Genetic Alterations and Cancer Cell Immortality

- Even after a tumor cell has escaped regulation by tumor suppressors or DNA repair proteins, it must overcome one more hurdle to unlimited proliferation: the intrinsic limitation on the number of cell divisions allowed to each cell.

- Ordinarily, a cell is restricted to about 50 to 70 mitotic divisions. After reaching this number, the cell typically becomes **senescent** and cannot continue to divide.

- Research has provided new insights on the mechanisms that count the number of cell divisions and has illustrated the ways tumor cells can circumvent the counting system.

- Each time a cell divides, the telomeres of chromosomes shorten slightly because DNA polymerase cannot replicate the tips of chromosomes.

- Once the telomere is reduced to a critical length, a signal is transmitted that causes the cell to become senescent. This process would place severe limitations on the proliferating cells in a tumor, preventing further clonal expansion.

- Many tumor cells overcome the process by activating a gene that encodes **telomerase,** a reverse transcriptase that replaces the telomeric segments that are normally lost during cell division.

- Activation of this enzyme, which is rarely present in normal cells but is found in 85% to 90% of tumor cells, is part of a process that allows a tumor cell to continue to divide without the restraint ordinarily imposed by telomere shortening.

- This uninhibited division allows the tumor to become large, and, by allowing continued DNA replication, it permits the accumulation of additional mutations that can further contribute to the aggressiveness of the tumor cell.

- Recent whole-genome sequencing studies have shown that somatic mutations in the promoter region of the *TERT* gene (which encodes a component of telomerase) are seen in 70% of melanomas.
- These mutations increase the expression of *TERT* and are likely to contribute to increased telomerase activity in melanoma cells.

INHERITED CANCER-CAUSING GENES

The *TP53* Gene

- Somatic mutations in the ***TP53* gene** are found in more than half of all human tumors, making this the most commonly altered cancer-causing gene.

- Somatic *TP53* mutations are seen, for example, in approximately 70% of colorectal tumors, as well as 40% of breast tumors and 60% of lung tumors.

- Approximately 80% to 90% of *TP53* mutations are concentrated in the portion of the gene that encodes a DNA-binding domain, typically preventing the p53 protein product from binding to DNA of other genes.
- *TP53* typically functions as a tumor suppressor gene (however, because *TP53* mutations often have a dominant negative effect, the 2-hit model does not typically apply to this tumor suppressor gene).

- Its protein product, p53, increases in quantity in response to cell damage (e.g., double-stranded DNA breaks caused by ionizing radiation). Acting as a transcription factor, p53 helps to regulate dozens of genes that affect cell growth, proliferation, and survival. For example, p53 binds to the promoter of *CDKN1A,* whose protein product, p21, blocks the inactivation of pRb (see Fig. 11-5).

