7 An Overview of Cancer Genes

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7.1 Nature of Genetic Mutations

When functioning properly, oncogenes and tumor suppressor genes prevent the development of cancer. The loss of function of both alleles is required for neoplastic transformation. In recent studies, it has been reported that approximately 80 DNA mutations alter the amino acid sequence in a typical cancer and of these mutations less than 15 are likely to result in the initiation, progression, or maintenance of the tumor [1]. Mutations that are causally involved in the tumorigenesis process and are positively selected for are called *driver* mutations. Whereas, mutations that provide no positive or negative selective advantage to the tumor, but are carried during cell division and expansion, are referred to as *passenger* mutations [1, 2]. Several types of mutations can occur and account for neoplastic transformation such as missense and nonsense mutations, insertions, deletions, duplications, frameshift mutations, and repeat expansions (Table 7.1). Interestingly, for example it has been noted that mutations converting 5′-CpG to 5′-TpG were more frequent in colorectal cancer than in breast cancers [1]. Along with genetic mutations, epigenetic dysregulation of genes also occurs in a wide variety of cancers. Epigenetics is defined as changes in genome function that occur without a change in the DNA sequence. Advances in our understanding of cancer oncogenes and tumor suppressor genes, and the mechanisms of their aberrant regulation, will provide insight to develop novel anticancer approaches and therapeutic strategies.

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7.2 Proto-oncogenes

The discovery of proto-oncogenes is one of the most fundamentally important findings of this century. Oncogenes are activated (frequently mutated) alleles of normally functioning wild-type genes (proto-oncogenes) that function in cell cycle progression or cellular proliferation. Activated or mutated proto-oncogenes promote unregulated cell cycle progression and cell proliferation, leading to cancer development. Proteins encoded by normal cellular proto-oncogenes function in all subcellular compartments including the nucleus, cytoplasm, and cell surface, and exert their function in most intracellular processes by acting as protein kinases, growth factors, growth factor receptors, or membrane associated signal transducers. Mutations in proto-oncogenes alter the normal structure and/or expression pattern, and the resulting oncogene acts in a dominant manner. That is, a mutation in only a single allele is required for activation of the proto-oncogene and/or loss of regulation of the proto-oncogene product. In genetic terms, this is typically referred to as a gain of function mutation.

7.2.1 Viral Oncogenes

The discovery of proto-oncogenes is rooted in the study of mammalian viruses, and in particular, retroviruses. In the earlier years of the twentieth century, radiation, chemicals, and viruses were shown to induce cancer in experimental animals, and later, transform cells in culture. The study of so called tumor viruses advanced quickly compared to studies of radiation-induced and chemical-induced carcinogenesis, for several reasons. Although both chemical carcinogens and radiation are potent inducers of neoplasia, it was found that tumor viruses could more efficiently and reproducibly transform cells in culture and induce tumors in experimental animals. Tumor viruses caused tumors to develop in a matter of days to weeks allowing rapid analysis following infection. Moreover, both radiation and chemical carcinogens act

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Table 7.1 Gene mutations in human cancer

Mutation	Description
Missense	A change in one DNA base pair that results in the substitution of one amino acid
Nonsense	A change in one DNA base pair that results in the substituting of one amino acid that encodes a stop codon
Insertion	Changes in the number of DNA base pairs in a gene by inserting additional DNA base pairs
Deletion	Changes in the number of DNA base pairs in a gene by removing a piece of DNA
Duplication	A piece of DNA that is aberrantly copied one or more times
Frameshift	The addition or loss of DNA base pairs that changes the reading frame
Repeat expansion	Short DNA sequences that are repeated a number of times in a row

randomly on the cellular genome. Examining the cellular genome to determine the carcinogenic effect of these agents at a level of individual genes or DNA segments was a daunting task. In contrast to the mammalian cellular genome, the small genome of the tumor viruses offered a less complex model for identification of specific sequences responsible for induction and progression of tumors, and a more efficient system for elucidation of molecular mechanisms governing neoplastic transformation.

