain of the E-cadherin (E-cad) cell–cell adhesion protein. **A,** In normal cells, the proteins glycogen synthase kinase 3β (GSK3β), APC, and axin function to promote degradation of free cytosolic β-cat, probably as a result of phosphorylation of the N-terminal sequences of β-cat by GSK3β. GSK3β activity and β-cat degradation are inhibited by activation of the wingless (Wnt) pathway, as a result of the action of the Frizzled receptor and disheveled (DSH) signaling protein. **B,** Mutation of APC in colorectal and other cancer cells results in accumulation of β-cat, binding to Tcf-4, and transcriptional activation of Tcf-4 target genes, such as *c-MYC*, *cyclin D1*, *MMP-7*, and *PPARΔ* (see text). **C,** Point mutations and small deletions in β-cat in cancer cells inhibit phosphorylation and degradation of β-cat by GSK3β and APC, with resultant activation of *c-MYC* and other Tcf-4 target genes. (Modified and reproduced with permission from Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. Science 1997;278:1043–8.)

on chromosome 17q21.[173] Subsequently, others found germ line *BRCA1* mutations substantially increase the risk not only of breast cancer but also of ovarian cancer.[174,175] Intensive research efforts were focused on the region of chromosome 17q harboring *BRCA1*, and the gene was ultimately identified by positional cloning approaches in 1994.[176,177]

Studies of germ line *BRCA1* mutations in breast cancer patients have yielded important results. In studies of families with four or more cases of breast and/or ovarian cancer diagnosed before age 60 years, germ line *BRCA1* mutations were identified in nearly 50% of families studied.[178–180] In fact, germ line *BRCA1* mutations may account for cancer predisposition in roughly 75% of families who manifest both breast and ovarian cancer.[178,180] Many distinct germ line *BRCA1* mutations have been identified, although most of the mutations result in the synthesis of a truncated BRCA1 protein.[178,180] Whereas most germ line *BRCA1* mutations have been identified in only one or a few families, some mutations have been found recurrently. The 11 most common mutations account for about 45% of the total *BRCA1* mutations observed.[179,180] In fact, the two most common mutations in *BRCA1* (185delAG and 5382insC) account for approximately 10% of the total. Of note, the 185delAG frameshift mutation at codon 185 of *BRCA1*, involving a deletion of two bases (adenine and guanine), has been identified in more than 20 Jewish families with familial breast or ovarian cancer. Moreover, population surveys of Ashkenazi Jews, chosen without regard to a family history of cancer, indicate that approximately 1% carry the 185delAG mutation.[179–181] Based on studies of families with germ line *BRCA1* mutations, the lifetime risks of breast cancer and ovarian cancer in those carrying an inactivating mutation are estimated to be 85% and 50%, respectively.[178,180] Whether particular germ line BRCA1 mutations confer a greater risk of breast and/or ovarian cancer than other mutations remains uncertain.

Because LOH of the *BRCA1* locus was found in roughly 50% of unselected breast cancers and 65 to 80% of unselected ovarian cancers,[180,182] *BRCA1* was hypothesized to have an important role in the development of sporadic breast and ovarian cancers. Surprisingly, very few sporadic cancers have been found to harbor detectable somatic mutations in *BRCA1*.[180,182] Somatic *BRCA1* mutations in sporadic breast cancer cases have not been described, and somatic *BRCA1* mutations have been identified only in approximately 5% of sporadic ovarian carcinomas.[182]

Although germ line mutations in the *BRCA1* gene underlie cancer predisposition in roughly 40 to 50% of families with multiple breast cancer cases, another highly penetrant autosomal dominant susceptibility gene termed *BRCA2* plays a critical role in a significant fraction of the families lacking *BRCA1* mutations. The *BRCA2* gene was mapped to chromosome 13q12–13 in 1994,[183] and identified by positional cloning strategies in 1995.[184] At present, many uncertainties about the nature, spectrum, prevalence, and significance of germ line and somatic mutations in the *BRCA2* gene remain. However, several points have been fairly well established. First, whereas germ line mutations in *BRCA1* and *BRCA2* appear to confer essentially similar lifetime risks of female breast cancer (ie, ~ 80%), the risk of ovarian cancer is reduced to approximately 10% in those with *BRCA2* mutations versus approximately 40 to 50% in those with *BRCA1* mutations. The risk of male breast cancer is markedly elevated in *BRCA2* mutation carriers, with a lifetime risk of approximately 6%. In contrast, there is no obvious risk of male breast cancer in *BRCA1* mutation carriers. There also appears to be an elevated risk of pancreatic and perhaps several other cancers in both male and female *BRCA2* mutation carriers.[180] LOH of the *BRCA2* locus at 13q12, but not at the *RB1* locus at 13q14, has been observed in some sporadic breast, pancreatic, head and neck, and other cancers, suggesting that *BRCA2* may be a target for somatic mutations in cancer. However, few somatic *BRCA2* mutations in sporadic cancers have been detected.[180]

