Oncogenes

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Since the early proposals of Boveri more than a century ago, much experimental evidence has confirmed that, at the molecular level, cancer is a result of lesions in the cellular deoxyribonucleic acid (DN)A. First, it has been observed that a cancer cell transmits to its daughter cells the phenotypic features characterizing the cancerous state. Second, most of the recognized mutagenic compounds are also carcinogenic, having as a target cellular DNA. Finally, the karyotyping of several types of human tumors, particularly those belonging to the hematopoietic system, led to the identification of recurrent qualitative and numerical chromosomal aberrations, reflecting pathologic re-arrangements of the cellular genome. Taken together, these observations suggest that the molecular pathogenesis of human cancer is due to structural and/or functional alterations of specific genes whose normal function is to control cellular growth and differentiation or, in different terms, cell birth and cell death.[1,2]

The identification and characterization of the genetic elements playing a role in the scenario of human cancer pathogenesis have been made possible by the development of DNA recombinant techniques during the last two decades. One milestone was the use of the DNA transfection technique that helped clarify the cellular origin of the "viral oncogenes." The latter were previously characterized as the specific genetic elements capable of conferring the tumorigenic properties to the ribonucleic acid (RNA) tumor viruses also known as retroviruses.[3,4] Furthermore, the transfection technique led to the identification of cellular transforming genes that do not have a viral counterpart. Besides the source of their original identification, viral or cellular genome, these transforming genetic elements have been designated as protooncogenes in their normal physiologic version and oncogenes when altered in cancer.[5,6] A second relevant experimental approach has regarded the identification and characterization of clonal and recurrent cytogenetic abnormalities in cancer cells, especially those derived from the hematopoietic system. Several oncogenes have been thus defined by molecular cloning of the chromosomal breakpoints, including translocations and inversions. Additional oncogenes have been identified through the analysis of chromosomal regions anomalously stained (homogeneously staining regions), representing gene amplification. Finally, the detection of chromosome deletions has been instrumental in the process of identification and cloning of a second class of cancer-associated genes, the tumor suppressors. Contrary to the oncogenes that are activated by dominant mutations and whose activity is to promote cell growth, tumor suppressors act in the normal cell as negative controllers of cell growth and are inactive in tumor cells. In general, therefore, the mutations inactivating tumor suppressor genes are of the recessive type.[7]

Recently, a third class of cancer-associated genes has been defined thanks to the analysis of tumors of a particular type; that is, tumors in which an inherited mutated predisposing gene plays a significant role. These tumors include cancers in patients suffering from hereditary nonpolyposis colorectal cancer syndromes.

The genes implicated in these tumors have been defined as mutator genes or genes involved in the DNA-mismatch repair process. Although not directly involved in the carcinogenesis process, these genes, when inactivated, expose the cells to a very high mutagenic load that eventually may involve the activation of oncogenes and the inactivation of tumor suppressors.[8]

In this chapter, the methods by which oncogenes were discovered will be first described. The various functions of cellular protooncogenes will then be presented, and the genetic mechanisms of protooncogene activation will be summarized. Finally, the role of specific oncogenes in the initiation and progression of human tumors will be discussed.

DISCOVERY AND IDENTIFICATION OF ONCOGENES

The first oncogenes were discovered through the study of retroviruses, RNA tumor viruses whose genomes are reverse-transcribed into DNA in infected animal cells.[9] During the course of infection, retroviral DNA is inserted into the chromosomes of host cells. The integrated retroviral DNA, called the provirus, replicates along with the cellular DNA of the host.[10] Transcription of the DNA provirus leads to the production of viral progeny that bud through the host cell membrane to infect other cells. Two categories of retroviruses are classified by their time course of tumor formation in experimental animals. Acutely transforming retroviruses can rapidly cause tumors within days after injection. These retroviruses can also transform cell cultures to the neoplastic phenotype. Chronic or weakly oncogenic retroviruses can cause tissue-specific tumors in susceptible strains of experimental animals after a latency period of many months. Although weakly oncogenic retroviruses can replicate in vitro, these viruses do not transform cells in culture.

Retroviral oncogenes are altered versions of host cellular protooncogenes that have been incorporated into the retroviral genome by recombination with host DNA, a process known as retroviral transduction.[11] This surprising discovery was made through study of the Rous sarcoma virus (RSV) (Figure 6-1). RSV is an acutely transforming retrovirus first isolated from a chicken sarcoma over 80 years ago by Peyton Rous.[12] Studies of RSV mutants in the early 1970s revealed that the transforming gene of RSV was not required for viral replication.[13–15] Molecular hybridization studies then showed that the RSV transforming gene (designated v-*src*) was homologous to a host cellular gene (c-*src*) that was widely conserved in eukaryotic species.[16] Studies of many other acutely transforming retroviruses from fowl, rodent, feline, and nonhuman primate species have led to the discovery of dozens of different retroviral oncogenes (see below and Table 6-1). In every case, these retroviral oncogenes are derived from normal cellular genes captured from the genome of the host. Viral oncogenes are responsible for the rapid tumor formation and efficient in vitro transformation activity characteristic of acutely transforming retroviruses.

In contrast to acutely transforming retroviruses, weakly oncogenic retroviruses do not carry viral oncogenes. These retroviruses, which include mouse mammary tumor virus (MMTV) and various animal leukemia viruses, induce tumors by a process called insertional mutagenesis (Figure 6-2).[8] This process results from integration of the DNA provirus into the host genome in infected cells. In rare cells, the provirus inserts near a protooncogene. Expression of the protooncogene is then abnormally driven by the transcriptional regulatory elements contained within the long terminal repeats of the provirus.[17,18] In these cases, proviral integration represents a mutagenic event that activates a protooncogene. Activation

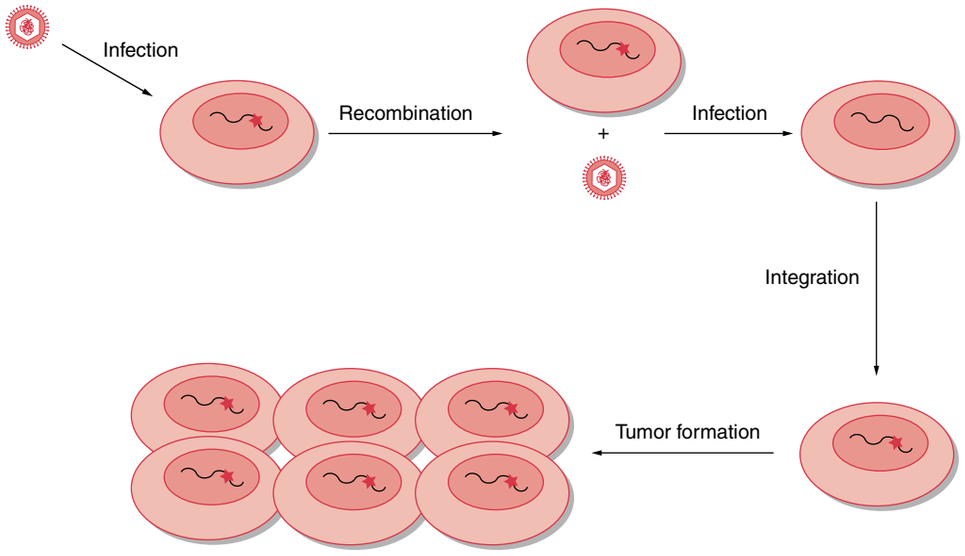

Figure 6-1 Retroviral transduction. A ribonucleic acid (RNA) tumor virus infects a human cell carrying an activated *src* gene (red star). After the process of recombination between retroviral genome and host deoxyribonucleic acid (DNA), the oncogene *c-src* is incorporated into the retroviral genome and is renamed *v-src*. When the retrovirus carrying *v-src* infects a human cell, the viral oncogene is rapidly transcribed and is responsible for the rapid tumor formation.

of the protooncogene then results in transformation of the cell, which can grow clonally into a tumor. The long latent period of tumor formation of weakly oncogenic retroviruses is therefore due to the rarity of the provirus insertional event that leads to tumor development from a single transformed cell. Insertional mutagenesis by weakly oncogenic retroviruses, first demonstrated in bursal lymphomas of chickens, frequently involves the same oncogenes (such as *myc*, *myb*, and *erb B*) that are carried by acutely transforming retroviruses.[19–21] In many cases, however, insertional mutagenesis has been used as a tool to identify new oncogenes, including *int-1*, *int-2*, *pim-1*, and *lck*.[22]

The demonstration of activated protooncogenes in human tumors was first shown by the DNA-mediated transformation technique.[23,24] This technique, also called gene transfer or transfection assay, verifies the ability of donor DNA from a tumor to transform a recipient strain of rodent cells called NIH 3T3, an immortalized mouse cell line (Figure 6-3).[25,26] This sensitive assay, which can detect the presence of single-copy oncogenes in a tumor sample, also enables the isolation of the transforming oncogene by molecular cloning techniques. After serial growth of the transformed NIH 3T3 cells, the human tumor oncogene can be cloned by its association with human repetitive DNA sequences. The first human oncogene isolated by the gene transfer technique was derived from a bladder carcinoma.[27,28] Overall, approximately 20% of individual human tumors have been shown to induce transformation of NIH 3T3 cells in gene-transfer assays. The value of transfection assay was recently reinforced by the laboratory of Robert Weinberg, which showed that the ectopic expression of the telomerase catalytic subunit (hTERT), in combination with the simian virus 40 large T product and a mutated oncogenic H-*ras* protein, resulted in the direct tumorigenic conversion of normal human epithelial and fibroblast cells.[29] Many of the oncogenes identified by gene-transfer studies are identical or closely related to those oncogenes transduced by retroviruses. Most prominent among these are members of the *ras* family that have been repeatedly isolated from various human tumors by gene transfer.[30,31] A number of new oncogenes (such as *neu*, *met*, and *trk*) have also been identified by the gene-transfer technique.[32,33] In many cases, however, oncogenes identified by gene transfer were shown to be activated by rearrangement during the experimental procedure and are not activated in the human tumors that served as the source of the donor DNA, as in the case of *ret* that was subsequently found genuinely rearranged and activated in papillary thyroid carcinomas.[34–36]