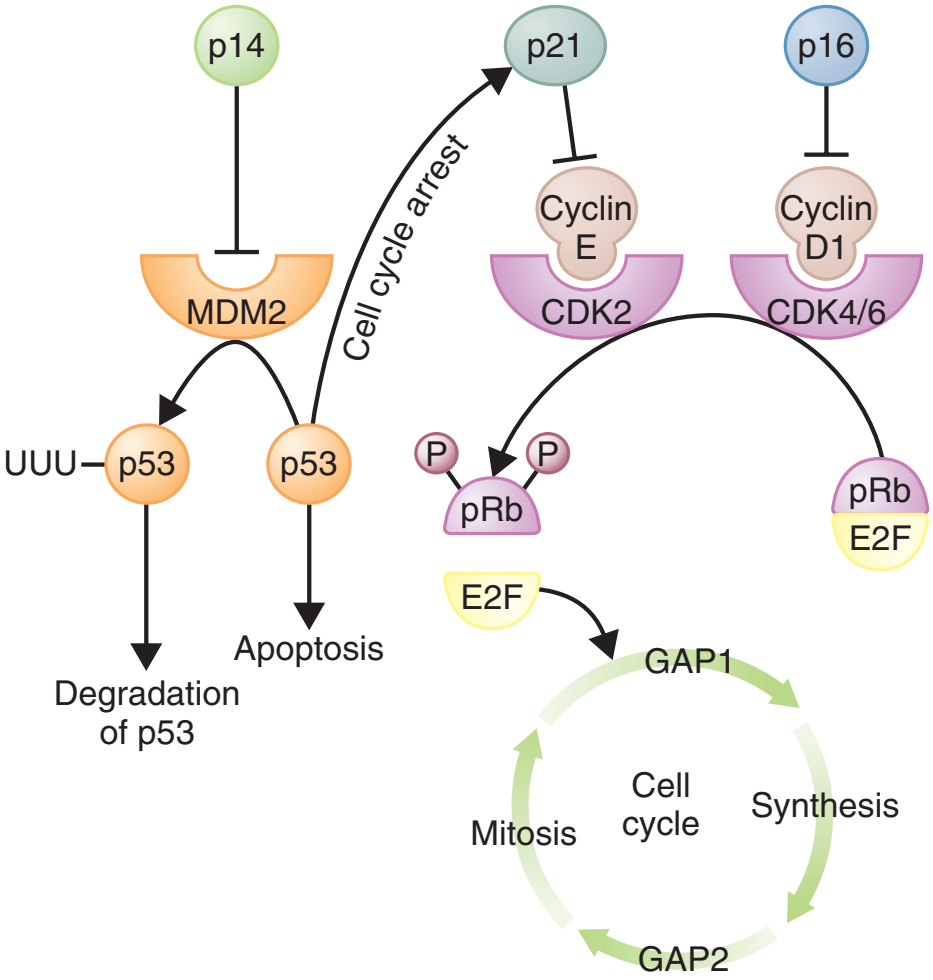


FIG 11-5 Regulation of the cell cycle is accomplished by a complex series of interactions among activators and repressors of the cycle. pRb acts as a master brake on the cell cycle by binding the E2F transcription complex, halting the cycle before S phase begins. The cyclin D–CDK4 complex inactivates pRb by phosphorylating it, thereby releasing the E2F complex and allowing the cell to progress through S phase. CDK inhibitors such as p16 and p21 inactivate CDKs, thus acting as another brake on the cycle. p53, acting through p21, can either halt the cell cycle or induce apoptosis in response to DNA damage. *CDK,* Cyclin-dependent kinase.

- When *TP53* is mutated, cells with damaged DNA can evade both repair and destruction, and continued replication of the damaged DNA can lead to tumor formation. For this to happen, other components of cell cycle control must also be compromised.

- For example, several DNA tumor viruses, such as the human papilloma virus that is responsible for most cases of cervical cancer, inactivate both pRb and p53. This produces cells that can neither repair their DNA nor undergo apoptosis in response to damage, leading in some cases to cancer.

- Carcinogenic substances can induce specific *TP53* mutations. Dietary ingestion of afla toxin B1, which can produce liver cancer, is associated with a mutation that produces an arginine-to-serine substitution at position 249 of the p53 protein.
- Exposure to benzopyrene, a powerful mutagen and carcinogen found in cigarette smoke, leads to alterations of specific *TP53* base pairs in lung tumors.
- This demonstrates a direct molecular link between cigarette smoking and lung cancer. Thus, examination of the type of *TP53* mutation seen in a tumor can provide clues to the identity of the causative carcinogenic agent.

- Germline mutations of *TP53* are responsible for an inherited cancer condition known as the Li–Fraumeni syndrome (LFS). This rare condition is transmitted in autosomal dominant fashion and involves breast and colon carcinomas, soft-tissue sarcomas, osteosarcomas, brain tumors, leukemia, and adrenocortical carcinomas.

- These tumors usually develop at early ages in LFS family members, and multiple primary tumors are commonly seen in persons with LFS. The demonstration of consistent *TP53* mutations in the constitutional DNA of patients with LFS confirmed the causative role of this gene.

- The inheritance of a mutated *TP53* gene greatly increases an individual's susceptibility to subsequent cell transformation and tumor development.
- Among LFS family members who inherit a mutated *TP53* gene, approximately 50% develop invasive cancer by 30 years of age, and more than 90% develop invasive cancer by age 70 years.

- *TP53* mutations account for only about 75% of LFS cases; some of the remaining cases are the result of mutations in another tumor suppressor gene, *CHEK2*. This gene encodes a kinase that normally phosphorylates p53 in response to ionizing radiation, resulting in the accumulation and activation of p53. Loss-of-function mutations in *CHEK2* result in a lack of p53 activation, causing LFS via the p53 pathway.

- *TP53* is medically important in at least two ways. First, the presence of *TP53* mutations in tumors, particularly those of the breast and colon, often signals a more-aggressive cancer with relatively poor survival prospects. It is thus a useful prognostic indicator. Second, *TP53* might ultimately prove important in tumor prevention.

- Laboratory experiments show that the insertion of a normal *TP53* gene into tumor cells can induce tumor regression by inducing abnormal cancer cells to undergo apoptosis. This has led to gene therapy protocols in which normal *TP53* copies are inserted into tumors in an effort to eliminate cancerous cells.

The Familial Adenomatous Polyposis Gene, *APC*

- Colorectal cancer (CRC) affects approximately 1 in 20 Americans, and, like most common cancers, it is more likely to occur in persons with a positive family history.

- One's risk of developing CRC is elevated two to three fold if a first-degree relative is affected; it increases to three to six fold if two first-degree relatives are affected. Approximately 2% to 5% of colon cancer cases are inherited as autosomal dominant syndromes, the two most important of which are: FAP and HNPCC.

- **Familial adenomatous polyposis (FAP)** is characterized by the appearance of large numbers of colonic adenomas, a type of polyp, in the second or third decade of life. Colonic adenomas are now understood to be the immediate precursors to colorectal cancer.

- The multiple adenomas of the patient with FAP therefore present a grave risk of early malignancy. Because early detection and removal of adenomatous polyps can significantly reduce the occurrence of cancer, it is important to understand the causative gene and its role in the development of polyps.

- The gene responsible for FAP was localized to the long arm of chromosome 5 by linkage analysis in families. Discovery of small, overlapping deletions in two unrelated patients provided the key to isolation of the disease-causing gene, termed ***APC* (adenomatous polyposis coli).**

- Among the genes that lay within the 100-kb region that was deleted in both patients, one showed apparent mutations in other patients. This mutation was seen in one patient but not in his unaffected parents (i.e., a de novo mutation), which helped to confirm the identification of the *APC* gene.