The *BRCA1* and *BRCA2* genes each encode a large nuclear protein. The amino acid sequences of the two proteins have only short regions of similarity with one another or other well-characterized proteins. Although their lack of obvious functional motifs stymied initial attempts to define the cellular functions of BRCA1 and BRCA2, several lines of evidence indicate that both proteins interact directly or indirectly with homologs of yeast Rad51, a protein that functions in the repair of double-stranded DNA breaks.[185–192] Moreover, the BRCA1, BRCA2, and Rad51 proteins all appear to be present in a stable multiprotein complex in the cell's nucleus. Consequently, it has been suggested that BRCA1 and BRCA2 may function in the response to or repair of DNA damage, particularly double-strand DNA breaks. Other findings imply that BRCA1 and perhaps BRCA2 may have a role in regulating transcription.[193] Although the DNA repair and transcription regulation functions may be distinct, it is entirely possible that the two functions are linked in a process sometimes referred to as transcription-coupled DNA repair.[189]

Despite these clues, many questions remain about the significance of the interactions of BRCA1 and BRCA2 with one another and their functions in DNA repair and/or transcriptional regulation. Again, like other tumor-suppressor genes with roles in site-specific predisposition to cancer and ubiquitous expression in adult tissues, it is not clear why germ line mutations in BRCA1 and BRCA2 markedly increase the risk of only selected cancer types (eg, breast and ovarian). One suggestion is that breast and certain other epithelial cells may be particularly susceptible to the type of DNA damage that arises in cells with BRCA1 or BRCA2 defects. Loss of BRCA1 or BRCA2 function would then lead to markedly increased rates of mutation acquisition only in certain cell types. Alternatively, the processes in which BRCA1 and BRCA2 function may have many back-up systems or fail-safe mechanisms in most normal cell types, but not in breast and selected other epithelial cell types. Yet, a third possible explanation for the tissue specificity of the cancers seen in BRCA1 and BRCA2 mutation carriers is that inactivation of either BRCA1 or BRCA2 is most often associated with a detrimental or even a lethal effect in stem cells other than those of the breast or ovary. Finally, though the observations thus far have implicated BRCA1 and BRCA2 predominantly in maintenance of genome integrity, other functions for the proteins are possible. For example, there a recent report suggests a link between the estrogen receptor and BRCA1 function, thus providing another potential explanation of this tumor suppressor's tissue specificity.[194]

WT1 GENE

Wilms tumor is the most common renal neoplasm of children, accounting for approximately 6% of all pediatric cancers.[195] Wilms tumor is similar to retinoblastoma in a number of ways, as both tumors can occur bilaterally or unilaterally, with single or multiple foci, and in a sporadic or inherited fashion. The two-mutation model originally proposed for retinoblastoma was also proposed to explain Wilms tumor.[196] Hereditary cases, however, are not as common for Wilms tumors as for retinoblastomas, and whereas almost all patients inheriting a mutation at the *RB1* locus develop a retinoblastoma, only approximately 50% of individuals carrying a germ line mutation predisposing to Wilms tumor develop the disease.[195]

Perhaps the first finding to offer insight into an inherited genetic basis for Wilms tumor was a report in 1964 describing six patients with Wilms tumor and sporadic aniridia (ie, congenital absence of the iris).[197] It was proposed that the simultaneous occurrence of these two very rare conditions might result from chromosomal aberrations affecting two or more loci, a situation now often referred to as a contiguous gene syndrome; mutation of one locus presumably leading to aniridia and mutation of another leading to Wilms tumor. This hypothesis was subsequently supported by the discovery of interstitial deletions of chromosome 11p13 in peripheral blood samples from children with the WAGR syndrome of Wilms' tumor: aniridia, genitourinary abnormalities, and mental retardation.[198] Cytogenetic studies of tumor tissues in a few cases of sporadic type Wilms tumors revealed deletions or translocations of chromosome band 11p13.[199,200] Subsequent studies of paired samples of Wilms tumor and normal cells from patients, using probes that detect restriction fragment length polymorphisms (RFLPs) on chromosome 11p, revealed that LOH of 11p occurred frequently in Wilms tumors of both the inherited and sporadic types.[201–204]

The *WT1* gene was identified in 1990 by virtue of mutations inactivating the gene in patients with the WAGR syndrome, as well as by

analysis of somatic mutations in the gene in tumors from a minority of patients with unilateral Wilms tumor and no associated congenital malformation.[205] *WT1* is encoded by 10 exons and its transcripts are subject to alternative splicing.[206,207] In contrast to the rather ubiquitous expression of the *RB1* and *p53* genes, high-level expression of the *WT1* gene appears to be restricted to embryonic kidney and a small subset of other tissues.[208,209] *WT1* messenger ribonucleic acids (mRNAs) encode proteins with molecular masses of 45,000 to 49,000 Daltons and 4 zinc-finger motifs. Based on its predicted amino acid sequence, the WT1 proteins were suspected from the outset to function in transcriptional regulation.[209] Several studies provide evidence to support this notion, although some WT1 isoforms may have a role in RNA processing, rather than in transcription regulation.[207,209] WT1 proteins suppress the transcriptional activity of promoter elements from a number of growth-inducing genes, including the genes for early growth response (EGR1), insulin-like growth factor-2 (IGF-2), and platelet-derived growth factor A chain (PDGFA), suggesting that WT1 may function in gene repression.[210] Other studies suggest that WT1 can activate or repress gene expression, depending on the cell type and promoter context.[211] Consistent with the notion that WT1 may have a physiologic function in transcriptional activation, recent work indicates that WT1 activates expression of amphiregulin, a member of the epidermal growth factor family.[212] Loss of amphiregulin expression may contribute to loss of appropriate differentiation during Wilms tumor development. Adding to the complex nature of WT1's role as a transcriptional regulator, recent studies suggest that certain WT1 splice variants have dramatically different effects in their ability to regulate gene expression.[213]