Chromosomal translocations have served as guideposts for the discovery of many new oncogenes.[37,38] Consistently recurring karyotypic abnormalities are found in many hematologic and solid tumors. These abnormalities include chromosomal rearrangements as well as the gain or loss of whole chromosomes or chromosome segments. The first consistent karyotypic abnormality identified in a human neoplasm was a characteristic small chromosome in the cells of patients with chronic myelogenous leukemia.[39] Later identified as a derivative of chromosome 22, this abnormality was designated the Philadelphia chromosome, after its city of discovery. The application of chromosome banding techniques in the early 1970s enabled the precise cytogenetic characterization of many chromosomal translocations in human leukemia, lymphoma, and solid tumors.[40] The subsequent development of molecular cloning techniques then enabled the identification of protooncogenes at or near chromosomal breakpoints in various neoplasms. Some of these protooncogenes, such as *myc* and *abl*, had been previously identified as retroviral oncogenes. In general, however, the cloning of chromosomal breakpoints has served as a rich source of discovery of new oncogenes involved in human cancer.

ONCOGENES, PROTOONCOGENES, AND THEIR FUNCTIONS Protooncogenes encode proteins that are involved in the control of cell growth. Alteration of the structure and/or expression of protooncogenes can activate them to become oncogenes capable of inducing in susceptible cells the neoplastic phenotype. Oncogenes can be classified into five groups based on the functional and biochemical properties of protein products of their normal counterparts (protooncogenes). These groups are (1) growth factors, (2) growth factor receptors, (3) signal transducers, (4) transcription factors, and (5) others, including programmed cell death regulators. Table 6-1 lists examples of oncogenes according to their functional categories.

GROWTH FACTORS Growth factors are secreted polypeptides that function as extracellular signals to stimulate the proliferation of target cells.[41,42] Appropriate target cells must possess a specific receptor in order to respond to a specific type of growth factor. A well-characterized example is platelet-derived growth factor (PDGF), an approximately 30 kDa protein consisting of two polypeptide chains.[43] PDGF is released from platelets during the process of blood coagulation. PDGF stimulates the proliferation of fibroblasts, a cell growth process that plays an important role in wound healing. Other well-characterized examples of growth factors include nerve growth factor, epidermal growth factor, and fibroblast growth factor.

The link between growth factors and retroviral oncogenes was revealed by study of the *sis* oncogene of simian sarcoma virus, a retrovirus first isolated from a monkey fibrosarcoma. Sequence analysis showed that *sis* encodes the beta chain of PDGF.[44] This discovery established the principle that inappropriately expressed growth factors could function as oncogenes. Experiments demonstrated that the constitutive expression of the *sis* gene product (PDGF-β) was sufficient to cause neoplastic transformation of fibroblasts but not of cells that lacked the receptor for PDGF.[45] Thus, transformation by *sis* requires interaction of the *sis* gene product with the PDGF receptor. The mechanism by which a growth factor affects the same cell that produces it is called autocrine stimulation .[46] The constitutive expression of the *sis* gene product appears to cause neoplastic transformation by the mechanism of autocrine stimulation, resulting in self-sustained aberrant cell proliferation. This model, derived from experimental animal systems, has been recently demonstrated in a human tumor. Dermatofibrosarcoma protuberans (DP) is an

Table 6-1 Oncogenes

Oncogene	Chromosome	Method of Identification	Neoplasm	Mechanism of Activation	Protein Function
Growth factors					
v-*sis*	22q12.3–13.1	Sequence homology	Glioma/fibrosarcoma	Constitutive production	B-chain PDGF
int2	11q13	Proviral insertion	Mammary carcinoma	Constitutive production	Member of FGF family
KS3	11q13.3	DNA transfection	Kaposi sarcoma	Constitutive production	Member of FGF family
HST	11q13.3	DNA transfection	Stomach carcinoma	Constitutive production	Member of FGF family
Growth factor receptors					
Tyrosine kinases: integral membrane proteins					
EGFR	7p1.1–1.3	DNA amplification	Squamous cell carcinoma	Gene amplification/increased protein	EGF receptor
v-*fms*	5q33–34 (FMS)	Viral homolog	Sarcoma	Constitutive activation	CSF1 receptor
v-*kit*	4q11–21 (KIT)	Viral homolog	Sarcoma	Constitutive activation	Stem-cell factor receptor
v-*ros*	6q22 (ROS)	Viral homolog	Sarcoma	Constitutive activation	?
MET	7p31	DNA transfection	MNNG-treated human osteocarcinoma cell line	DNA rearrangment/ligand-independent constitutive activation (fusion proteins)	HGF/SF receptor
TRK	1q32–41	DNA transfection	Colon/thyroid carcinomas	DNA rearrangment/ligand-independent constitutive activation (fusion proteins)	NGF receptor
NEU	17q11.2–12	Point mutation/DNA amplification	Neuroblastoma/breast carcinoma	Gene amplification	?
RET	10q11.2	DNA transfection	Carcinomas of thyroid; MEN2A, MEN2B	DNA rearrangment/point mutation (ligand-independent constitutive activation/fusion proteins)	GDNF/NTT/ART/PSP receptor
Receptors lacking protein kinase activity					
mas	6q24–27	DNA transfection	Epidermoid carcinoma	Rearrangment of 5' noncoding region	Angiotensin receptor
Signal transducers					
Cytoplasmic tyrosine kinases					
SRC	20p12–13	Viral homolog	Colon carcinoma	Constitutive activation	Protein tyrosine kinase
v-*yes*	18q21–3 (YES)	Viral homolog	Sarcoma	Constitutive activation	Protein tyrosine kinase
v-*fgr*	1p36.1–36.2 (FGR)	Viral homolog	Sarcoma	Constitutive activation	Protein tyrosine kinase
v-f*es*	15q25–26 (FES)	Viral homolog	Sarcoma	Constitutive activation	Protein tyrosine kinase
ABL	9q34.1	Chromosome	CML	DNA rearrangment translocation (constitutive activation/fusion proteins)	Protein tyrosine kinase
Membrane-associated G proteins					
H-*RAS*	11p15.5	Viral homolog/ DNA transfection	Colon, lung, pancreas carcinmoas	Point mutation	GTPase
RAS	12p11.1–12.1	Viral homolog/ DNA transfection	AML, thyroid carcinoma, melanoma	Point mutation	GTPase
N-*RAS*	1p11–13	DNA transfection	Carcinoma, melanoma	Point mutation	GTPase
gsp	20	DNA sequencing	Adenomas of thyroid	Point mutation	Gs α
gip	3	DNA sequencing	Ovary, adrenal carcinoma	Point mutation	Gi α
GTPase exchange factor (GEF)					
Dbl	Xq27	DNA transfection	Diffuse B-cell lymphoma	DNA rearrangment	GEF for Rho and Cdc42Hs
Vav	19p13.2	DNA transfection	Hematopoietic cells	DNA rearrangment	GEF for Ras?
Serine/threonine kinases: cytoplasmic					
v-*mos*	8q11 (MOS)	Viral homolog	Sarcoma	Constitutive activation	Protein kinase (ser/thr)
v-*raf*	3p25 (RAF-1)	Viral homolog	Sarcoma	Constitutive activation	Protein kinase (ser/thr)
pim-1	6p21 (PIM-).	Insertional mutagenesis	T-cell lymphoma	Constitutive activation	Protein kinase (ser/thr)
Cytoplasmic regulators					
v-*crk*	17p13 (CRK)	Viral homolog		Constitutive tyrosine phosphorilation of cellular substrates (eg, paxillin)	SH-2/SH-3 adaptor
Trancription Factors					
v-*myc*	8q24.1 (MYC)	Viral homolog	Carcinoma, myelocytomatosis	Deregulated activity	Transcription factor
N-*MYC*	2p24	DNA amplification	Neuroblastoma; lung carcinoma	Deregulated activity	Transcription factor
L-*MYC*	1p32	DNA amplification	Carcinoma of lung	Deregulated activity	Transcription factor
v-*myb*	6q22–24	Viral homolog	Myeloblastosis	Deregulated activity	Transcription factor
v-*fos*	14q21–22	Viral homolog	Osteosarcoma	Deregulated activity	Transcription factor API
v-*jun*	p31–32	Viral homolog	Sarcoma	Deregulated activity	Transcription factor API
v-*ski*	1q22–24	Viral homolog	Carcinoma	Deregulated activity	Transcription factor
v-*rel*	2p12–14	Viral homolog	Lymphatic leukemia	Deregulated activity	Mutant NFKB
v-*ets-1*	11p23–q24	Viral homolog	Erythroblastosis	Deregulated activity	Transcription factor
v-*ets-2*	21q24.3	Viral homolog	Erythroblastosis	Deregulated activity	Transcription factor
v-*erbA1*	17p11–21	Viral homolog	Erythroblastosis	Deregulated activity	T3 Transcription factor
v-*erbA2*	3p22–24.1	Viral homolog	Erythroblastosis	Deregulated activity	T3 Transcription factor
Others					
BCL2	18q21.3	Chromosomal translocation	B-cell lymphomas	Constitutive activity	Antiapoptotic protein
MDM2	12q14	DNA amplification	Sarcomas	Gene amplification/increased protein	Complexes with p53

AML = acute myeloid leukemia; CML = chronic myelogenous leukemia; CSF = colony stimulating factor; DNA = deoxyribonucleic acid; EGF = epidermal growth factor; FGF = fibroblast growth factor; GTPase = guanosine triphosphatase; HGF = hepatocyte growth factor; NGF = nerve growth factor; PDGF = platelet-derived growth factor.