- *APC* is a tumor suppressor gene, and both copies of *APC* must be inactivated in a cell for tumor progression to begin. Persons who inherit an *APC* mutation (the first "hit") typically experience somatic loss-of-function mutations in hundreds of their colonic epithelial cells, giving rise to multiple adenomas.

- In some cases, loss of function of *APC* occurs because of hypermethylation of *APC*'s promoter region, which results in reduced transcription.
- **Hypermethylation**, as well as other changes in gene regulation (Box 11-1) has been observed in the inactivation of a number of tumor suppressor and DNA repair genes, including those associated with retinoblastoma *(RB1),* breast/ ovarian cancer *(BRCA1),* hereditary nonpolyposis colorectal cancer *(MLH1),* malignant melanoma *(CDKN2A),* and von Hippel–Lindau disease *(VHL).*

- Identification of the *APC* gene has been important in diagnosing and managing CRC in families with FAP.

- Somatic ***APC* mutations** are found in 85% of *all* sporadic, noninherited cases of colon cancer. These somatic *APC* mutations (i.e., those that disable both copies of the gene in a colonic cell) are among the earliest alterations that give rise to colon cancer.

- *APC* mutations are not sufficient by themselves to complete the progression to metastatic disease. As shown in Figure 11-8, other genes are also altered.

- For example, gain-of-function mutations are seen in the *KRAS* gene in approximately 50% of colon tumors. This gene encodes a signal transduction molecule, and a gain-of-function mutation increases signaling and thus cellular proliferation.
- Loss-of-function mutations in the *TP53* gene are also seen in more than 50% of colorectal tumors and usually occur relatively late in the pathway to cancer. Ordinarily, p53 would be activated in response to mutations like those of *APC* and *KRAS,* leading to DNA repair or apoptosis. Cells that lack p53 activity are free to continue along the path to malignancy in spite of their damaged DNA.
- Still another tumor suppressor gene, *SMAD4,* is also frequently mutated in the CRC pathway.

- Extensive studies have revealed at least **three ways** in which the APC protein acts as a tumor suppressor.

- Perhaps most importantly, it participates in the phosphorylation and degradation of β-catenin, a key molecule in the Wnt signal transduction pathway. Among other things, this pathway is involved in activation of the Myc transcription factor. By reducing β-catenin levels, APC dampens signals that lead to cellular proliferation.

- *APC* mutations are also thought to affect cell-to-cell and cell-to-matrix adhesion properties (this is important because alteration of cell adhesion control permits cells to invade other tissues and to metastasize to other sites). Again, this activity is mediated through β-catenin, which interacts with a cell-surface molecule (E-cadherin) whose loss of function leads to abnormal cell adhesion properties.

- Finally, APC is expressed in the microtubules that pull chromosomes apart during meiosis. Alterations in APC result in altered microtubule activity, such that aneuploidies and chromosome breaks arise during mitosis. Thus, *APC* mutations also promote cancer by increasing genomic instability.

The Hereditary Nonpolyposis Colon Cancer Genes

- Hereditary nonpolyposis colon cancer (HNPCC, or Lynch syndrome), a second form of inherited colon cancer, accounts for up to 5% of all colorectal cancer cases.

- HNPCC is an autosomal dominant, high-penetrance cancer syndrome, with a lifetime colorectal cancer risk of 50% to 80% in heterozygotes. In addition, the risk of endometrial cancer among females with HNPCC is approximately 40% to 60%, and the risk of ovarian cancer is 5% to 10%.

- Cancers of the small bowel, stomach, brain, pancreas, renal pelvis, and ureter are seen in a smaller percentage of mutation carriers.

- HNPCC patients do not have polyposis; they typically have a relatively small number of polyps. Also, polyps in HNPCC patients are more likely to occur in the proximal colon, whereas those of FAP patients are more likely to be concentrated in the distal colon.

- Approximately 40% to 60% of HNPCC cases are caused by mutations in a gene called ***MSH2***, and another 25% to 30% of cases are caused by mutations in the ***MLH1*** gene.
- Mutations in three other genes, ***PMS2, MSH6,* and *EPCAM***, help to account for a small percentage of additional cases.

- Each of these genes is known to play an important role in **DNA mismatch repair** in many different organisms. Inactivation of both alleles of any one of these genes increases the genome-wide mutation rate in an affected cell by as much as 1000-fold. This increased rate of mutation results in the alteration of a number of cellular regulatory genes, thus leading to an increased incidence of cancer.

- A characteristic feature of tumors from HNPCC patients is a **high degree of instability** of microsatellite loci, which generates many new microsatellite alleles. Such microsatellite instability is also seen in about 15% of sporadic colorectal carcinomas, but somatic loss-of-function mutations in the HNPCC genes seem to occur only infrequently in these tumors.

- The most common alteration seen in these sporadic tumors is **hypermethylation** of the ***MLH1* gene**, resulting in its inactivation.

- A comparison of FAP and HNPCC reveals interesting **differences** in the way each syndrome leads to colon cancer.

- In FAP, an inherited *APC* mutation results in hundreds of polyps, each of which has a relatively low probability of incurring all of the other genetic alterations required for progression to metastatic cancer. But, because the number of polyps is large, there is a high probability (almost 100%) that at least one of them will produce a cancerous tumor.
- In HNPCC, the number of polyps is much smaller (hence the term *nonpolyposis*), but, because of a relative lack of DNA repair, each polyp has a high probability of experiencing the multiple alterations necessary for tumor development.