WT1 inactivation clearly contributes to Wilms tumor development in those with the WAGR syndrome and approximately 10% of apparently sporadic Wilms tumors have detectable somatic mutations in the *WT1* gene.[214] Nevertheless, much evidence indicates that Wilms tumors arise through mutations in genes other than *WT1*. First, the chromosome 11p allelic losses seen in Wilms tumor frequently involve band 11p15, but not band 11p13, where the *WT1* gene resides.[214–216] Second, the 11p15 region harbors a gene responsible for Beckwith-Wiedemann syndrome (BWS), a congenital disorder in which affected individuals manifest hyperplasia of the kidneys, endocrine pancreas, and other internal organs, macroglossia, and hemihypertrophy.[217,218] Those affected by BWS are also at increased risk for the development of embryonic tumors, such as hepatoblastoma and Wilms tumor. Finally, linkage studies of three families with dominant inheritance of Wilms tumor exclude linkage of the susceptibility locus in these families to any part of chromosome 11p.[219,220] On the whole, the data suggest that germ line mutations in any one of at least three different genes (ie, *WT1*, the BWS gene, and at least one nonchromosome 11p gene) can predispose to Wilms tumor. Whether a combination of inherited and somatic mutations in more than one of these genes is ultimately required for the transformation of a developing kidney cell into a Wilms tumor, or whether alternative genetic pathways for the development of Wilms' tumors exist, remains to be established. The genetic heterogeneity seen in Wilms tumor provides an important contrast to the apparently less-complex genetic pathway of retinoblastoma.

NF1 AND NF2 GENES

***NF1* GENE** von Recklinghausen or type 1 neurofibromatosis (NF1) is a dominantly inherited syndrome with variable disease manifestations, but the consistent feature is that tissues derived from the neural crest are most commonly affected. In addition to the nearly uniform development of neurofibromas, NF1 patients are at elevated risk of developing pheochromocytomas, schwannomas, neurofibrosarcomas, and primary brain tumors.[221–223] The *NF1* gene was initially localized to the pericentromeric region of chromosome 17q by linkage analyses.[224,225] Subsequently, karyotype studies of two NF1 patients identified germ line chromosomal rearrangements involving band 17q11.[226,227] In further work, both patients were found to have genetic alterations of a localized region of band 17q11. Intensive positional cloning efforts in this chromosome region led to the identification of the *NF1* gene in 1991.[228–230] The *NF1* gene is large, spanning roughly 350 kb of DNA, and it encodes a protein product with a molecular mass of about 300 kilodaltons (kDa).[222,223,231] Although germ line mutations in the *NF1* gene are believed to underlie the development of the associated disease features in all or very nearly all NF1 patients, specific germ line *NF1* mutations have been identified in approximately one-half to two-thirds of NF1 patients.[222,223,231,232] Difficulties in identifying germ line mutations in the *NF1* gene in the remaining NF1 patients may be a result of the inherent inefficiencies and insensitivity associated with mutation detection strategies in such a large gene.

In addition to germ line *NF1* mutations in those patients with NF1, the *NF1* gene is affected by somatic mutations in a fraction of colon cancers, melanomas, neuroblastomas, and bone marrow cells from patients with the myelodysplastic syndrome.[223,231,233–235] Consistent with its presumed tumor-suppressor role, the mutations inactivate *NF1*. Studies of leukemias arising in pediatric neurofibromatosis patients provide the clearest evidence that both copies of the *NF1* gene are inactivated during tumorigenesis,[236] as predicted by the Knudson model. Like the *RB1*, *p53*, and *APC* genes, the *NF1* gene is expressed ubiquitously. Thus, as for other inherited cancer syndromes, the basis for the tissue specificity of the malignant tumors seen in neurofibromatosis patients (predominantly neurofibrosarcomas, leukemias, and primary brain tumors) remains puzzling. The *NF1* protein product, termed neurofibromin, is a cytoplasmic protein with high similarity to guanosine triphosphate (GTPase)-activating proteins (GAPs).[223,237–239] Perhaps the best studied GAP is Ras-GAP, which markedly enhances the GTPase activity of the wild-type K-Ras, H-Ras, and N-Ras proteins. Although the means through which *NF1* defects alter cell growth is not well understood, it is likely that inactivation of neurofibromin function leads to alterations in signaling pathways regulated by small Ras-like G proteins. Interestingly, NF1 null mice do not develop neurofibromas, but NF1 null, p53 null mice do, implicating p53 loss as a necessary step in the pathogenesis of this disease.[240,241]