7.2.1.1 DNA Viruses

Some of DNA viruses and one class of RNA viruses (the retroviruses) have been shown to have oncogenic potential. The Shope papilloma virus was one of the first DNA tumor viruses to be described [3]. It causes benign papillomas that can progress to malignant carcinomas in cottontail rabbits. Papillomaviruses, along with other classes of DNA viruses such as the hepatitis B viruses, have the ability to transform cells in their natural host. Most other DNA viruses, including adenoviruses, simian virus 40 (SV40), and polyomaviruses lack transforming ability. In natural hosts, cells infected with these DNA viruses undergo cell death rather than transformation as a consequence of viral replication. However, these later viruses demonstrate their oncogenic potential in heterologous, nonpermissive species in which viruses cannot replicate. Each class of DNA virus has led to remarkable discoveries in proto-oncogenesis [4].

7.2.1.2 RNA Retroviruses

The RNA retroviruses represent the class of tumor viruses that has contributed the most to our understanding of mammalian carcinogenesis. Retroviruses are the only currently known RNA viruses to have oncogenic potential. A feature common to these viruses is the ability to replicate in infected cells via a provirus intermediate. The proviral intermediate is generated through the action of a retroviral enzyme termed reverse transcriptase, which synthesizes a DNA copy of the retroviral RNA genome. RNA to DNA reverse transcription is obligatory for RNA retroviral replication in infected mammalian cells. The DNA transcript of the retroviral genome incorporates into the cellular genome where it replicates along with cellular DNA. The RNA polymerase enzyme of the host cells transcribes the provirus DNA, generating new RNA virions and retroviral mRNAs needed for synthesis of viral proteins. Importantly, unlike most of the DNA viruses, retroviruses are not cytotoxic or cytocidal to the host cells. This reflects the nature of the retroviral lifecycle, where new retroviral particles are released from the cell by budding rather than by cell lysis. Thus, RNA viruses can transform the same cells in which they replicate. The recombination event that occurs between the retroviral DNA (provirus) and host DNA as part of the replication cycle has significant implications for neoplastic transformation and tumor development [5].

The first oncogenic retrovirus discovered was the Rous sarcoma virus (RSV). Peyton Rous inoculated chickens with a chicken sarcoma cellular extract and was able to demonstrate efficient transmission of an agent that propagated tumor growth [6]. Subsequent studies demonstrated that RSV had transforming properties in cultured cells. This was found to be in contrast to another well-studied retrovirus, the avian leukosis virus (ALV). ALV maintained the ability to induce tumors following inoculation in chickens (albeit after months, and not days to weeks compared to RSV), but did not demonstrate the ability to transform cells in culture [4]. The differences in induction efficiency in animals (in vivo activity) and ability to transform cells in culture (in vitro activity) form the basis for dividing retroviruses into two groups: (1) the acutely transforming oncogenic retroviruses, and (2) the weakly oncogenic or non-transforming retroviruses.

7.2.2 Cellular and Retroviral Oncogene Discovery

The differences between the acutely and weakly or non-transforming retroviruses are extremely important and provided clues towards recognition of the first proto-oncogene. In comparing RSV and ALV genomes, RSV was shown to be 1.5 kb greater in size than ALV. This additional segment was correctly postulated to be responsible for the rapid transforming properties of RSV. In 1971, Peter Vogt isolated RSV mutants that had the weakly oncogenic properties of ALV [7]. These weakly oncogenic RSV mutants were approximately the same size as ALV, did not have the ability to transform cultured cells, and did not efficiently induce sarcomas in animals, but maintained retroviral replication capabilities. The missing 1.5 kb sequence in these mutant RSV genomes was subsequently demonstrated to be required not only for initiation but

also for maintenance of neoplastic transformation. Because different RSV mutants were not complimentary and did not lead to neoplastic transformation in cell culture, it was concluded that a single gene could be responsible for both in vitro transformation and in vivo oncogenesis. The first retroviral oncogene was named v-*src* for its sarcoma inducing action. Since then over 30 viral oncogenes have been discovered in over 40 transforming viruses [4, 8, 9].