- Consequently, the average age of onset of colorectal cancer in HNPCC is only about 10 years later than that of FAP.

Inherited Breast Cancer

- The lifetime prevalence of breast cancer in women is 1 in 8, and a woman's risk of developing breast cancer doubles if a first-degree relative is affected.
- Two genes, ***BRCA1*** and ***BRCA2,*** have been identified as major contributors to inherited breast cancer.

- Population-based studies show that only a small percentage of all breast cancers—approximately 1% to 3%—can be attributed to inherited mutations in *BRCA1* or *BRCA2.*
- Among women with breast cancer who also have a positive family history of the disease, the percentage with inherited mutations in either of these genes increases to approximately 20% to 30%.
- Among affected women who have a positive family history of both breast and ovarian cancer, 60% to 80% have inherited a *BRCA1* or *BRCA2* mutation.

- Inherited mutations in these genes are also more common among women with early-onset breast cancer and among those with bilateral breast cancer.

- Women who inherit a mutation in *BRCA1* experience a 50% to 80% lifetime risk of developing breast cancer; the lifetime risk for those who inherit a *BRCA2* mutation is slightly lower, averaging approximately 50%.

- *BRCA1* mutations also increase the risk of ovarian cancer among women (40-50% lifetime risk, which is substantially higher than the lifetime risk of 1/70 in the general female population). These mutations also confer a modestly increased risk of prostate and colon cancers.
- *BRCA2* mutations confer an increased risk of ovarian cancer (20% lifetime risk).

- Approximately 6% of males who inherit a *BRCA2* mutation develop breast cancer, which represents a 70-fold increase over the risk in the general male population.

- Most *BRCA1* and *BRCA2* mutations result in truncated mRNA or protein products and a consequent loss of function.

- Affected persons inherit one copy of a *BRCA1* or *BRCA2* mutation and then experience a somatic loss of the remaining normal allele in one or more cells (following the two-hit model for tumor suppressor genes).
- Somatic mutations affecting these genes are seldom seen in sporadic (noninherited) breast tumors.

- *BRCA1* and *BRCA2* are both large genes, and they exhibit extensive allelic heterogeneity (approximately 2000 distinct mutations, most of which are deletions or nonsense or frameshift mutations, have been reported for each gene).

- This poses challenges for genetic diagnosis, which is done primarily by direct DNA sequencing of the coding and regulatory regions of both genes. Typically, a panel of approximately one dozen breast cancer-associated risk genes are now tested for disease-causing mutations.

- Although *BRCA1* and *BRCA2* share no significant DNA sequence similarity, they both participate in the DNA repair process.

- The protein product of *BRCA1* is phosphorylated (and thus activated) by the ATM and CHEK2 kinases in response to DNA damage (Fig. 11-10).
- The *BRCA1* protein product binds to the *BRCA2* product, which in turn binds to RAD51, a protein involved in the repair of double-stranded DNA breaks.
- *BRCA1* and *BRCA2* thus participate in an important DNA repair pathway, and their inactivation results in incorrect DNA repair and genomic instability.

- In addition to their roles in the RAD51 pathway, *BRCA1* and *BRCA2* help to suppress tumor formation through their interactions with p53, pRb, and Myc, and by helping to maintain genomic stability.