***NF2* GENE** Neurofibromatosis type 2 (NF2, also known as central neurofibromatosis) is an autosomal dominant disorder that is distinct from NF1 on both genetic and clinical grounds.[223,242,243] A hallmark of NF2 is the occurrence of bilateral schwannomas that affect the vestibular branch of the eighth cranial nerve (acoustic neuromas). NF2 patients are also at elevated risk for meningiomas, spinal schwannomas, and ependymomas. The *NF2* gene was mapped to chromosome 22q by a combination of linkage analyses and LOH studies,[244–246] and cloned, in 1993, via positional cloning approaches.[247,248] Germ line mutations inactivating the *NF2* gene were observed in those patients with NF2, and somatic *NF2* mutations were also seen in a subset of sporadic (non–NF2-associated) schwannomas and meningiomas. Somatic *NF2* mutations in most other tumor types appear to be infrequent. However, preliminary studies indicate that the *NF2* gene may be frequently affected by somatic mutations in malignant mesotheliomas,[249] despite this tumor type not being seen at increased frequency in patients with NF2.[242] The *NF2* gene encodes a protein with strong similarity to a cytoskeletal protein family thought to act as linker proteins between integral membrane proteins and scaffolding proteins of the filamentous submembrane lattice.[248] Consequently, *NF2* gene alterations might contribute to tumor development, at least in part, via effects on cell shape, cell–cell interactions, and/or cell movement. Mouse studies confirm the importance of NF2 in tumor development. Although NF2 null mice typically develop osteosarcomas and not schwannomas, recent studies that used a conditional NF2 inactivation system in mouse Schwann cells appear to recapitulate the disease phenotype seen in humans.[250]

VHL GENE

von Hippel-Lindau (VHL) syndrome is a rare dominant disorder predisposing affected individuals to the development of hemangioblastomas of the central nervous system and retina, as well as renal carcinomas of clear cell type and pheochromocytomas.[251–253] The *VHL* gene was mapped to chromosome 3p by linkage analysis. Similar to the situation with many other inherited

cancer genes, LOH studies established that the *VHL* gene behaves as a typical tumor-suppressor gene, with both alleles inactivated during tumorigenesis.[253,254] Positional cloning efforts identified the *VHL* gene in 1993.[255] Germ line mutations inactivating one *VHL* allele are seen in the majority of individuals in families displaying features of the VHL syndrome.[251–253] As with some other inherited cancer syndromes, preliminary genotype–phenotype relationships have been observed. Specifically, a certain class of *VHL* germ line mutations is associated with the development of renal cancer only, a second class of germ line mutations is linked to predisposition to both renal cancer and pheochromocytoma, and yet a third mutation class is associated only with pheochromocytoma.[253] Somatic mutations in the *VHL* gene are also seen in upwards of 80% of sporadic renal cell carcinomas of the clear cell type, but not in renal cell carcinomas of other histopathologic types (eg, papillary type).[251,253] Approximately 20% of sporadic clear cell renal cancers do not carry a detectable mutation in the *VHL* gene. However, in many of these cases, the *VHL* gene may be inactivated by methylation of its transcriptional regulatory sequences,[256] a mechanism described earlier because of its association with inactivation of the *INK4a* locus in certain tumors. In tumor types other than clear cell renal cancer, inactivation of the *VHL* gene by somatic mutations or other mechanisms (eg, promoter methylation) appears to be uncommon.[253]

The *VHL* gene encodes a 213-amino-acid protein that was initially found to form a complex with the B and C subunits of the elongin or SIII transcriptional elongation factor complex, a protein complex that may regulate transcriptional elongation by RNA polymerase II.[252,257–259] More recent studies show that the VHL and the elongin B and C proteins are present in several multiprotein complexes in the cytoplasm. One such complex may regulate proteolytic degradation of other cellular proteins.[260,261] Recent studies suggest a link between VHL loss of function and the tumor's characteristic neoangiogenic features.[262,263] The *VHL* gene product apparently degrades hypoxia-inducing factor (HIF)-alpha under normal conditions of oxygenation. Presumably in the absence of VHL, HIF-α, which is a transcription factor, will modulate the transcription of genes that are normally expressed (or repressed) only under conditions of hypoxia. This would likely include those genes that are involved with angiogenesis. Further detailed biochemical and cell biology studies on VHL and renal cell carcinomas are likely to offer definitive insights into the function of the VHL protein in normal and neoplastic cells, and the basis for the tumor spectrum seen in individuals carrying germ line *VHL* mutations.

DNA REPAIR PATHWAY GENES

At the outset of the chapter, tumor-suppressor genes were defined as those genes inactivated by germ line or somatic mutations in cancer. It was also emphasized that DNA damage-recognition and repair genes constitute a subset of the tumor-suppressor gene class, because they are affected by inactivating mutations in cancer. Whereas tumor-suppressor genes such as *RB1*, *p53*, *APC*, and *INK4a* appear to have active roles in regulating cell growth and/or apoptosis, the DNA damage-recognition and repair genes can arguably be viewed as having more passive roles in processes controlling growth. Distinguishing between what constitutes a growth-regulating tumor-suppressor gene versus a DNA repair-type tumor-suppressor gene may be difficult because some tumor-suppressor genes, including perhaps *p53, BRCA1,* and *BRCA2*, may ultimately be established to have functions in both growth control and DNA repair. Nevertheless, based on present data, there is a reasonable basis to suggest that loss-of-function mutations in both alleles of certain DNA repair pathway genes, such as the DNA mismatch repair genes, probably do not directly alter cell growth. Rather, inactivation of DNA mismatch repair activity likely contributes to cancer via an increased frequency of mutations in other cellular genes, particularly genes that are rate determining in tumor development.