Similar to the discovery of the first oncogene, the discovery of the origin of retroviral oncogenes had monumental implications. The extra 1.5 kb of nucleic acid in RSV was not necessary for viral replication/growth. It was not clear where the apparently extraneous nucleic acid segment originated. The answer was obtained through the study of retroviral tumors of the very rare animal that developed tumors after being infected by a non-transforming retrovirus. These previously non-transforming retroviruses were found to have incorporated new genetic material in their RNA genome corresponding to a new oncogene which conferred capability for neoplastic transformation. The portion of the proviral genome corresponding to the newly recognized oncogene was used to probe for similar sequences in host cells. This analysis demonstrated that genes possessing the capability for neoplastic transformation were conserved among several different species. This observation suggested that host cell DNA could be incorporated into the genome of a retrovirus during recombination in the infected cell. Further study of the cellular homologues of retroviral oncogenes showed that they are normal cellular genes that encode proteins involved in various aspects of cellular homeostasis, including cell proliferation and differentiation. The normal cellular counterpart of the retroviral oncogenes is referred to as cellular proto-oncogenes. The current paradigm holds that viral oncogenes originate from cellular proto-oncogenes, and that these genes have been altered in a manner which confers the ability to induce cellular neoplastic transformation in infected cells [4, 8]. In like manner, cellular proto-oncogenes can be activated in various ways (point mutation, deletion, amplification, or rearrangement) that result in the synthesis of an oncogenic protein product [4, 8].

The discovery of the ability of genes to induce tumors in animals and humans linked the study of transforming retroviruses with the field of molecular biology of human cancers. However, it is clear that most human cancers are not caused by infection with transforming viruses. Shortly after it was established that specific virus associated genes could cause cellular transformation, alterations in cellular proto-oncogenes were found to be responsible for human tumors. The first instance linking the possibility of a human proto-oncogene with cancer, when retroviral involvement could be eliminated, was reported in 1981 by two groups, who showed that DNA extracted from a human bladder carcinoma cell line (EJ) could induce transformation in an immortalized but non-transformed mouse cell line NIH 3T3 [9, 10]. In 1982, the first human activated proto-oncogenes were isolated and identified from the EJ bladder carcinoma cell line and a human lung carcinoma. These genes were cellular homologs of the Harvey-*ras* and Kirsten-*ras* retroviral oncogenes, both of which had previously been shown to induce rat sarcomas [11]. The discovery of proto-oncogenes solidified the link between genes and cancer, and ushered in an era of genetic discovery focused on identification of genetic abnormalities that contribute to the development of human neoplasms.

7.2.3 Mechanisms of Activation of Cellular Proto-oncogenes

Cellular proto-oncogenes must become activated in order to express oncogenic potential leading to neoplastic transformation. Activation of cellular proto-oncogenes typically involves chromosomal translocation, amplification, or point mutation. The changes that result can be broadly categorized into (1) changes to the structure of a proto-oncogene which result in an abnormal gene product with aberrant function (examples include the *bcr-abl* translocation and c-*ras* point mutations, described below) and (2) changes to the regulation of gene expression resulting in aberrant expression or inappropriate production of the structurally normal growth-promoting protein (examples include translocations involving c-*myc*, amplification involving N-*myc* in neuroblastomas, and some point mutations in c-*ras*).

7.2.3.1 Proto-oncogene Activation Through Chromosomal Translocation

Translocation leading to structural alteration of bcr-abl. Evolving techniques in cytogenetics over the last century have led to increased resolution of individual chromosomes. Abnormalities in chromosomes were known to occur in neoplastic cells from at least 1914, when Boveri noted somatic alterations in the genetic material of sea urchin eggs fertilized by two sperm. The abnormal cells looked similar to tumors, and he hypothesized that cancer might result from cellular aberrations that produced abnormal mitotic figures [12]. However, it was not clear whether chromosomal abnormalities represented primary oncogenic events, or accumulated errors secondary to neoplastic transformation. Initially, the plethora of chromosomal abnormalities favored the latter scenario, as no consistent chromosomal abnormality was identified upon examination of many tumors and similar tumors from different individuals. That changed in 1960 when Nowell and Hungerford described the first reproducible tumor-specific chromosomal aberration in chronic myelogenous leukemia [13]. They observed the presence of a shortened chromosome 22, subsequently named the Philadelphia chromosome after the city in which it was dis-

covered. It was found in cancer cells from over 90% of patients with chronic myelogenous leukemia (CML). This observation suggested that (1) the abnormality may have imparted some form of growth advantage over other cells and may be causally related to the development of tumors, and (2) other neoplasms may also harbor their own specific chromosomal or genetic aberrations. Since the first recognition of common chromosomal abnormalities in specific human tumors, numerous translocations involving important genes have been characterized (Table 7.2).