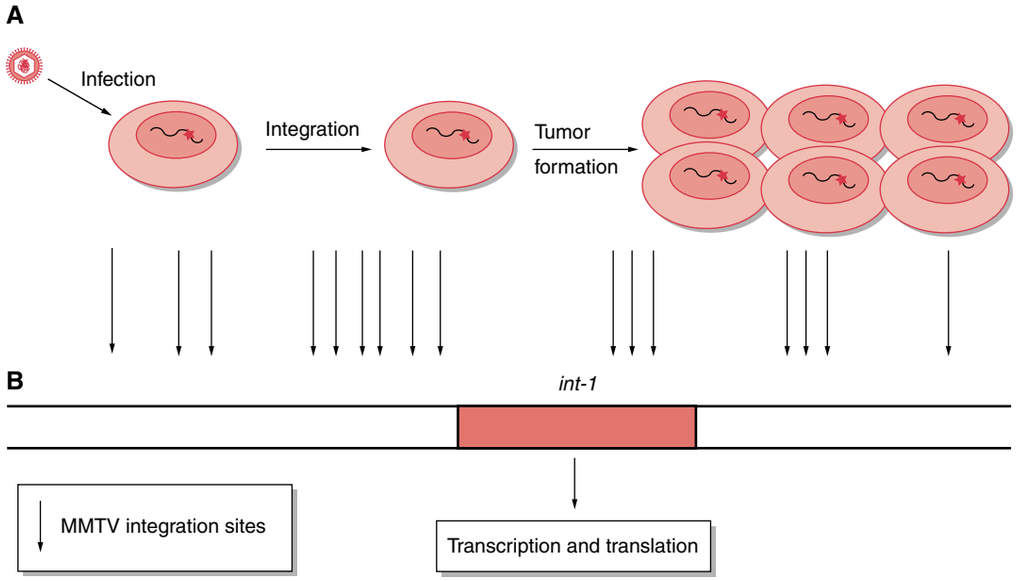

Figure 6-2 Insertional mutagenesis. **A,** The process is independent of genes carried by the retrovirus. Retrovirus, for example, mouse mammary tumor virus (MMTV), infects a human cell. The proviral deoxyribonucleic acid (DNA) is integrated into the host genome in infected cells. Rarely, the provirus inserts near a protooncogene (eg, *int-1*) and activates the protosoncogene. Activated protooncogene results in cell transformation and in tumor formation. ***B*,** Sites of integration of MMTV retrovirus near the protooncogene *int-1*. All sites determine *int-1* activation.

infiltrative skin tumor that was demonstrated to present specific cytogenetic features: reciprocal translocation and supernumerary ring chromosomes, involving chromosomes 17 and 22.[47,48] Molecular cloning of the breakpoints revealed a fusion between the collagen type Ia1 (*COL1A1*) gene and PDGF-β gene. The fusion gene resulted in a deletion of PDGF-β exon 1 and a constitutive release of this growth factor.[49] Subsequent experiments of gene transfer of DPs genomic DNA into NIH 3T3 cells directly demonstrated the occurrence of an autocrine mechanism by the human rearranged PDGF-*b* gene involving the activation of the endogenous PDGF receptor.[50,51] Another example of a growth factor that can function as an oncogene is *int-2*, a member of the fibroblast growth factor family. *Int-2* is sometimes activated in mouse mammary carcinomas by MMTV insertional mutagenesis.[52]

Growth Factor Receptors Some viral oncogenes are altered versions of normal growth factor receptors that possess intrinsic tyrosine kinase activity.[53] Receptor tyrosine kinases, as these growth factor receptors are collectively known, have a characteristic protein structure consisting of three principal domains: (1) the extracellular ligand-binding domain, (2) the transmembrane domain, and (3) the intracellular tyrosine kinase catalytic domain (see Figure 6-2). Growth factor receptors are molecular machines that transmit information in a unidirectional fashion across the cell membrane. The binding of a growth factor to the extracellular ligand-binding domain of the receptor results in the activation of the intracellular tyrosine kinase catalytic domain. The recruitment and phosphorylation of specific cytoplasmic proteins by the activated receptor then trigger a series of biochemical events generally leading to cell division.

Because of the role of growth factor receptors in the regulation of normal cell growth, it is not surprising that these receptors constitute an important class of protooncogenes. Examples include *erb B*, *erb B-2*, *fms*, *kit*, *met*, *ret*, *ros*, and *trk*. Mutation or abnormal expression of growth factor receptors can convert them into oncogenes.[54] For example, deletion of the ligand-binding domain of *erb B* (the epidermal growth factor receptor) is thought to result in constitutive activation of the receptor in the absence of ligand binding.[55] Point mutation in the tyrosine kinase domain or of the extracellular domain and deletion of intracellular regulatory domains can also result in the constitutive activation of receptor tyrosine kinases. Increased expression through gene amplification and abnormal expression in the wrong cell type are additional mechanisms through which growth factor receptors may be involved in neoplasia. The identification and study of altered growth factor receptors in experimental models of neoplasia have contributed much to our understanding of the normal regulation of cell proliferation.

Signal Transducers Mitogenic signals are transmitted from growth factor receptors on the cell surface to the cell nucleus through a series of complex interlocking pathways collectively referred to as the signal transduction cascade.[56] This relay of information is accomplished in part by the stepwise phosphorylation of interacting proteins in the cytosol. Signal transduction also involves guanine nucleotide-binding proteins and second messengers such as the adenylate cyclase system.[57] The first retroviral oncogene discovered, *src*, was subsequently shown to be involved in signal transduction.

Many protooncogenes are members of signal transduction pathways.[58,59] These consist of two main groups: nonreceptor protein kinases and guanosine triphosphate (GTP)-binding proteins. The nonreceptor protein kinases are subclassified into tyrosine kinases (eg, *abl*, *lck*, and *src*) and serine/threonine kinases (eg, *raf-1*, *mos*, and *pim-1*). GTP-binding proteins with intrinsic GTPase activity are subdivided into monomeric and heterotrimeric groups.[60] Monomeric GTP-binding proteins are members of the important *ras* family of protooncogenes that includes H-*ras*, K-*ras*, and N-*ras*.[61] Heterotrimeric GTP-binding proteins (G proteins) implicated as protooncogenes currently include *gsp* and *gip*. Signal transducers are often converted to oncogenes by mutations that lead to their unregulated activity, which in turn leads to uncontrolled cellular proliferation.[62]

Transcription Factors Transcription factors are nuclear proteins that regulate the expression of target genes or gene families.[63] Transcriptional regulation is mediated by protein binding to specific DNA sequences or DNA structural motifs, usually located upstream of the target gene. Transcription factors often belong to multigene families that share common DNA-binding domains such as zinc fingers. The mechanism of action of transcription factors also involves binding to other proteins, sometimes in heterodimeric complexes with specific partners. Transcription factors are the final link in the signal transduction pathway that converts extracellular signals into modulated changes in gene expression.

Many protooncogenes are transcription factors that were discovered through their retroviral homologs.[64] Examples include *erb A*, *ets*, *fos*, *jun*, *myb*, and c-*myc*. Together, *fos* and *jun* form the AP-1 transcription factor, which positively regulates a number of target genes whose expression leads to cell division.[65,66] *Erb A* is the receptor for the T3 thyroid hormone, triiodothyronine.[67] Protooncogenes that function as transcription factors are often activated by chromosomal translocations in hematologic and solid neoplasms.[68] In certain types of sarcomas, chromosomal translocations cause the formation of fusion proteins involving the association of *EWS* gene with various partners and resulting in an aberrant tumor-associated transcriptional activity. Interestingly, a role of the adenovirus *E1A* gene in promoting the formation of fusion transcript fli1/ews in normal human fibroblasts was recently reported.[69] An important example of a protooncogene with a transcriptional activity in human hematologic tumors is the c-*myc* gene, which helps to control the expression of genes leading to cell proliferation.[70] As will be discussed later in this chapter, the c-*myc* gene is frequently activated by chromosomal translocations in human leukemia and lymphoma.

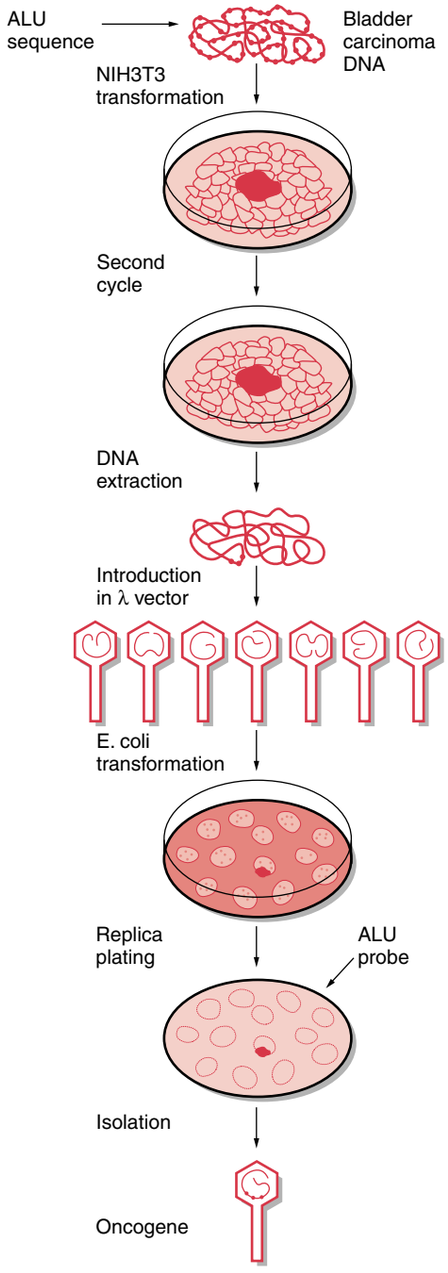

Figure 6-3 Transfection assay. Deoxyribonucleic acid (DNA) from a tumor (eg, bladder carcinoma) was used to transform a rodent immortalized cell line (NIH 3T3). After serial cycles, DNA from transformed cells was extracted and then inserted into λ vector, which was subsequently used to transform an appropriate *Escherichia coli* strain. Using a specific probe (*ALU*), it was possible to isolate and then characterize the involved human oncogene.