Several recessive cancer predisposition syndromes resulting from inactivation of genes that function in DNA damage recognition and repair have been well described, including ataxia-telangiectasia (AT), Bloom syndrome, xeroderma pigmentosum, and Fanconi anemia. In each case, the specific cancer types and DNA-damaging agents that increase cancer risk are essentially distinct. Although AT heterozygotes may have a subtly increased risk of breast cancer,[264] in other recessive cancer syndromes, only homozygotes appear to have a clearly increased cancer risk. This observation contrasts sharply with the picture in the dominant cancer predisposition syndromes discussed earlier (eg, inherited retinoblastoma, familial adenomatous polyposis, NF1, and NF2), where heterozygotes have a clearly elevated cancer risk. Furthermore, as discussed earlier, the basis for increased cancer risk in an individual with a dominant cancer syndrome attributable to a germ line tumor-suppressor mutation (eg, *RB1* or *APC* mutation) is that cancers arise following inactivation of the remaining normal copy of the gene by a second "hit" in somatic cells (ie, the Knudson hypothesis). Therefore, it seems reasonable to argue that second "hits" in tumor-suppressor genes of the type that underlie dominant cancer syndromes must have considerably more potent effects on initiating cancer development than second "hits" in tumor-suppressor genes of the type that underlie recessive cancer syndromes.

In light of these considerations and because recessive cancer syndromes are quite rare, our discussion of the role of DNA repair genes in cancer focuses on DNA mismatch repair gene mutations and HNPCC. The DNA mismatch repair (MMR) genes are also worthy of a more complete discussion because the genes appear to be inactivated in a considerable subset of sporadic cancers, including roughly 10 to 20% of colorectal, endometrial, and gastric cancers.

DNA Mismatch Repair Gene Defects and HNPCC As for breast cancer, familial clustering of colon cancer has long been recognized, with perhaps 10 to 20% of all colon cancers attributable to the inheritance of a gene defect with a strong effect on cancer risk. Germ line *APC* mutations are responsible for 0.5 to 1% of colorectal cancer cases in the Western world, and HNPCC is responsible for approximately 2 to 4%.[265–268] Diagnosis of HNPCC is problematic when only clinical criteria is used. First, overt clinical findings prior to cancer diagnosis, such as the florid intestinal polyposis seen in individuals with FAP, are lacking in individuals with HNPCC. Second, there is always a likelihood of chance clustering of cancer within a family for a common malignancy such as colorectal cancer. Nevertheless, diagnostic criteria for identifying those individuals and families most likely to be affected by HNPCC have been determined.[265–268] Representative diagnostic criteria include (1) exclusion of familial polyposis; (2) colorectal cancer in at least three relatives, one of them being a first-degree relative of the others; (3) two or more successive generations affected; and (4) at least one of the affected individuals being younger than 50 years of age at the time of diagnosis. Even though not all individuals affected by HNPCC meet these criteria, the criteria are useful for excluding familial aggregations of colorectal cancer that are likely to have a genetic basis distinct from that underlying the majority of HNPCC cases.[266,267]

Several genes responsible for HNPCC have been identified, including two on chromosome 2p (*MSH2* and *MSH6*) and another on chromosome 3p (*MLH1*). Together, germ line mutations in the *MSH2* and *MLH1* genes account for virtually all classic HNPCC cases.[266–270] The protein products of the *MSH2* and *MLH1* genes appear to have critical roles in the recognition and repair of DNA mismatches (Figure 7-10). A number of other gene-encoding proteins that function in mismatch repair have been identified, and mutations inactivating the *PMS2* and *MSH6* genes have been seen in a small fraction of those with HNPCC.[266–271]

In cells with one normal and one mutant allele of a DNA mismatch repair gene, DNA repair is minimally impaired, if at all. However, inactivation of the remaining allele can occur as a result of somatic mutation in an initiated cell population during the earliest stages of tumor development. Once the cell acquires impaired mismatch repair function, for instance, as a result of inactivation of both alleles of either *MSH2* or *MLH1*, hundreds of errors/mutations may arise and fail to be repaired during each cell-division cycle. Because these mutations preferentially arise in mononucleotide, dinucleotide, and trinucleotide repeat tracts (ie, microsatellite sequence tracts) the phenotype is

often referred to as the microsatellite instability (MSI) phenotype.

Germ line mutations in the known MMR genes have only been detected in 2 to 4% of colorectal cancer patients, although approximately 10 to 15% of all colon cancers display the MSI phenotype.[267–270,272–274] It is clear that only a small fraction of the sporadic colorectal cancers with the MSI phenotype develop as the result of a germ line mutation in a known mismatch repair gene. Somatic mutations in mismatch repair genes have been found in some sporadic colorectal cancers with the MSI phenotype.[275] In most sporadic cases, however, inactivation of the *MLH1* gene occurs in association with methylation of its promoter.[276,277] The basis for the inactivation and the molecular mechanism(s) underlying the methylation are unknown.