Table 7.2 Chromosomal translocation breakpoints and genes

Type	Affected gene	Disease	Rearranging gene
Non-fusions/hematopoietic Tumors			
Basic-helix-loop-helix			
t(8;14)(q24;q32)	c-*myc* (8q24)	BL, BL-ALL	IgH, IgL
t(2;8)(p12;q24)			
t(8;22)(q24;q11)			
t(8;14)(q24;q11)	c-*myc* (8q24)	T-ALL	TCRα
t(8;12)(q24;q22)	c-*myc* (8q24)	B-CLL/ALL	
	BTG (12q22		
t(7;19)(q35;p13)	*lyl1* (19p13)	T-ALL	TCRβ
t(1;14)(p32;q11)	*tal1*/SCL	T-ALL	TCRα
t(7;9)(q35;q34)	*tal2* (9q34)		
LIM proteins			
t(11;14)(p15;q11)	RBTN1/Ttg1 (11p15)	T-ALL	TCRδ
t(11;14)(p13;q11)	RBTN2/Ttg2 (11p13)	T-ALL	TCRδ/α/β
t(7;11)(q35;p13)			
Homeobox protein			
t(10;14)(q24;q11)	*hox11* (10q24)	T-ALL	TCRα/β
t(7;10)(q35;q24)			
Zinc-finger protein			
t(3;14)(q27;q32)	*Laz3*/*bcl6* (3q27)	NHL/DLCL	IgH
t(3;4)(q27;p11)	*Laz3*/*bcl6* (3q27)	NHL	
Others			
t(11;14)(q13;q32)	*bcl1* (*PRAD-1*) (11q13)	B-CLL and others	IgH, IgL
t(14;18)(q32;21)	*bcl2* (18q21)	FL	TCR-Cα
inv14 and t(14;14)(q11;32)	*TCL-1* (14q32.1)	T-CLL	IgH
t(10;14)(q24;q32)	*lyt-10* (10q24)	B lymphoma	IgH
t(14;19)(q32;q13.1)	*bcl3* (19q13.1)	B-CLL	IgH
t(5;14)(q31;q32)	*IL3* (5q31)	Pre-B-ALL	TCRβ
t(7;9)(q34;q34.3)	*tan1* (9q34.3)	T-ALL	TCRα
t(1;7)(p34;q34)	*lck* (1p34)	T-ALL	TCRα
t(X;14)(q28;11)	*C6.1B* (Xq28)	T-PLL	

Type	Affected gene	Protein domain	Fusion protein	Disease
Gene fusions/ hematopoietic tumors				
inv 14(q11;q32)	*TCR*α (14q11)	TCR-Cα	VH-TCR-Cα	T/B-cell lymphoma
	VH (14q32)	lg VH		
t(9;22)(q34;q11)	*CABL* (9q34)	Tyrosine kinase	Serine + tyrosine kinase	CML/ALL
	bcr (22q11)	Serine kinase		
t(1;19)(q23;p13.3)	*PBX1* (1q23)	HD	AD + HD	Pre-B-ALL
	E2A (19p13.3)	AD-bHLH		
t(17;19)(q22;p13)	*HLF* (17q22)	bZIP	AD + bZIP	Pro-B-ALL
	E2A (19p13)	AD-b-HLH		
t(15;17)(q21-q11-22)	*PML* (15q21)	Zinc finger	Zn-finger + RAR DNA	APL
	*RAR*α (17q21)	Retinoic acid receptor-α	and ligand binding	

(continued)

Table 7.2 (continued)