PROGRAMMED CELL DEATH REGULATION Normal tissues exhibit a regulated balance between cell proliferation and cell death. Programmed cell death is an important component in the processes of normal embryogenesis and organ development. A distinctive type of programmed cell death, called apoptosis, has been described for mature tissues.[71] This process is characterized morphologically by blebbing of the plasma membrane, volume contraction, condensation of the cell nucleus, and cleavage of genomic DNA by endogenous nucleases into nucleosome-sized fragments. Apoptosis can be triggered in mature cells by external stimuli such as steroids and radiation exposure. Studies of cancer cells have shown that both uncontrolled cell proliferation and failure to undergo programmed cell death can contribute to neoplasia and insensitivity to anticancer treatments.

The only protooncogene thus far shown to regulate programmed cell death is *bcl-2*. *Bcl-2* was discovered by the study of chromosomal translocations in human lymphoma.[72,73] Experimental studies show that *bcl-2* activation inhibits programmed cell death in lymphoid cell populations.[74] The dominant mode of action of activated *bcl-2* classifies it as an oncogene. The *bcl-2* gene encodes a protein localized to the inner mitochondrial membrane, endoplasmic reticulum, and nuclear membrane. The mechanism of action of the *bcl-2* protein has not been fully elucidated, but studies indicate that it functions in part as an antioxidant that inhibits lipid peroxidation of cell membranes.[75] The normal function of *bcl*-2 requires interaction with other proteins, such as *bax*, also thought to be involved in the regulation of programmed cell death (Figure 6-4). It is unlikely that *bcl-2* is the only apoptosis gene involved in neoplasia although additional protooncogenes await identification.

MECHANISMS OF ONCOGENE ACTIVATION

The activation of oncogenes involves genetic changes to cellular protooncogenes. The consequence of these genetic alterations is to confer a growth advantage to the cell. Three genetic mechanisms activate oncogenes in human neoplasms: (1) mutation, (2) gene amplification, and (3) chromosome rearrangements. These mechanisms result in either an alteration of protooncogene structure or an increase in protooncogene expression (Figure 6-5). Because neoplasia is a multistep process, more than one of these mechanisms often contribute to the genesis of human tumors by altering a number of cancer-associated genes. Full expression of the neoplastic phenotype, including the capacity for metastasis, usually involves a combination of protooncogene activation and tumor suppressor gene loss or inactivation.

MUTATION Mutations activate protooncogenes through structural alterations in their encoded proteins. These alterations, which usually involve critical protein regulatory regions, often lead to the uncontrolled, continuous activity of the mutated protein. Various types of mutations, such as base substitutions, deletions, and insertions, are capable of activating protooncogenes.[76] Retroviral oncogenes, for example, often have deletions that contribute to their activation. Examples include deletions in the amino-terminal ligand-binding domains of the *erb B*, *kit*, *ros*, *met*, and *trk* oncogenes.[6] In human tumors, however, most characterized oncogene mutations are base substitutions (point mutations) that change a single amino acid within the protein.

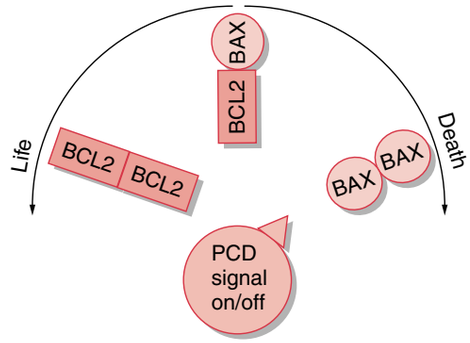

Figure 6-4 Effect of *bcl-2* activity on the control of the cell life. In the presence of *BAX* only, the cell goes to apoptosis; *bcl-2* regulates the cycle of the cell by the interaction with *BAX*. When *bcl-2* is overexpressed, the cell cycle is deregulated and the apoptosis is prevented, eventually leading to tumor formation. This is an important cause for tumor formation. PCD = programmed cell death or (apoptosis).

Point mutations are frequently detected in the *ras* family of protooncogenes (K-*ras*, H-*ras*, and N-*ras*).[77] It has been estimated that as many as 15% to 20% of unselected human tumors may contain a *ras* mutation. Mutations in K-*ras* predominate in carcinomas. Studies have found K-*ras* mutations in about 30% of lung adenocarcinomas, 50% of colon carcinomas, and 90% of carcinomas of the pancreas.[78] N-*ras* mutations are preferentially found in hematologic malignancies, with up to a 25% incidence

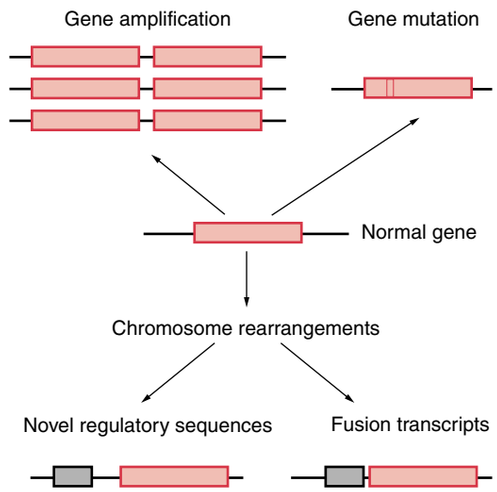

Figure 6-5 Schematic representation of the main mechanisms of oncogene activation (from protooncogenes to oncogenes). The normal gene (protooncogene) is depicted with its transcibed portion (rectangle). In the case of gene amplification, the latter can be duplicated 100-fold, resulting in an excess of normal protein. A similar situation can occur when following chromosome rearrangements such as translocation, the transcription of the gene is now regulated by novel regulatory sequences belonging to another gene. In the case of point mutation, single aminoacid substitutions can alter the biochemical properties of the gene product, causing, in the example, its constitutive enzymatic activation. Chromosome rearrangements, such as translocation and inversion, can then generate fusion transcripts resulting in chimeric oncogenic proteins.

in acute myeloid leukemias and myelodysplastic syndromes.[79,80] The majority of thyroid carcinomas have been found to have *ras* mutations distributed among K-*ras*, H-*ras*, and N-*ras*, without preference for a single *ras* family member but showing an association with the follicular type of differentiated thyroid carcinomas.[81,82] The majority of *ras* mutations involve codon 12 of the gene, with a smaller number involving other regions such as codons 13 or 61.[83] *Ras* mutations in human tumors have been linked to carcinogen exposure. The consequence of *ras* mutations is the constitutive activation of the signal-transducing function of the *ras* protein.

Another significant example of activating point mutations is represented by those affecting the *ret* protooncogene in multiple endocrine neoplasia type 2A syndrome (MEN2A).

Germline point mutations affecting one of the cysteines located in the juxtamembrane domain of the *ret* receptor have been found to confer an oncogenic potential to the latter as a consequence of the ligand-independent activation of the tyrosine kinase activity of the receptor. Experimental evidences have pointed out that these mutations involving cysteine residues promote *ret* homodimerization via the formation of intermolecular disulfide bonding, most likely as a result of an unpaired number of cysteine residues.[84,85]

Table 6-2 Oncogene Amplification in Human Cancers

Tumor Type	Gene Amplified	Percentage
Neuroblastoma	*MYCN*	20–25
Small-cell lung cancer	*MYC*	15–20
Glioblastoma	*ERB B-1* (EGFR)	33–50
Breast cancer	*MYC*	20
	ERB B-2 (EGFR2)	~20
	FGFR1	12
	FGFR2	12
	CCND1 (cyclin D1)	15–20
Esophageal cancer	*MYC*	38
	CCND1 (cyclin D1)	25
Gastric cancer	K-*RAS*	10
	CCNE (cyclin E)	15
Hepatocellular cancer	*CCND1* (cyclin D1)	13
Sarcoma	*MDM2*	10–30
	CDK4	11
Cervical cancer	*MYC*	25–50
Ovarian cancer	*MYC*	20–30
	ERB B-2 (EGFR2)	15–30
	AKT2	12
Head and neck cancer	*MYC*	7–10
	ERB B-1(EGFR)	10
	CCND1(cyclin D1)	~50
Colorectal cancer	*MYB*	15–20
	H-*RAS*	29
	K-*RAS*	22

Gene Amplification Gene amplification refers to the expansion in copy number of a gene within the genome of a cell. Gene amplification was first discovered as a mechanism by which some tumor cell lines can acquire resistance to growth-inhibiting drugs.[86] The process of gene amplification occurs through redundant replication of genomic DNA, often giving rise to karyotypic abnormalities called double-minute chromosomes (DMs) and homogeneous staining regions (HSRs).[87] DMs are characteristic minichromosome structures without centromeres. HSRs are segments of chromosomes that lack the normal alternating pattern of light- and dark-staining bands. Both DMs and HSRs represent large regions of amplified genomic DNA containing up to several hundred copies of a gene. Amplification leads to the increased expression of genes, which in turn can confer a selective advantage for cell growth.