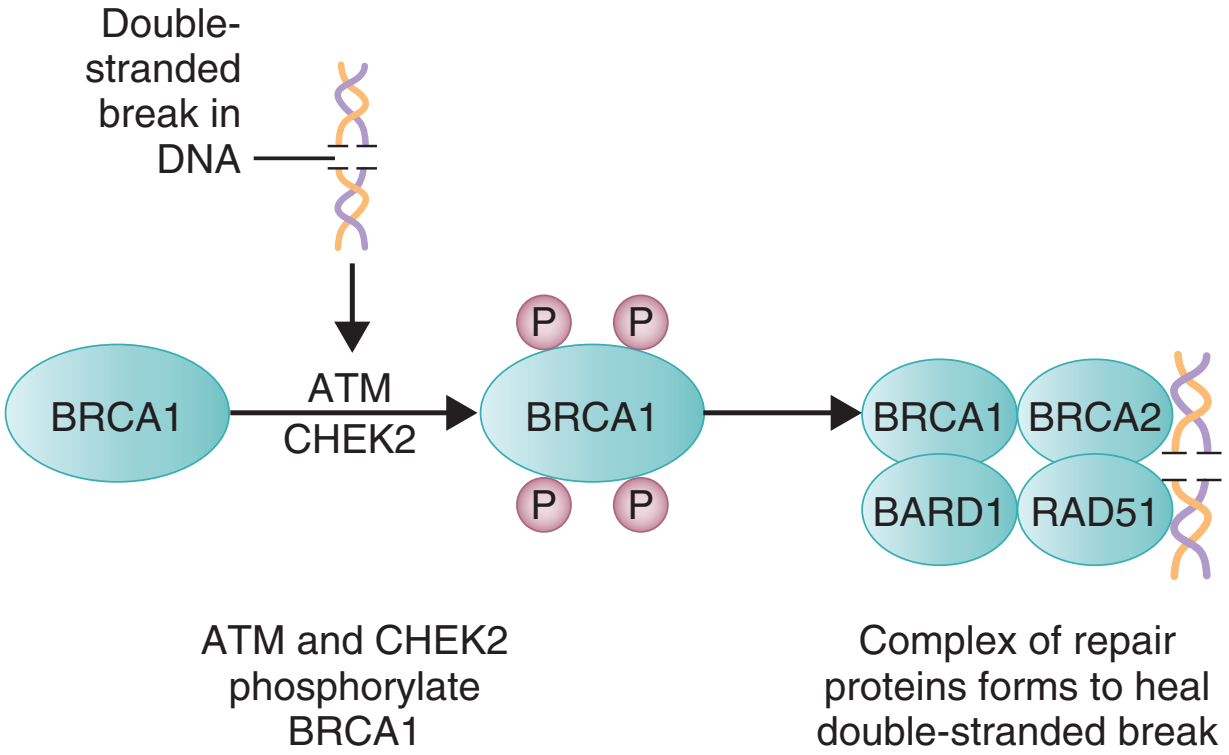


FIG 11-10 The roles of BRCA1 and BRCA2 in DNA repair. BRCA1 is phosphorylated by ATM and CHEK2 in response to double-stranded DNA breaks (produced, for example, by ionizing radiation). BRCA1 binds to BRCA2, which interacts with RAD51 to form a complex involved in DNA repair.

- Although *BRCA1* and *BRCA2* mutations are the most common known causes of familial breast cancer, this disease can also be caused by inherited mutations in several other tumor suppressor genes.

- Germline mutations in a tumor suppressor gene called *PTEN* are responsible for Cowden disease, which is characterized by multiple benign tumors and an increased susceptibility to breast cancer.
- The risk of breast cancer among heterozygous carriers of mutations in the *ATM* gene is approximately double that of the general population.
- Mutations in the *PALB2* gene, which forms a complex with *BRCA1* and *BRCA2* in repairing double-stranded DNA breaks, also double the risk of breast cancer.

- It is estimated that the major breast cancer genes, such as *BRCA1, BRCA2, PTEN, PALB2,* and *CHEK2,* account for less than 25% of the overall inherited predisposition to breast cancer.

- Other breast cancer–causing genes are likely to exist, but their individual effects on cancer risk are thought to be relatively small. Large-scale genome-wide association and DNA sequencing studies have now identified multiple additional inherited genetic variants that contribute small increases in breast cancer risk (Fig. 11-11).
- For example, a variant in the gene that encodes fibroblast growth factor receptor 2 (FGFR2) increases breast cancer risk by about 25%.