Many of the mutations arising in cells with MMR deficiency are likely to be detrimental to cell growth or even lethal. A small fraction of the total mutations that arise presumably activate oncogenes or inactivate tumor-suppressor genes. Some genes are preferentially mutated in MMR-deficiency cancers, presumably because these mutations confer a selective growth advantage. For instance, genes that contain repetitive DNA sequences, such as microsatellite tracts, might be expected to be targets of mutation in these cancers and data support this prediction. An example of a gene containing a mononucleotide repeat tract in its coding sequence, and that is frequently inactivated in colorectal cancers with MMR-deficiency, is the type II receptor for transforming growth factor-β (TGF-β).

The TGF-β type II receptor is a compelling candidate tumor-suppressor gene, as both copies of the gene are inactivated by mutations in more than 90% of MSI colorectal cancers.[278,279] The TGF-β cytokine is known to inhibit the growth of many epithelial cells. Intriguingly, a recent study has suggested that germ line mutations in the cytoplasmic domain of the TGF-β type II receptor is associated with HNPCC,[280] although it will be important to confirm this observation in additional HNPCC kindreds. Finally, a downstream effector of the TGF-β pathway, Smad4 (also called DPC4), was recently identified as a tumor-suppressor gene. *SMAD4* is somatically mutated in 45 to 50% of pancreatic cancers, in 10 to 20% of colorectal cancers, and in a very small fraction of other cancers.[281–283] Germ line inactivating mutations in *SMAD4* are found in a major fraction of patients with juvenile polyposis syndrome (JPS).[284] Those patients with JPS develop benign (hamartomatous, not adenomatous) polyps of the intestinal tract and are at increased risk of colorectal and gastric cancer.

Another recently suggested candidate for somatic inactivation in MMR-deficient colorectal cancers is the *BAX* gene,[285] which is a potential p53-regulated gene encoding a Bcl-2-related pro-apoptotic protein. Finally, there are data suggesting that gain-of-function mutations in β-*catenin* arise preferentially in MSI colon cancers,[164,165,286] although the β-*catenin* mutations

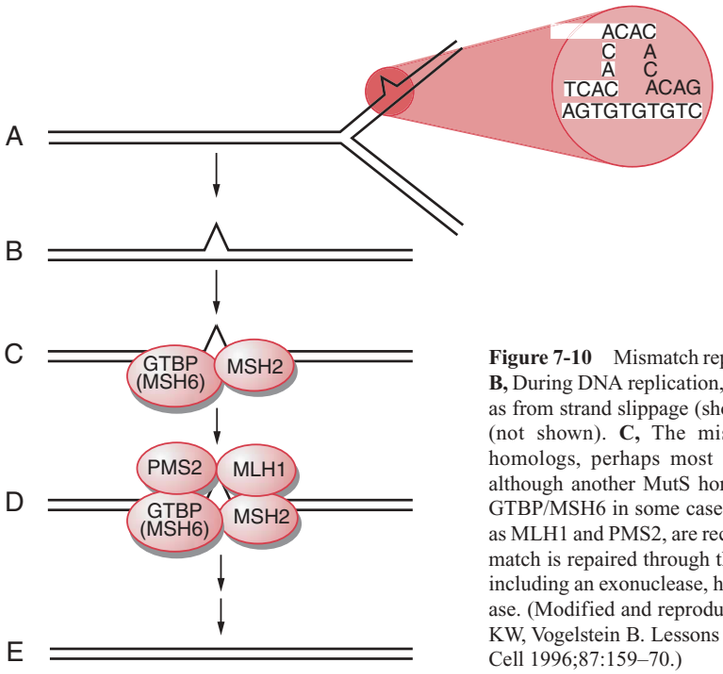

Figure 7-10 Mismatch repair pathway in human cells. **A** and **B,** During DNA replication, DNA mismatches may arise, such as from strand slippage (shown) or misincorporation of bases (not shown). **C,** The mismatch is recognized by MutS homologs, perhaps most often MSH2 and GTBP/MSH6, although another MutS homolog, MSH3, may substitute for GTBP/MSH6 in some cases. **D** and **E,** MutL homologs, such as MLH1 and PMS2, are recruited to the complex and the mismatch is repaired through the action of a number of proteins, including an exonuclease, helicase, DNA polymerase, and ligase. (Modified and reproduced with permission from Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–70.)

are not present in a microsatellite tract. These mutation comparisons emphasize that it is the *pathways* rather than the specific genes that are best considered as targets of mutations. In MMR-proficient cancers, *APC* is much more frequently mutated than β-*catenin*, while the reverse is true for MMR-deficient cancers; these mutations have similar effects on the pathways through which APC and β-*catenin* control growth. Similarly, *p53* is more often mutated than BAX and *SMAD4* is more often mutated than *TGF-β RII* in MMR-proficient cancers, while the reverse is true for MMR-deficient cancers. This is one example of the ways in which the spectrum of somatic mutations in specific cancers can provide important clues to pathogenesis.