The frequent observation of DMs and HSRs in human tumors suggested that the amplification of specific protooncogenes may be a common occurrence in neoplasia.[88] Studies then demonstrated that three protooncogene families–*myc*, *erb B*, and *ras*–are amplified in a significant number of human tumors (Table 6-2). About 20% to 30% of breast and ovarian cancers show c-*myc* amplification, and an approximately equal frequency of c-*myc* amplification is found in some types of squamous cell carcinomas.[89] N-*myc* was discovered as a new member of the *myc* protooncogene family through its amplification in neuroblastomas.[90] Amplification of N-*myc* correlates strongly with advanced tumor stage in neuroblastoma (Table 6-3), suggesting a role for this gene in tumor progression.[91,92] L-*myc* was discovered through its amplification in small-cell carcinoma of the lung, a neuroendocrine-derived tumor.[93] Amplification of *erb B*, the epidermal growth factor receptor, is found in up to 50% of glioblastomas and in 10% to 20% of squamous carcinomas of the head and neck.[77] Approximately 15% to 30% of breast and ovarian cancers have amplification of the *erbB-2* (*HER-2/neu*) gene. In breast cancer, *erbB-2* amplification correlates with advanced stage and poor prognosis.[94] Members of the *ras* gene family, including K-*ras* and N-*ras*, are sporadically amplified in various carcinomas.

Chromosomal Rearrangements Recurring chromosomal rearrangements are often detected in hematologic malignancies as well as in some solid tumors.[37,95,96] These rearrangements consist mainly of chromosomal translocations and, less frequently, chromosomal inversions. Chromosomal rearrangements can lead to hematologic malignancy via two different mechanisms: (1) the transcriptional activation of protooncogenes or (2) the creation of fusion genes. Transcriptional activation, sometimes referred to as gene activation, results from chromosomal rearrangements that move a protooncogene close to an immunoglobulin or T-cell receptor gene (see Figure 6-5). Transcription of the protooncogene then falls under control of regulatory elements from the immunoglobulin or T-cell receptor locus. This circumstance causes deregulation of protooncogene expression, which can then lead to neoplastic transformation of the cell.

Fusion genes can be created by chromosomal rearrangements when the chromosomal breakpoints fall within the loci of two different genes. The resultant juxtaposition of segments from two different genes gives rise to a composite structure consisting of the head of one gene and the tail of another. Fusion genes encode chimeric proteins with transforming activity. In general, both genes involved in the fusion contribute to the transforming potential of the chimeric oncoprotein. Mistakes in the physiologic rearrangement of immunoglobulin or T-cell receptor genes are thought to give rise to many of the recurring chromosomal rearrangements found in hematologic malignancy.[97] Examples of molecularly characterized chromosomal rearrangements in hematologic and solid malignancies are given in Table 6-4. In some cases, the same protooncogene is involved in several different translocations (ie, *c-myc*, *ews*, and *ret*).

Gene Activation The t(8;14)(q24;q32) translocation, found in about 85% of cases of Burkitt lymphoma, is a well-characterized example of the transcriptional activation of a protooncogene. This chromosomal rearrangement places the c-*myc* gene, located at chromosome band 8q24, under control of regulatory elements from the immunoglobulin heavy chain locus located at 14q32.[98] The resulting transcriptional activation of c-*myc*, which encodes a nuclear protein involved in the regulation of cell proliferation,

Table 6-3 Neuroblastoma

Benign ganglioneuromas	0/64(0%)	100
Low stages	31/772 (4%)	90
Stage 4-S	15/190 (8%)	80
Advanced stages	612/1.974 (31%)	30
Total	658/3000 (22%)	50

MYCN copy numbers are correlated with stage and survival in neuroblastoma.

Table 6-4 Molecularly Characterized Chromosome Rearrangments in Tumors

Affected Gene	Rearrangements	Disease	Protein Type
Hematopoietic tumors			
Gene fusion			
c-ABL (9q34)	t(9:22) (q34:q11)	CML	Tyrosine kinase activated by BCR
BCR (22q11)		and acute leukemia	
PBX-1(1q23)	t(1:19)(q23:p13.3)	Acute pre–B-cell leukemia	Homeodomain
E2A(19p13.3).		HLH	
PML(15q21)	t(15:17) (q21:q11–22)	Acute myeloid leukemia	Zinc finger
RAR(17q21)			
CAN(6p23)	t(6:9) (p23:q34)	Acute myeloid leukemia	No homology
DEK(9q34).			
REL	ins(2:12) (p13:p11.2–14)	Non-Hodgkin lymphoma	NF(κ)B family
NRG			No homology
Oncogenes juxtaposed with IG loci			
c-MYC	t(8:14) (q24:q32)	Burkitt lymphoma; BL-ALL	HLH domain
	t(2:8) (p12:q24)		
	t(8:22) (q24:q11)		
BCL1 (PRADI?)	t(11:14) (q13:q32)	B-cell chronic lymphocyte leukemia	PRADI-GI cyclin
BCL-2	t(14:18) (q32:21)	Follicular lymphoma	Inner mitochondrial membrane
BCL-3	t(14:19) (q32:q13.1)	Chronic B-cell leukemia	CDC10 motif
IL-3	t(5:14) (q31:q32)	Acute pre-B-cell leukemia	Growth factor
Oncogenes juxtaposed with TCR loci			
c-MYC	t(8:14) (q24:q11)	Acute T-cell leukemia	HLH domain
LYLA	t(7:19) (q35:p13)	Acute T-cell leukemia	HLH domain
TALA/SCL/TCL-5	t(1:14) (q32:q11)	Acute T-cell leukemia	HLH domain
TAL-2	t(7:9) (q35:q34)	Acute T-cell leukemia	HLH domain
Rhombotin 1/Ttg-1	t(11:14) (p15:q11)	Acute T-cell leukemia	LIM domain
Rhombotin 2/Ttg-2	t(11:14) (p13:q11)	Acute T-cell leukemia	LIM domain
	t(7:11) (q35:p13)		
HOX 11	t(10:14) (q24:q11)	Acute T-cell leukemia	Homeodomain
	t(7:10) (q35:q24)		
TAN-1	t(7:9) (q34:q34.3)	Acute T-cell leukemia	Notch homologue
TCL-1	t(7q35-14q32.1)	B-cell chronic lymphocitic leukemia	
	or inv		
	t(14q11-14q32.1)		
	or inv		
*S*olid Tumors			
Gene fusions in sarcomas			
FLI1,EWS	t(11:22) (q24:q12)	Ewing sarcoma	Ets transcription factor family
ERG,EWS	t(21:22) (q22:q12)	Ewing sarcoma	Ets transcription factor family
ATV1,EWS	t(7:21) (q22:q12)	Ewing sarcoma	Ets transcription factor family
ATF1,EWS	t(12:22) (q13:q12)	Soft-tissue clear cell sarcoma	Transcription factor
CHN,EWS	t(9:22) (q22 31:q12)	Myxoid chondrosarcoma	Steroid receptor family
WT1,EWS	t(11:22) (p13:q12)	Desmoplastic small round cell tumor	Wilms tumor gene
SSX1,SSX2,SYT	t(X:18) (p11.2:q11.2)		Synovial sarcoma HLH domain
PAX3,FKHR	t(2:13) (q37:q14)	Alveolar rhabdomysarcoma	Homeobox homologue
PAX7,FKHR	t(1:13) (q36:q14)	Rhabdomyosarcoma	Homeobox homologue
CHOP,TLS	t(12:16) (q13:p11)	Myxoid liposarcoma	Transcription factor
var,HMG1-C	t(var:12) (var:q13-15)	Lipomas	HMG DNA-binding protein
HMG1-C?	t(12:14) (q13-15)	Leiomyomas	HMG DNA-binding protein
Gene fusions in thyroid carcinomas			
RET/ptc1	inv(10) (q11.2:q2.1)	Papillary thyroid carcinomas	Tyrosine kinase actived by H4
RET/ptc2	t(10:17) (q11.2:q23)	Papillary thyroid carcinomas	Tyrosine kinase actived by RIa(PKA)
RET/ptc3	inv(10) (q11.2)	Papillary thyroid carcinomas	Tyrosine kinase actived by ELE1
TRK	inv(1) (q31:q22-23)	Papillary thyroid carcinomas	Tyrosine kinase actived by TPM3
TRK–T1(T2)	inv(1) (q31:q25)	Papillary thyroid carcinomas	Tyrosine kinase actived by TPR
TRK –T3	t(1q31:3)	Papillary thyroid carcinomas	Tyrosine kinase actived by TFG
Haematopoietic and solid tumors			
Oncogenes juxtaposed with other loci			
PTH deregulates PRAD1	inv(11)(p15:q13)	Parathyroid adenoma	PRADI-GI cyclin
BTG1 deregulates MYC	t(8:12)(q24:q22)	B-cell chronic lymphocytic	MYC-HLH domain

HLH = helix loop helix structural domain; HMG = high mobility group; H4; ELE1; IG = immunoglobulin; TPR and TFG = partially uncharacterized genes with a dimerizing coiled-coil domain; RIa = regulatory subunit of PKA enzyme; TCR = T-cell receptor; TPM3 = isoform of nonmuscle tropomyosin.