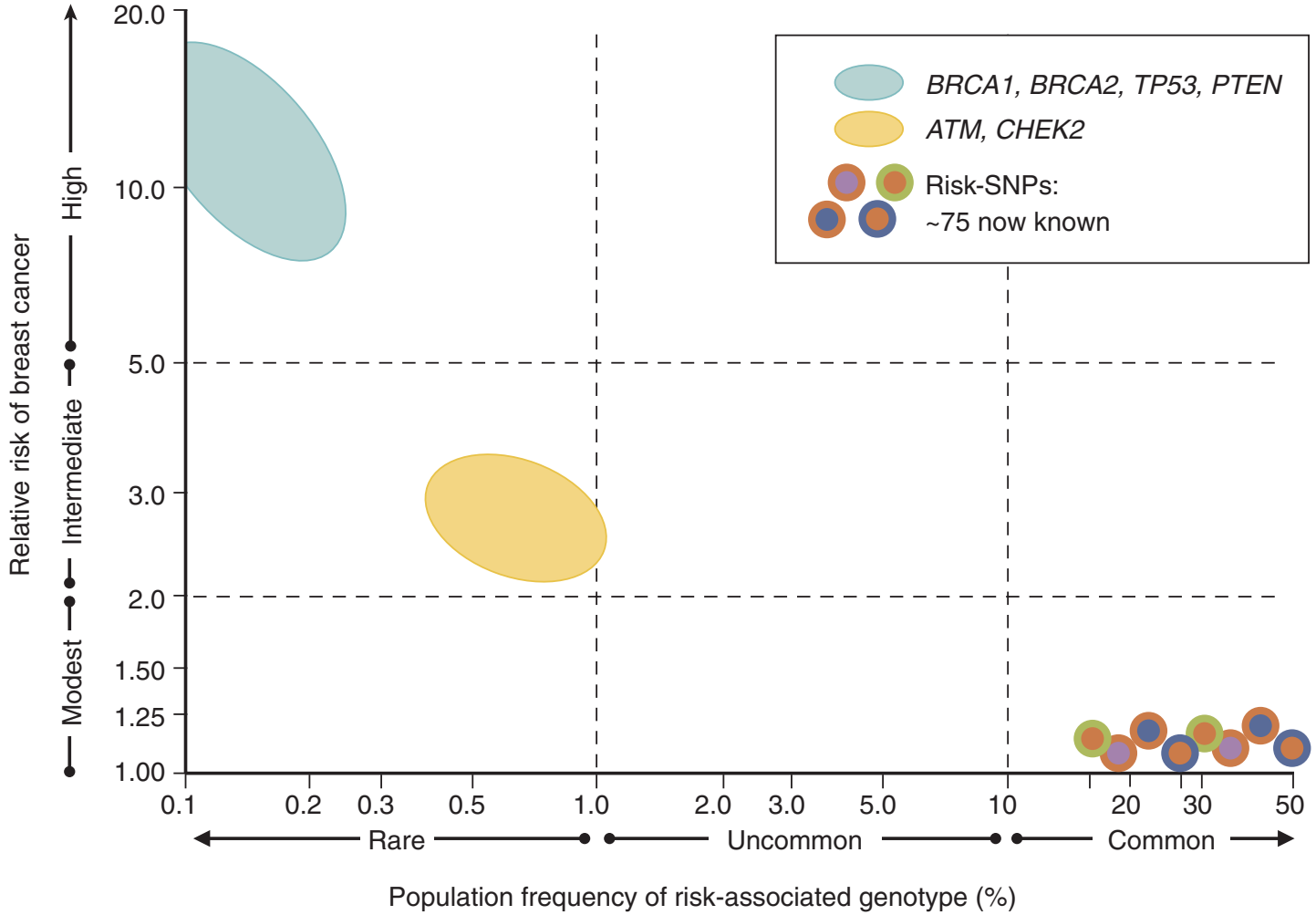


FIG 11-11 The relationship between the relative risk of breast cancer due to a cancer-associated variant (*y*-axis) and the frequency of the variant in the population (*x*-axis). Inherited disease-causing variants in *BRCA1* and *BRCA2* are collectively rare in the population, but they confer a large increase in risk among those who have the variants. Risk alleles in *ATM* and *CHEK2* have higher frequencies, but they confer only a two- to threefold increase in risk. Dozens of common SNP alleles are associated with an increase in breast cancer risk, but they each confer an additional risk of only 5% to 20%. (Courtesy Sean Tavtigian, PhD, Huntsman Cancer Institute, University of Utah Health Sciences Center.)

- Most studies indicate that the clinical course of breast cancer among patients with *BRCA1* or *BRCA2* mutations is not substantially different from that of other breast cancer patients.

- However, the substantial increase in risk of ovarian cancer (which has a high mortality rate and is difficult to detect early), has led to the recommendation that women who have a causal *BRCA1* or *BRCA2* mutation should undergo a prophylactic oophorectomy after they have completed their child-bearing years. This reduces ovarian cancer risk by about 90%, and because it reduces estrogen levels, it reduces breast cancer risk by about 50%.

- Bilateral prophylactic mastectomy, an option chosen by some *BRCA1* and *BRCA2* mutation carriers, reduces the risk of breast cancer by approximately 90%.

Familial Melanoma

- Largely as a result of increased exposure to ultraviolet radiation, the incidence of melanoma has increased approximately 20-fold in the United States during the past 70 years.
- It is now one of the most common cancers, with 76,000 new cases per year.
- The risk of developing melanoma increases by a factor of 2 when a first-degree relative is affected. The risk increases further, to approximately sixfold, when the first-degree relative is affected before 50 years of age.
- It is estimated that approximately 10% of melanoma cases occur in inherited, familial forms.

- Germline mutations in the *CDKN2A* gene are estimated to be involved in 20% to 40% of familial melanoma cases. *CDKN2A* encodes two different proteins, both of which are important components of the cell cycle.
- The first protein, p16, is a cyclin-dependent kinase inhibitor which interacts negatively with a cyclin-dependent kinase (CDK4) that phosphorylates and down-regulates the pRb protein (see Fig. 11-5).