CANDIDATE TUMOR-SUPPRESSOR GENES

The tumor-suppressor genes discussed above and others summarized in Table 7-1 are distinguished by the fact that germ line-inactivating mutations in the genes are associated with inherited cancer predisposition. The link between germ line mutation and elevated cancer risk provides incontrovertible evidence of the gene's role in tumorigenesis. Other findings, such as the demonstration in sporadic cancers of LOH of one tumor-suppressor gene allele accompanied by somatic mutation of the remaining allele, offer evidence for a more widespread role for many of the inherited cancer genes. Although the tumor-suppressor genes in Table 7-1 are definitively linked to inherited cancer syndromes, it is possible that germ line mutations in other bona fide tumor-suppressor genes may be associated with minimal cancer risk. Tumor-suppressor genes of this type could still be frequently inactivated by somatic mutations in sporadic forms of cancer, although their principal role might relate to tumor progression rather than tumor initiation. Such genes are enumerated in Table 7-2. Because the evidence in favor of these genes' involvement does not include predisposition when inherited in mutant form, they should largely be viewed as "candidate" tumor-suppressor genes rather than as bona fide tumor-suppressor genes. Further functional and biochemical data is required to change their status from "candidate" to "culprit."

An increasing number of genes with decreased or absent expression in cancers are being discovered. These genes are sometimes termed tumor suppressors solely on the basis simply of their reduced expression and are not listed in Table 7-2. Other genes with the capability to antagonize the tumorigenic or in vitro growth properties when overexpressed in cancer cell lines have also been termed tumor suppressors and are also not listed in Table 7-2. Some of these genes may, indeed, have critical roles in growth regulation. However, the altered expression of many genes in cancers simply reflects the altered growth and differentiation properties of cancer cells and their abnormal microenvironment when compared to normal cells in the tissue or organ from which the cancer arose. Moreover, many genes that play no role in cancer can dramatically alter cell growth when expressed exogenously at high and nonphysiologic levels. In the end, the mutational and functional evidence should be carefully weighed before concluding that a gene has a causal role in tumorigenesis and whether it should appropriately be designated as a tumor-suppressor gene.

Table 7-1 Selected Tumor Suppressor Genes and Associated Protein Function

Gene	Associated inherited cancer syndrome	Cancers with somatic mutations	Presumed function of protein
RB1	Familial retinoblastoma	Retinoblastoma, osteosarcoma, SCLC, breast, prostate, bladder, pancreas, esophageal, others	Transcriptional regulator; E2F binding
TP53	Li-Fraumeni syndrome	Approximately50% of all cancers (rare in some types, such as prostate carcinoma and neuroblastoma)	Transcription factor; regulates cell cycle and apoptosis
[a]*INK4a*			
p16	Familial melanoma, Familial pancreatic carcinoma	Approximately 25-30% of many different cancer types (eg, breast, lung, pancreatic, bladder)	Cyclin-dependent kinase inhibitor (ie, Cdk4 and Cdk6)
$p19^{ARF}$	?Familial melanoma?	Approximately 15% of many different cancer types	Regulates Mdm-2 protein stability and hence p53 stability; alternative reading frame of *p16*/*INK4a* gene
APC	Familial adenomatous polyposis coli (FAP), Gardner syndrome, Turcot syndrome	Colorectal, desmoid tumors, thyroid cancers, stomach cancers	Regulates levels of β-catenin protein in the cytosol; binding to microtubules
BRCA1	Inherited breast and ovarian cancer	Ovarian (~10%), rare in breast cancer	DNA repair; complexes with Rad 51 and BRCA2; transcriptional regulation
BRCA2	Inherited breast (both female and male), pancreatic cancer, ?others?	Rare mutations in pancreatic, ?others/	DNA repair; complexes with Rad 51 and BRCA1
WT-1	WAGR, Denys-Drash Syndrome	Wilms' tumor	Transcription factor
NF-1	Neurofibromatosis type 1	Melanoma, neuroblastoma	p21ras-GTPase
NF-2	Neurofibromatosis type 2	Schwannoma, meningioma, ependymoma	Juxtamembrane link to cytoskeleton
VHL	von-Hippel Lindau syndrome	Renal (clear cell type), hemangioblastoma	Regulator of protein stability
MEN-1	Multiple endocrine neoplasia type 1 Endocrine tumors of the pancreas	Parathyroid adenoma, pituitary adenoma, enocrine tumors of the pancreas	Not known
PTCH	Gorlin syndrome, hereditary basal cell carcinoma syndrome	Basal cell skin carcinoma, medulloblastoma	Transmembrane receptor for sonic hedgehog factor; negative regulator of smoothened protein
PTEN/*MMAC1*	Cowden syndrome; sporadic cases of juvenile polyposis syndrome	Glioma, breast, prostate, follicular thyroid carcinoma, head and neck squamous carcinoma	Phosphoinositide 3-phosphatase; protein tyrosine phosphatase
DPC4	Familial juvenile polyposis syndrome	Pancreatic(~50%), approximately 10–15% of colorectal cancers, rare in others	Transcriptional factor in TGF-β signaling pathway
E-CAD	Familial diffuse-type gastric cancer; lobular breast cancer	Gastric (diffuse type), lobular breast carcinoma, rare in other types (eg, ovarian)	Cell-cell adhesion molecule
LKB1/*STK1*	Peutz-Jeghers syndrome	Rare in colorectal, not known in others	Serine/threonine protein kinase
SNF5/*INI1*	Rhabdoid predisposition syndrome (renal or extra-renal malignant rhabdoid tumors), choroid plexus carcinoma medulloblastoma; central primitive neuroectodermal tumors)	Rare in rhabdoid tumors, choroid plexus carcinoma, medulloblastoma	Member of the SWI/SNF chromatin ATP-dependent remodeling complex
EXT1	Hereditary multiple exostoses	Not known	Glycosyltransferase; heparan sulfate chain elongation
EXT2	Hereditary multiple exostoses	Not known	Glycosyltransferase; heparan sulfate chain elongation
TSC1	Tuberous sclerosis	Not known	Not known; cytoplasmic vesicle localization
TSC2	Tuberous sclerosis	Not known	Putative GTPase activating protein for Rap1 and rab5; Golgi localization
MSH2, *MLH1* *PMS1*, *PMS2*, *MSH6*	Hereditary non-polyposis colorectal cancer	Colorectal, gastric, endometrial	DNA mismatch repair