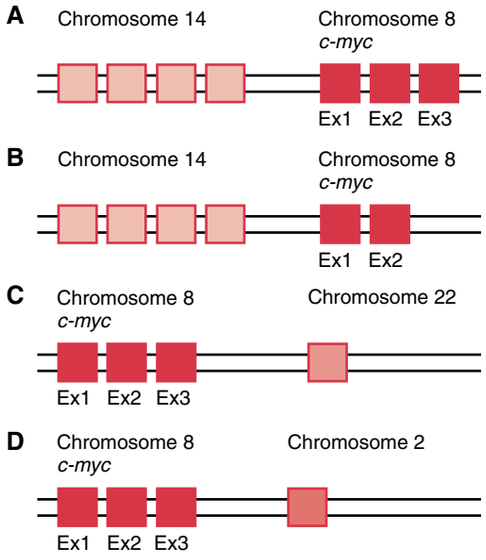

Figure 6-6 C-*myc* translocations found in Burkitt lymphoma. **A,** t(8;14)(q24;q32) translocation involving the locus of immunoglobulin heavy-chain gene located at 14q32. **B,** t(8;14)(q24;q32) translocation where only 2 exons (Ex) of c-*myc* are translocated under regulatory elements from the immunoglobulin heavy-chain locus located at 14q32. **C,** t(8;22)(q24;q11) translocation involving the *l* locus of immunoglobulin light-chain gene at 22q11. **D,** t(2;8)(p12;q24) translocation involving the κ locus of immunoglobulin light-chain gene located at 2p12.

plays a critical role in the development of Burkitt lymphoma.[99] The c-*myc* gene is also activated in some cases of Burkitt lymphoma by translocations involving immunoglobulin light-chain genes.[100,101] These are t(2;8)(p12;q24), involving the κ locus located at 2p12, and t(8;22)(q24;q11), involving the κ locus at 22q11 (Figure 6-6). Although the position of the chromosomal breakpoints relative to the c-*myc* gene may vary considerably in individual cases of Burkitt lymphoma, the consequence of the translocations is the same: deregulation of c-*myc* expression, leading to uncontrolled cellular proliferation.

In some cases of T cell acute lymphoblastic leukemia (T-ALL), the c-*myc* gene is activated by the t(8;14)(q24;q11) translocation. In these cases, transcription of c-*myc* is placed under the control of regulatory elements within the T-cell receptor α locus located at 14q11.[102] In addition to c-*myc*, several protooncogenes that encode nuclear proteins are activated by various chromosomal translocations in T-ALL involving the T-cell receptor α or β locus. These include *HOX11*, *TAL1*, *TAL2*, and *RBTN1/Tgt1*.[103–105] The proteins encoded by these genes are thought to function as transcription factors through DNA-binding and protein-protein interactions. Overexpression or inappropriate expression of these proteins in T cells is thought to inhibit T-cell differentiation and lead to uncontrolled cellular proliferation.

A number of other protooncogenes are also activated by chromosomal translocations in leukemia and lymphoma. In most follicular lymphomas and some large cell lymphomas, the *bcl-2* gene (located at 18q21) is activated as a consequence of t(14;18)(q32;q21) translocations.[72,73] Overexpression of the *bcl-2* protein inhibits apoptosis, leading to an imbalance between lymphocyte proliferation and programmed cell death.[74] Mantle cell lymphomas are characterized by the t(11;14)(q13;q32) translocation, which activates the *cyclin d1* (*bcl-1*) gene located at 11q13.[106,107] Cyclin D1 is a G1 cyclin involved in the normal regulation of the cell cycle. In some cases of T cell chronic lymphocytic leukemia and prolymphocytic leukemia, the *tcl-1* gene at 14q32.1 is activated by inversion or translocation involving chromosome 14.[108] The *tcl-1* gene product is a small cytoplasmic protein whose function is not yet known.

Gene Fusion The first example of gene fusion was discovered through the cloning of the breakpoint of the Philadelphia chromosome in chronic myelogenous leukemia (CML).[109] The t(9;22)(q34;q11) translocation in CML fuses the c-*abl* gene, normally located at 9q34, with the *bcr* gene at 22q11 (Figure 6-7).[110] The *bcr/abl* fusion, created on the der(22) chromosome, encodes a chimeric protein of 210 kDa, with increased tyrosine kinase activity and abnormal cellular localization.[111] The precise mechanism by which the *bcr/abl* fusion protein contributes to the expansion of the neoplastic myeloid clone is not yet known. The t(9;22) translocation is also found in up to 20% of cases of acute lymphoblastic leukemia (ALL). In these cases, the breakpoint in the *bcr* gene differs somewhat from that found in CML, resulting in a 185 kDa bcr/abl fusion protein.[112] It is unclear at this time why the slightly smaller bcr/abl fusion protein leads to such a large difference in neoplastic phenotype.

In addition to c-*abl*, two other genes encoding tyrosine kinases are involved in distinct gene fusion events in hematologic malignancy. The t(2;5)(p23;q35) translocation in anaplastic large cell lymphomas fuses the *NPM* gene (5q35) with the *ALK* gene (2p23).[113] *ALK* encodes a membrane-spanning tyrosine kinase similar to members of the insulin growth factor receptor family. The NPM protein is a nucleolar phosphoprotein involved in ribosome assembly. The *NPM/ALK* fusion creates a chimeric oncoprotein in which the ALK tyrosine kinase activity may be constitutively activated. The t(5;12)(q33;p13) translocation, characterized in a case of chronic myelomonocytic leukemia, fuses the *tel* gene (12p13) with the tyrosine kinase domain of the PDGF receptor *b* gene (PDGFR-*b* at 5q33).[114] The *tel* gene is thought to encode a nuclear DNA-binding protein similar to those of the *ets* family of protooncogenes.

Gene fusions sometimes lead to the formation of chimeric transcription factors.[68,95] The t(1;19)(q23;p13) translocation, found in childhood pre-B-cell ALL, fuses the *E2A* transcription factor gene (19p13) with the *PBX1* homeodomain gene (1q23).[115] The E2A/PBX1 fusion protein consists of the amino-terminal transactivation domain of the E2A protein and the DNA-binding homeodomain of the PBX1 protein. The t(15;17)(q22;q21) translocation in acute promyelocytic leukemia (PML) fuses the *PML* gene (15q22) with the *RARA* gene at 17q21.[116] The PML protein contains a zinc-binding domain called a RING finger that may be involved in protein-protein interactions. *RARA* encodes the retinoic acid alpha-receptor protein, a member of the nuclear steroid/thyroid hormone receptor superfamily. Although retinoic acid binding is retained in the fusion protein, the PML/RARA fusion protein may confer altered DNA-binding specificity to the RARA ligand complex.[117] Leukemia patients with the *PML/RARA* gene fusion respond well to retinoid treatment. In these cases, treatment with all-*trans* retinoic acid induces differentiation of PML cells.

The *ALL1* gene, located at chromosome band 11q23, is involved in approximately 5% to 10% of acute leukemia cases overall in children and adults.[118,119] These include cases of ALL, acute myeloid leukemia, and leukemias of mixed cell lineage. Among leukemia genes, *ALL1* (also called *MLL* and *HRX*) is unique because it participates in fusions with a large number of different partner genes on the various chromosomes. Over 20 different reciprocal translocations involving the *ALL1* gene at 11q23 have been reported, the most common of which are those involving chromosomes 4, 6, 9, and 19.[120] In approximately 5% of cases of acute leukemia in adults, the *ALL1* gene is fused with a portion of itself.[121] This special type of gene fusion is called self-fusion.[122] Self-fusion of the *ALL1* gene, which is thought to occur through a somatic recombination mechanism, is found in high incidence in acute leukemias with trisomy 11 as a sole cytogenetic abnormality. The *ALL1* gene encodes a large protein with DNA-binding motifs, a transactivation domain, and a region with homology to the *Drosophila trithorax* protein (a regulator of homeotic gene expression).[123,124] The various partners in *ALL1*

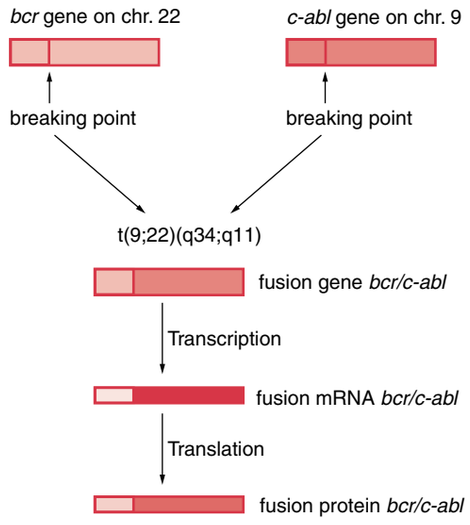

Figure 6-7 Gene fusion. The t(9;22)(q34;q11) translocation in chronic myelogenous leukemia (CML) determines the fusion of the c-*abl* gene with the *bcr* gene. Such a gene fusion encodes an oncogenic chimeric protein of 210 kDa. Chr = chromosome.

fusions encode a diverse group of proteins, some of which appear to be nuclear proteins with DNA-binding motifs.[125,126] The ALL1 fusion protein consists of the aminoterminus of ALL1 and the carboxyl terminus of one of a variety of fusion partners. It appears that the critical feature in all ALL1 fusions, including self-fusion, is the uncoupling of the ALL1 amino-terminal domains from the remainder of the ALL1 protein.