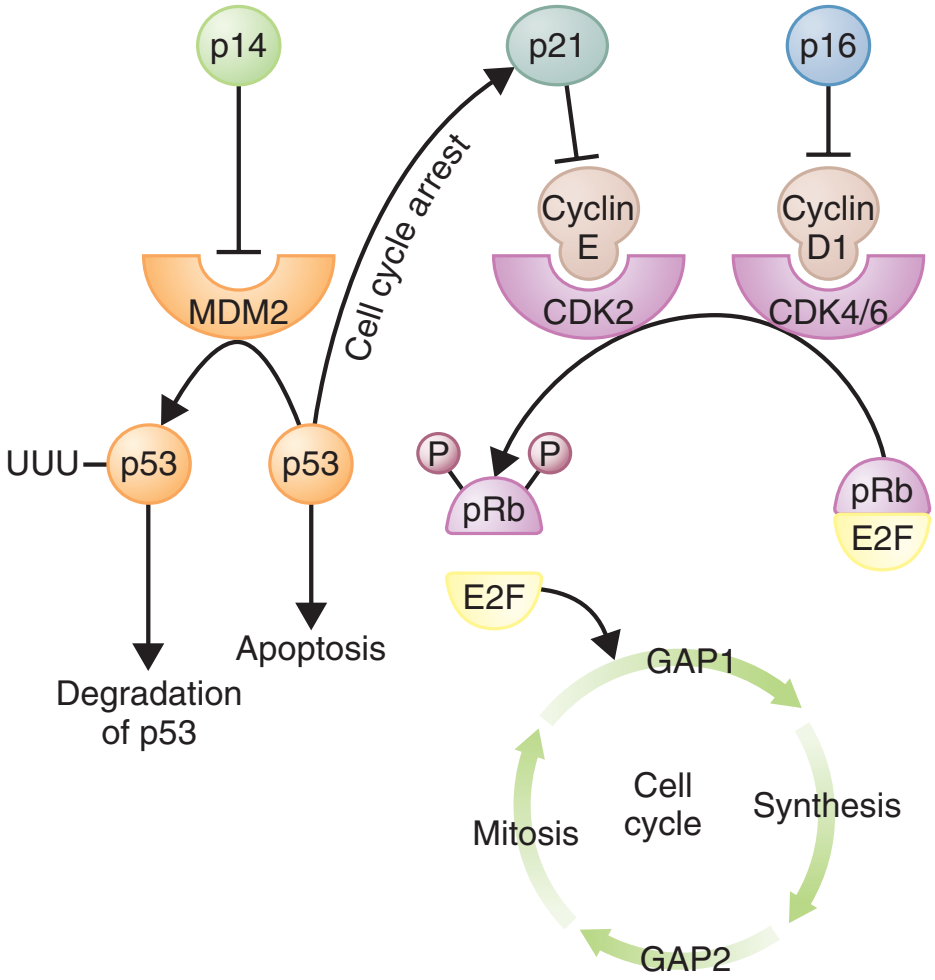


FIG 11-5 Regulation of the cell cycle is accomplished by a complex series of interactions among activators and repressors of the cycle. pRb acts as a master brake on the cell cycle by binding the E2F transcription complex, halting the cycle before S phase begins. The cyclin D–CDK4 complex inactivates pRb by phosphorylating it, thereby releasing the E2F complex and allowing the cell to progress through S phase. CDK inhibitors such as p16 and p21 inactivate CDKs, thus acting as another brake on the cycle. p53, acting through p21, can either halt the cell cycle or induce apoptosis in response to DNA damage. *CDK,* Cyclin-dependent kinase.

- Inherited mutations in the gene that encodes CDK4 can also result in familial melanoma. These rare gain-of-function mutations convert the cyclin-dependent kinase from a proto-oncogene to an activated oncogene. The activated CDK4 down-regulates pRb, resulting again in a lack of cell cycle control and tumor formation.

- Melanoma provides an example in which the same tumor type can result from either the activation of a proto-oncogene *(CDK4)* or the loss of a tumor suppressor gene *(CDKN2A).*

- *CDKN2A* plays a role not only in familial melanoma but also in most sporadic melanomas, in which somatic loss-of-function mutations of this gene lead to inactivation of the p16 tumor suppressor protein.
- About 50% of sporadic melanomas contain somatic deletions of *CDKN2A,* and loss-of-function point mutations are seen in another 5% to 10% of melanomas.

- **Hypermethylation** of the promoter region of *CDKN2A,* which down-regulates the gene, is seen in 1/4 to 3/4 of all melanomas.

- Somatic mutations in other genes are also seen in sporadic melanomas. Approximately half of these tumors contain somatic gain-of-function mutations in *BRAF,* a gene that encodes a kinase involved in the RAS signal-transduction pathway (drugs that inhibit BRAF are now being used to treat melanoma).

- In addition, one of the RAS genes, *NRAS,* is mutated in 15% to 30% of sporadic melanomas. Approximately 10% of melanomas have somatic *TP53* mutations, and about 6% have somatic *RB1* mutations (persons who inherit an *RB1* mutation also have an increased risk of melanoma).

IS GENETIC INHERITANCE IMPORTANT IN COMMON CANCERS?

- The term "**common cancers**" is often used to designate those cancers, such as carcinomas of the breast, colon, or prostate, that are not usually part of an inherited cancer syndrome (e.g., Li–Fraumeni syndrome or familial adenomatous polyposis).

- Recall that germline mutations in genes such as *BRCA1* or *APC,* although very important in aiding our understanding of the basis of carcinogenesis, are responsible for only a small proportion of breast or colon cancer cases.