Type	Affected gene	Protein domain	Fusion protein	Disease
t(11;17)(q23;q21.1)	*PLZF* (11q23)	Zinc-finger	Zn-finger + RAR DNA	APL
	*RAR*α (17q21)	Retinoic acid receptor-α	and ligand binding	
t(4;11)(q21;q23)	*mll* (11q23)	A-T hook/Zn-finger	A-T hook + Ser-pro	ALL/PreB-ALL/ANLL
	AF4 (4q21)	Ser-Pro rich		
t(9;11)(q21;q23)	*AF9/MLLT3* (9p22)	(Ser-Pro rich)	A-T hook + (Ser-pro)	ALL/PreB-ALL/ANLL
	mll (11q23)	A-T hook/Zn-finger		
t(11;19)(q23;p13)	*mll* (11q23)	A-T hook/Zn-finger	A-T hook + Ser-pro	Pre-B-ALL/
	ENL (19p13)	Ser-Pro rich		T-ALL/ANLL
t(X;11)(q13;q23)	*AFX1* (Xq13)	(Ser-Pro rich)	A-T hook + (Ser-pro)	T-ALL
	mll (11q23)	A-T hook/Zn-finger		
t(1;11)(p32;q23)	*AF1P* (1p32)	Eps-15 homolog	A-T hook + ?	ALL
	mll (11q23)	A-T hook/Zn-finger		
t(6;11)(q27;q23)	*AF6* (6q27)	Myosin homolog	A-T hook + ?	ALL
	mll (11q23)	A-T hook/Zn-finger		
t(11;17)(q23;q21)	*mll* (11q23)	A-T hook/Zn-finger	A-T hook + leucine zipper	AML
	AF17 (17q21)	Cys-rich/leucine zipper		
t(8;21)(q22;q22)	*eto/MTG8* (8q22)	Zn-finger	DNA binding +	AML
	*aml1/CBF*α (21q22)	DNA binding Zn-fingers		
		Runt homology		
t(3;21)(q26;q22)	*evi-1* (3q26)	Zn-finger	DNA binding +	CML
	aml1 (21q22)	DNA binding	Zn-fingers	
t(3;21)(q26;q22)	*EAP* (3q26)	Sn Protein	DNA binding +	Myelodysplasia
	aml1 (21q22)	DNA binding	out-of-frame EAP	
t(16;21)(p11;q22)	*FUS* (16p11)	Gln-Ser-Tyr/Gly-rich/	Gln-Ser-Tyr +	Myeloid
		RNA binding	DNA binding	
	erg (21q22)	Ets-like DNA binding		
t(6;9)(p23;q34)	*dek* (6p23)	?	? + ZIP	AML
	can (9q34)	ZIP		
9;9?	*set* (9q34)	?	? + ZIP	AUL
	can (9q34)	ZIP		
t(4;16)(q26;p13)	*IL2* (4q26)	IL2	IL2/TM	T-lymphoma
	BMC (16p13.1)	?/TM domain		
inv(2;2)(p13;p11.2-p14)	*rel* (2p13)	DNA binding-activator	DNA binding + ?	NHL
	NRG (2p11.2-p12)	?		
inv(16)(p13;q22)	Myosin *MYH11* (16p13)		DNA binding?	AML
	*CBF*β (16q22)			
t(5;12)(q33;p13)	*PDGFb* (5q33)	Receptor kinase	Kinase + DNA binding	CMML
	TEL (12p13)	Ets-like DNA binding		
t(2;5)(p23;q35)	*NPM* (5q35)	Nucleolar phosphoprotein	N-terminus NPM	NHL
	ALK (2p23)	Tyrosine kinase	+ kinase	
Gene fusions/solid tumors				
inv10(q11.2;q21)	*ret* (10q11.2)	Tyrosine kinase	Unk + tyrosine kinase	Papillary thyroid
	D10S170 (q21)	Uncharacterized		Carcinoma
t(11;22)(q24;q12)	*fli1* (11q24)	Ets-like DNA binding	Gin-Ser-Tyr + DNA binding	Ewing's sarcoma
	ews (22q12)	Gin-Ser-Tyr/Gly-rich/RNA binding		
t(21;22)(?;q12)	*erg* (21q22)	Ets-like DNA binding	Gin-Ser-Tyr + DNA binding	Ewing's sarcoma
	ews (22q12)	Gin-Ser-Tyr/Gly-rich/RNA binding		

(continued)

Table 7.2 (continued)