Solid tumors, especially sarcomas, sometimes have consistent chromosomal translocations that correlate with specific histologic types of tumors.[127] In general, translocations in solid tumors result in gene fusions that encode chimeric oncoproteins. Studies thus far indicate that in sarcomas, the majority of genes fused by translocations encode transcription factors.[128] In myxoid liposarcomas, the t(12;16)(q13;p11) fuses the *FUS* (*TLS*) gene at 16p11 with the *CHOP* gene at 12q13.[129] The FUS protein contains a transactivation domain that is contributed to the FUS/CHOP fusion protein. The CHOP protein, which is a dominant inhibitor of transcription, contributes a protein-binding domain and a presumptive DNA-binding domain to the fusion. Despite knowledge of these structural features, the mechanism of action of the FUS/CHOP oncoprotein is not yet known. In Ewing sarcoma, the t(11;22)(q24;q12) fuses the *EWS* gene at 22q12 with the *FLI1* gene at 11q24.[130] Like FUS, the EWS protein contains three glycine-rich segments and an RNA-binding domain. The FLI1 protein contains an *ets*-like DNA-binding domain. The EWS/FLI1 fusion protein combines a transactivation domain from EWS with the DNA-binding domain of FLI1. In alveolar rhabdomyosarcoma, the t(2;13)(q35;q14) fuses the *PAX3* gene at 2q35 with the *FKHR* gene at 13q14.[131] The PAX3 protein, a transcription factor that activates genes involved in development, is a paired-box homeodomain protein with two distinct DNA-binding domains. The FKHR protein encodes a conserved DNA-binding motif (the forkhead domain) similar to that first identified in the *Drosophila* forkhead homeotic gene. The PAX3/FKHR fusion protein is a chimeric transcription factor containing the PAX3 DNA-binding domains, a truncated forkhead domain, and the carboxy-terminal FKHR regions.

In DP, an infiltrating skin tumor, both a reciprocal translocation t(17;22)(q22;q13) and supernumerary ring chromosomes derived from the t(17;22) have been described.

Although early successful studies in this field have been performed with lymphomas and leukemia, as we have discussed before, the first chromosomal abnormality in solid tumors to be characterized at the molecular level as a fusion protein was an inversion of chromosome 10 found in papillary thyroid carcinomas.[132] In this tumor, two main recurrent structural changes have been described, including inv(10) (q112.2; q21.2), as the more frequent alteration, and a t(10;17)(q11.2;q23). These two abnormalities represent the cytogenetic mechanisms which activate the protooncogene *ret* on chromosome 10, forming the oncogenes RET/*ptc1* and RET/*ptc2*, respectively. Alterations of chromosome 1 in the same tumor type have then been associated to the activation of NTRK1 (chromosome 1), an NGF receptor which, like RET, forms chimeric fusion oncogenic proteins in papillary thyroid carcinomas.[133] A comparative analysis of the oncogenes originated from the activation of these two tyrosine kinase receptors has allowed the identification and characterization of common cytogenetic and molecular mechanisms of their activation. In all cases, chromosomal rearrangements fuse the tK portion of the two receptors to the 5' end of different genes that, due to their general effect, have been designated as activating genes. In the majority of cases, the latter belong to the same chromosome where the related receptor is located, 10 for RET and 1 for NTRK1.

Furthermore, although functionally different, the various activating genes share the following three properties: (1) they are ubiquitously expressed; (2) they display domains demonstrated or predicted to be able to form dimers or multimers; (3) they translocate the tK-receptor-associated enzymatic activity from the membrane to the cytoplasm.

These characteristics can explain the mechanism(s) of oncogenic activation of *ret* and *NTRK1* protooncogenes. In fact, following the fusion of their tK domain to activating gene, several things happen: (1) *ret* and *NTRK1*, whose tissue-specific expression is restricted to subsets of neural cells, become expressed in the epithelial thyroid cells; (2) their dimerization triggers a constitutive, ligand-independent transautophosphorylation of the cytoplasmic domains and as a consequence, the latter can recruit SH2 and SH3 containing cytoplasmic effector proteins, such as Shc and Grb2 or phospholipase C (PLCγ), thus inducing a constitutive mitogenic pathway; (3) the relocalization in the cytoplasm of *ret* and *NTRK1* enzymatic activity could allow their interaction with unusual substrates, perhaps modifying their functional properties.

In conclusion, in PTCs, the oncogenic activation of *ret* and *NTRK1* protooncogenes following chromosomal rearrangements occurring in breakpoint cluster regions of both protooncogenes could be defined as an ectopic, constitutive, and topologically abnormal expression of their associated enzymatic (tK) activity.[134]

ONCOGENES IN THE INITIATION AND PROGRESSION OF NEOPLASIA

Human neoplasia is a complex multistep process involving sequential alterations in protooncogenes (activation) and in tumor suppressor genes (inactivation). Statistical analysis of the age incidence of human solid tumors indicates that five or six independent mutational events may contribute to tumor formation.[135] In human leukemias, only three or four mutational events may be necessary, presumably involving different genes.

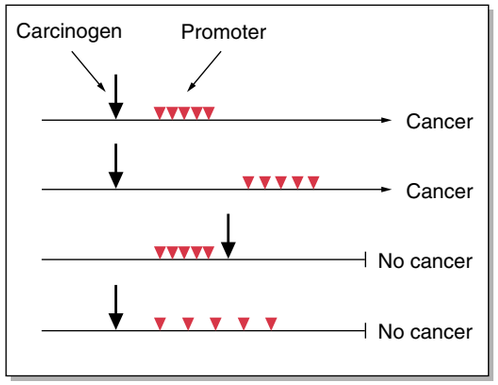

Figure 6-8 A model of exposure to a mutagen and to a tumor promoter. Cancer develops exclusively when the exposure to promoter follows the exposure to carcinogen (mutagen; eg, 7,12-dimethyl-benzanthracene [DMBA]) and only when the intensity of the exposure to promoter is higher than a threshold.

The study of chemical carcinogenesis in animals provides a foundation for our understanding the multistep nature of cancer.[136] In the mouse model of skin carcinogenesis, tumor formation involves three phases, termed initiation, promotion, and progression. Initiation of skin tumors can be induced by chemical mutagens such as 7,12-dimethyl-benzanthracene (DMBA) (Figure 6-8). After application of DMBA, the mouse skin appears normal. If the skin is then continuously treated with a promoter, such as the phorbol ester TPA, precancerous papillomas will form. Chemical promoters such as TPA stimulate growth but are not mutagenic substances. Over a period of months of continuous application of the promoting agent, some of the papillomas will progress to skin carcinomas. Treatment with DMBA or TPA alone does not cause skin cancer. Mouse papillomas initiated with DMBA usually have H-*ras* oncogenes with a specific mutation in codon 61 of the H-*ras* gene. The mouse skin tumor model indicates that initiation of papillomas is the result of mutation of the H-*ras* gene in individual skin cells by the chemical mutagen DMBA. For papillomas to appear on the skin, however, growth of mutated cells must be continuously stimulated by a promoting agent. Additional unidentified genetic changes must then occur for papillomas to progress to carcinoma.

Although a single oncogene is sufficient to cause tumor formation by some rapidly transforming retroviruses such as RSV, transformation by a single oncogene is not usually seen in experimental models of cancer. Other rapidly transforming retroviruses carry two different oncogenes that cooperate in producing the neoplastic phenotype. One well-characterized example of this type of cooperation is the avian erythroblastosis virus, which carries the *erb A* and *erb B* oncogenes.[137] Cooperation between oncogenes can also be demonstrated by in vitro transformation studies using nonimmortalized cell lines. For example, studies have shown cooperation between the nuclear myc protein and the cytoplasmic-membrane-associated ras protein in

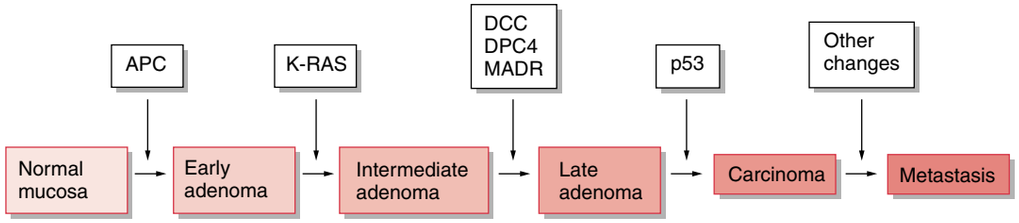

Figure 6-9 Colorectal cancer development. Colorectal cancer results from a series of pathologic changes that transform normal colonic epithelium into invasive carcinoma. Specific genetic events, shown by vertical arrows, accompany this multistep process.

the transformation of rat embryo fibroblasts.[138] As previously reported, a cooperation between SV40 large T product and mutated H-*ras* gene also have been found necessary to transform normal human epithelial and fibroblast cells provided that they constitutively expressed the catalytic subunit of telomerase enzyme, indicating a more complex pattern in the neoplastic conversion of human cells.