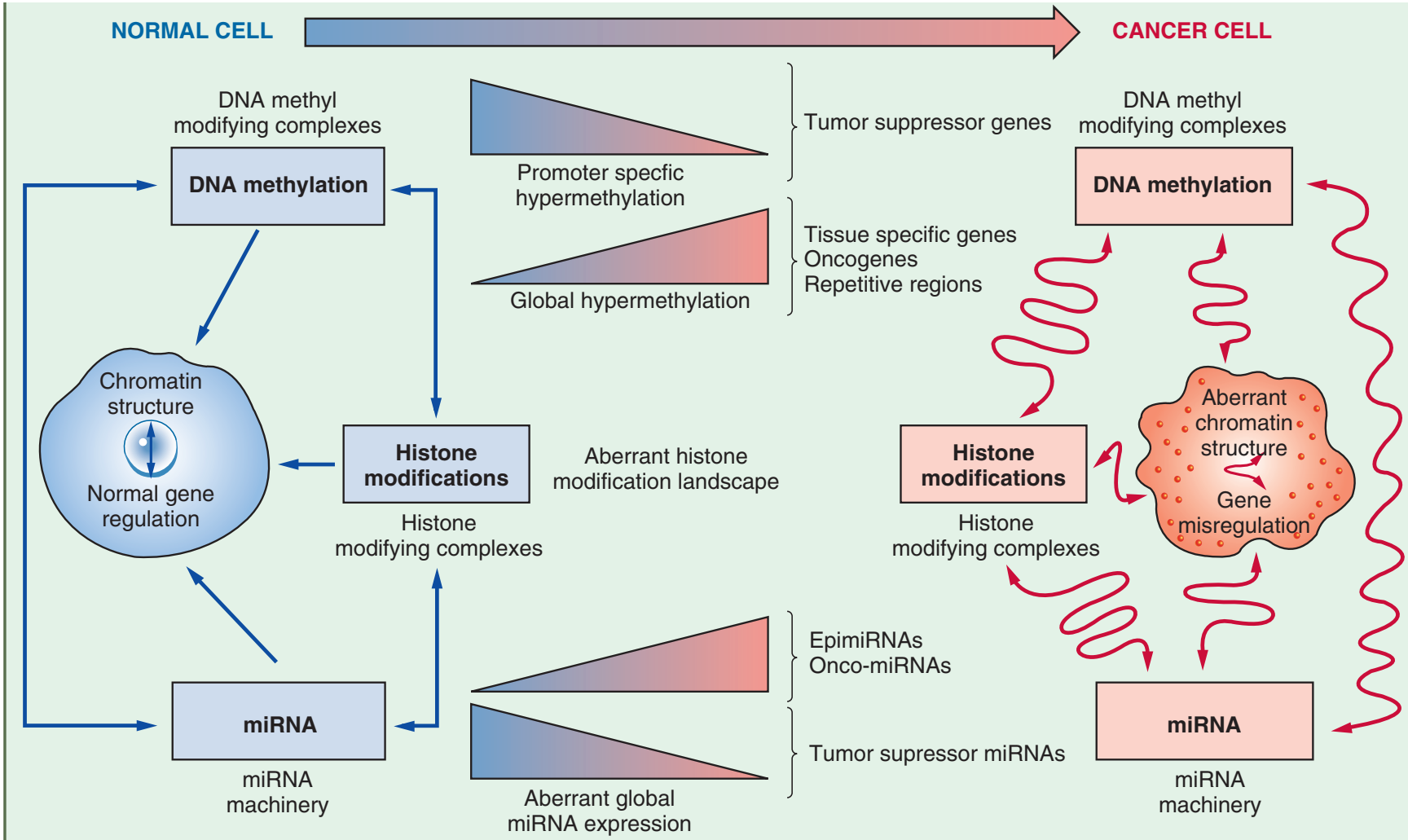
- Yet these cancers do cluster in families. Typically, the presence of one affected first-degree relative increases one's risk of developing a common cancer by a factor of two or more. It is likely that additional genes, as well as nongenetic factors that are shared in families, contribute to this increased familial risk

BOX 11-1 Epigenetics and Cancer

Epigenetics is defined as the study of changes in gene expression or phenotype caused by mechanisms other than variation in DNA sequences. Examples of epigenetic changes include methylation, histone modification, and mRNA-binding by microRNAs. As discussed in Chapter 2, methylation of a gene's promoter region, along with histone hypoacetylation, are associated with chromatin condensation, which inhibits the binding of transcription factors to a gene's promoter. Consequently, expression of the gene (i.e., transcription to mRNA) is reduced. Methylation and histone modification are involved in the processes of genomic imprinting (see Chapter 5) and X inactivation (see Chapter 6). Epigenetic alterations can cause persons with the same DNA sequences (i.e., identical twins) to have quite different disease profiles.

Epigenetic alterations are important in cancer because they can alter the expression of many cancer-associated genes. Tumor cells typically exhibit widespread hypomethylation (decreased methylation), which can increase the activity of oncogenes. Hypomethylation increases as tumors progress from benign neoplasms to malignancy. In addition, the promoter regions of tumor-suppressor genes (e.g., *RB1* and *BRCA1*) are often hypermethylated, which decreases their rate of transcription and their ability to inhibit tumor formation. Hypermethylation also is seen in specific subgroups of microRNA genes. When these microRNA genes are methylated, their mRNA targets are over-expressed, and this overexpression has been associated with tumorigenesis.

Except through gene therapy (see Chapter 13), mutations in DNA sequences cannot be altered. Epigenetic modifications, however, can be reversed through the administration of therapeutic drugs. For example, 5-azacytidine, a demethylating agent, has been used to treat leukemia and myelodysplastic syndrome. Another class of drugs, histone deacetylase (HDAC) inhibitors, counteracts the histone hypoacetylation that can silence the activity of tumor-suppressor genes. HDAC inhibitors have been used in the treatment of T-cell lymphomas. A challenge in developing drugs that modulate epigenetic alterations is to target only the genes responsible for a specific cancer.



Global epigenomic alterations and cancer. Oncogenesis involves accumulated genetic alterations combined with the epigenetic changes: DNA methylation, histone modifications, and miRNAs. In cancer cells, tumor-suppressor genes become hypermethylated and with histone modifications cause abnormal gene silencing. The gene silencing of tumor-suppressor genes results in tumor progression. Global hypomethylation leads to chromosomal instability and fragility. Additionally, these modifications create abnormal mRNA and miRNA expression, which leads to activation of oncogenes and silencing of tumor-suppressor genes. (From McCance KL, Huether