[a]Note that the *INK4a* gene encodes two distinct protein products as a result of alternative splicing (see text).

Table modified from Table 1 of Fearon ER. Tumor suppressor genes. In: Vogelstein B, Kinzler KW, editors The genetic basis of human cancer, 2nd ed. McGraw-Hill: New York, NY. 1999: p. 233.

SUMMARY

There is now overwhelming evidence that mutations in tumor-suppressor genes are the major molecular determinants of most common human cancers. However, the first convincing experimental evidence that tumorigenesis might result, at least in part, from the inactivation of normal cellular genes with essential roles in growth regulation was presented only 30 years ago. Additional evidence for the existence of tumor-suppressor genes and their importance in tumorigenesis emerged gradually from somatic cell genetic and epidemiologic studies, as well as from studies, using cytogenetic and molecular genetic techniques, of chromosome losses in tumor cells. In the last 15 years, more than 30 tumor-suppressor genes have been identified by molecular cloning techniques. In some cases, these genes are inactivated in the germ line and their inactivation predisposes to cancer. Far more frequently, these same tumor-suppressor genes are inactivated by somatic mutations during tumor development. Although we have learned much about tumor-suppressor genes, a great deal of work remains. A more complete description of tumorigenesis will undoubtedly emerge with the identification of additional tumor-suppressor genes, the detailed characterization of their normal cellular functions, and the elucidation of the frequency and spectrum of mutations and other mechanisms that inactivate these genes and their protein products in human tumors. The findings will not only provide new insights into cancer pathogenesis, but should also prove critical in improving the diagnosis and management of patients with cancer.

Table 7-2 Selected Candidate Tumor-Suppressor Genes and Their Encoded Proteins

Gene	Cancers with somatic mutations	Protein Function	Comments
TGF-β type II R	RER+ colorectal and gastric cancer, head and neck, lung, and esophageal squamous cell carcinoma	TGF-β receptor component	Both alleles inactivated in RER+ cancers with mutations; mutations infrequent in non-RER+ cancers; germline variant allele proposed to be associated with "HNPCC-like" phenotype
BAX	RER+ colorectal	Pro-apoptotic factor	Mutations are heterozygous (1 allele) in the majority of cancers; ? genetically unstable microsatellite tract vs. specific target for inactivation?
FHIT	Lung, cervical, renal, others	Dinucleoside polyphosphate hydrolase	Mutations detected in ~5-10% of cancers; majority of mutations affect non- coding sequences; aberrant splicing and reduced RNA and protein levels are common; ? genetically unstable locus vs. specific target for inactivation?
α-*CAT*	Some prostate and lung, ?others	Links E-cadherin cell adhesion complex to cytoskeleton	Mutations present in a small fraction of cancers
DCC	Some colorectal, neuroblastoma, male germ cell cancer, gliomas, ?others ?	Netrin-1 receptor component; regulates cell migration and apoptosis	Mutations rarely detected; decreased or absent expression is seen in > 50% of a variety of cancer types
MADR2/SMAD2	Some colorectal	Transcription factor/signaling molecule in TGF-β pathway	Mutations in < 5% of colorectal and other cancers (e.g., gastric)
CDX2	Rare mutations in colorectal	Homeobox transcription factor	CDx2 +/- knockout mice are predisposed to intestinal tumors; decreased Cdx2 protein expression in human and rodent colorectal tumors
MKK4	Rare mutations in pancreas, lung, breast, and colorectal; ?others	Stress- and cytokine-induced protein kinase	
PP2R1B	Lung, colorectal	Subunit of serine/threonine protein phosphatase 2A	Mutations are heterozygous in some cases
MCC	Rare mutations in colorectal	Not known	Mutations in about 5–10% of sporadic colorectal cancers

Table corresponds to Table 2 of Fearon ER. Tumor suppressor genes. In: Vogelstein B, Kinzler KW, eds. The Genetic Basis of Human Cancer, 2nd Edition. McGraw-Hill: New York, NY. In press, 1999. p. 234

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