Collaboration between two different general categories of oncogenes (eg, nuclear and cytoplasmic) can often be demonstrated but is not strictly required for transformation.[139] The production of transgenic mice expressing a single oncogene such as *myc* has also demonstrated that multiple genetic changes are necessary for tumor formation. These transgenic mice strains, in fact, generally show an increased incidence of neoplasia and the tumors that result frequently are clonal, implying that other events are necessary.The production of transgenic mice expressing a single oncogene such as *myc* has also demonstrated that multiple genetic changes are necessary for tumor formation.[140]

Cytogenetic studies of the clonal evolution of human hematologic malignancies have provided much insight into the multiple steps involved in the initiation and progression of human tumors.[141] The evolution of CML from chronic phase to acute leukemia is characterized by an accumulation of genetic changes seen in the karyotypes of the evolving malignant clones. The early chronic phase of CML is defined by the presence of a single Philadelphia chromosome. The formation of the *bcr/abl* gene fusion as a consequence of the t(9;22) translocation is thought to be the initiating event in CML.[110] The biologic progression of CML to a more malignant phenotype corresponds with the appearance of additional cytogenetic abnormalities such as a second Philadelphia chromosome, isochromosome 17, or trisomy 8.[142] These karyotypic changes are thought to reflect additional genetic changes

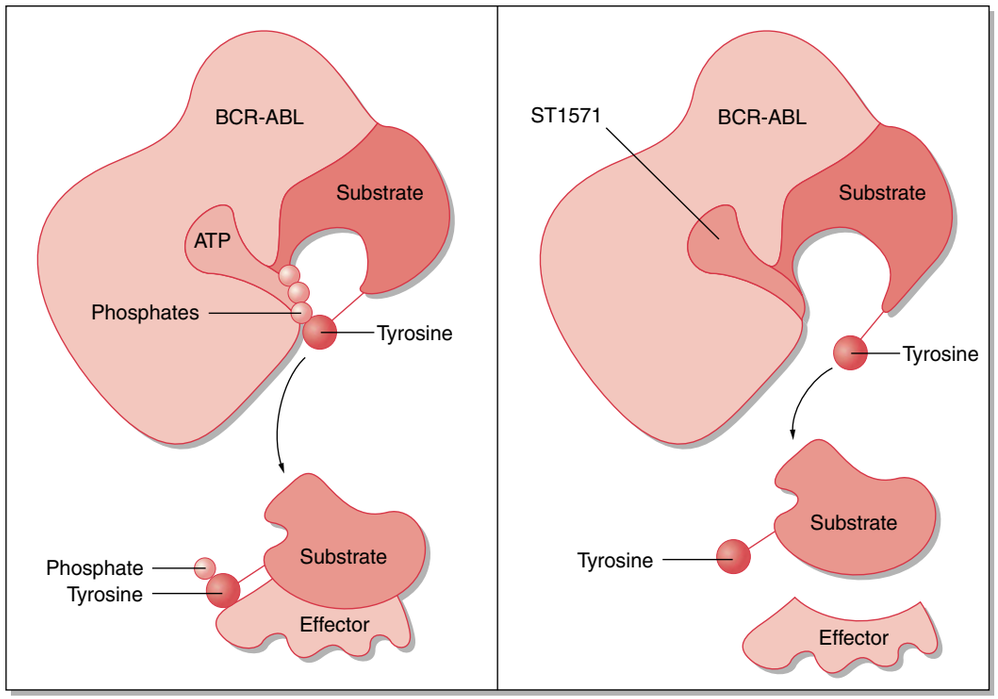

Figure 6-10 Mode of action of STI571. The effect of ATP binding on the oncoprotein BCR-ABL (left): the fusion protein binds the molecule of ATP in the kinase pocket. Afterwards, it can phosphorylate a substrate, that can interact with the downstream effector molecules. When STI571 is present (right), the oncoprotein binds STI571 in the kinase pocket (competing with ATP); therefore the substrate cannot be phosphorylated.

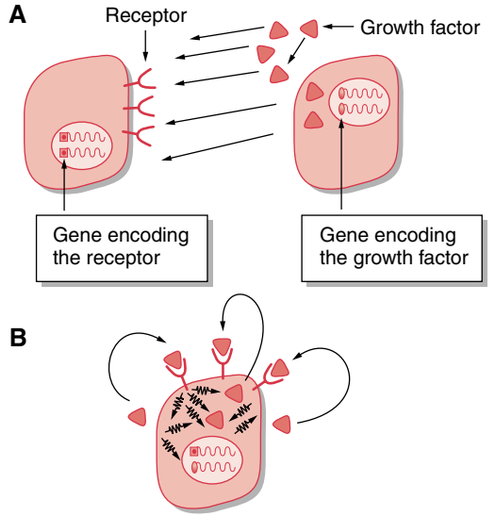

Figure 6-11 Paracrine and autocrine stimulation. **A,** A growth factor produced by the cell on the right stimulates another cell carrying the appropriate receptor (left) on cell membrane. This process is named paracrine stimulation. **B,** A growth factor is produced by the same cell expressing the corresponding receptor. This process is designated autocrine stimulation.

involving an increase in oncogene dosage and loss or inactivation of tumor suppressor genes. Although the karyotypic changes in evolving CML are somewhat variable from patient to patient, the accumulation of genetic changes always correlates with progression from differentiated cells of low malignancy to undifferentiated cells of high malignancy.

The initiation and progression of human neoplasia involve the activation of oncogenes and the inactivation or loss of tumor suppressor genes. The mechanisms of oncogene activation and the time course of events, however, vary among different types of tumors. In hematologic malignancies, soft-tissue sarcomas and the papillary type of thyroid carcinomas, initiation of the malignant process predominantly involves chromosomal rearrangements that activate various oncogenes.[95] Many of the chromosomal rearrangements in leukemia and lymphoma are thought to result from errors in the physiologic process of immunoglobulin or T-cell receptor gene rearrangement during normal B-cell and T-cell development. Late events in the progression of hematologic malignancies involve oncogene mutation, mainly of the *ras* family, inactivation of tumor suppressor genes such as *p53*, and sometimes additional chromosomal translocations.[143]

In carcinomas such as colon and lung cancer, the initiation of neoplasia has been shown to involve oncogene and tumor suppressor gene mutations.[144] These mutations are generally thought to result from chemical carcinogenesis, especially in the case of tobacco-related lung cancer, where a novel tumor suppressor gene (designated FHIT) has been found to be inactivated in the majority of cancers, particularly in those from smokers.[145,146] In preneoplastic ade-

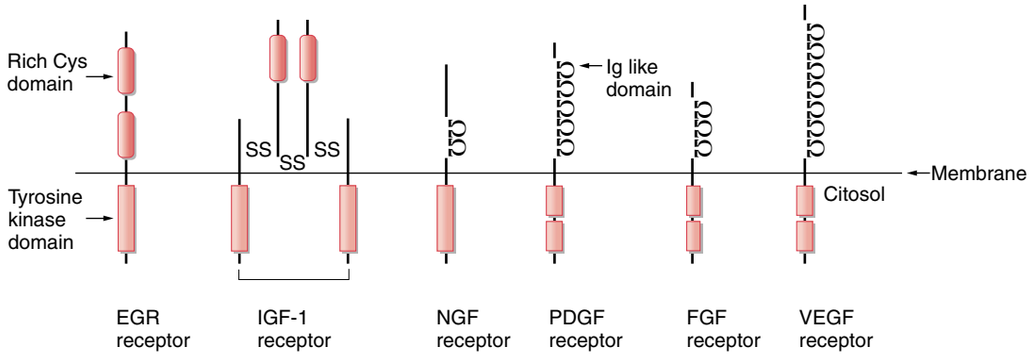

Figure 6-12 Representative examples of tyrosine kinase receptor families. EGF = epidermal growth factor; FGF = fibroblast growth factor; Ig= immunoglobulin; IGF1 = insulinlike growth factor; PDGF = platelet-derived growth factor; VEGF = vascular endothelial growth factor.

nomas of the colon, the K-*ras* gene is often mutated.[147] Progression of colon adenomas to invasive carcinoma frequently involves inactivation or loss of the *DCC* and *p53* tumor suppressor genes (see Figure 6-9). Gene amplification is often seen in the progression of some carcinomas and other types of tumors. Amplification of the *erb B-2* oncogene may be a late event in the progression of breast cancer.[94] Members of the *myc* oncogene family are frequently amplified in small-cell carcinoma of the lung.[93] As mentioned previously, amplification of N-*myc* strongly correlates with the progression and clinical stage of neuroblastoma.[92] Although there is variability in the pathways of human tumor initiation and progression, studies of various types of malignancy have clearly confirmed the multistep nature of human cancer.

SUMMARY AND CONCLUSIONS

The initiation and progression of human neoplasia is a multistep process involving the accumulation of genetic changes in somatic cells. These genetic changes consist of the activation of cooperating oncogenes and the inactivation of tumor suppressor genes, which both appear necessary for a complete neoplastic phenotype. Oncogenes are altered versions of normal cellular genes called protooncogenes. Protooncogenes are a diverse group of genes involved in the regulation of cell growth. The functions of protooncogenes include growth factors, growth factor receptors, signal transducers, transcription factors, and regulators of programmed cell death. Protooncogenes may be activated by mutation, chromosomal rearrangement, or gene amplification. Chromosomal rearrangements that include translocations and inversions can activate protooncogenes by deregulation of their transcription (eg, transcriptional activation) or by gene fusion. Tumor suppressor genes, which also participate in the regulation of normal cell growth, are usually inactivated by point mutations or truncation of their protein sequence coupled with the loss of the normal allele.

The discovery of oncogenes represented a breakthrough in our understanding of the molecular and genetic basis of cancer. Oncogenes have also provided important knowledge concerning the regulation of normal cell proliferation, differentiation, and programmed cell death. The identification of oncogene abnormalities has provided tools for the molecular diagnosis and monitoring of cancer. Most important, oncogenes represent potential targets for new types of cancer therapies. It is more than a hope that a new generation of chemotherapeutic agents directed at specific oncogene targets will be developed. The goal of these new drugs will be to kill cancer cells selectively while sparing normal cells. One promising approach entails using specific oncogene targets to trigger programmed cell death. One example of the accomplishment of such a goal is represented by the inhibition of the tumor-specific tyrosine kinase bcr/abl in CML by imatinib (Gleevec or STI571)[148](see Figure 6-10). The same compound has been proven active also in a different tumor type, gastrointestinal stromal tumor, where it inhibits the tyrosine kinase receptor c-kit.[149] Our rapidly expanding knowledge of the molecular mechanisms of cancer holds great promise for the development of better combined methods of cancer therapy in the near future.

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