Type	Affected gene	Protein domain	Fusion protein	Disease
t(12;22)(q13;q12)	*AFT1* (12q13)	bZIP	Gin-Ser-Try-bZIP	Melanoma
	ews (22q12)	Gin-Ser-Tyr/Gly-rich/RNA binding		
t(12;16)(q13;p11)	*CHOP* (12q13)	(DNA binding?)/ZIP	Gin-Ser-Tyr	Liposarcoma
	FUS (16p11)	Gin-Ser-Tyr/Gly-rich/RNA binding		
t(2;13)(q35;q14)	*PAX3* (2q35)	Paired box/homeodomain	+ (DNA binding?)/ZIP	Rhabdomyosarcoma
	FKHR (13q14)	Forkhead domain		
t(X;18)(p11.2;q11.2)	*SYT* (18q11.2)	None identified	PB/HD + DNA binding	Synovial sarcoma
	SSX (Xp11.2)	None identified		

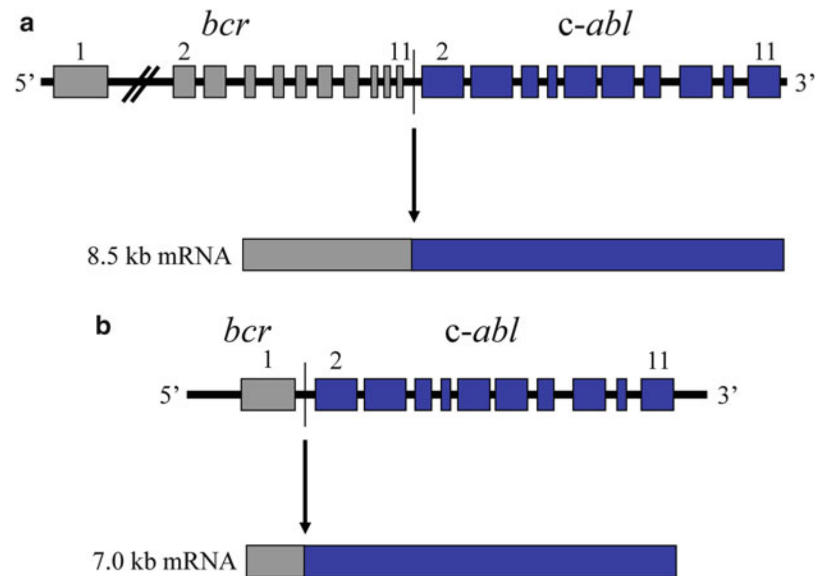

Fig. 7.1 *bcr-abl* translocation in chronic myelogenous leukemia. The c-*abl* proto-oncogene on chromosome 9 is translocated to the breakpoint cluster region (*bcr*) of chromosome 22. The result is a novel tyrosine kinase which functions independently of normal regulatory elements. (**a**) The t(9;22) that is commonly observed in chronic myelogenous leukemia (CML). (**b**) The t(9;22) that is commonly observed in acute lymphocytic leukemia.

Consequent to rapid advances in cytogenetic resolution techniques, Rowley in 1973 [14] found that the Philadelphia chromosome actually resulted from a reciprocal translocation involving the long arms of chromosomes 9 (9q34) and 22 (22q11). Analysis of the affected region on chromosome 9 revealed a proto-oncogene, c-*abl* [15], which when translocated to chromosome 22 generates a fusion gene. The c-*abl* proto-oncogene has 11 exons that encode for a 145 kDa protein with tyrosine kinase activity. The chromosomal breakpoint within the c-*abl* gene consistently involves one of two alternatively spliced exons. Breakpoints along the functional gene on chromosome 22 are clustered near the center in a 6 kb region termed the breakpoint cluster region (*bcr*). Upon translocation, nearly the entire c-*abl* proto-oncogene is placed under *bcr* promoter activity. Transcription and splicing yield a long mRNA transcript encoding a chimeric 210 kDa protein that expresses increased tyrosine kinase activity, likely because it is less responsive to normal regulatory elements (Fig. 7.1). A similar translocation exists in some acute lymphoblastic leukemias, although the breakpoint occurs further upstream in the *bcr* gene which results in a smaller chimeric protein (190 kDa), which has also been shown to have increased tyrosine kinase activity [16–20].

Translocation leading to dysregulation of c-myc. Investigation into the role of the c-*myc* proto-oncogene in neoplastic transformation led to development of a model of proto-oncogene activation based upon insertional mutagenesis. This mechanism of proto-oncogene activation emerged from studies of acutely transforming retroviruses and weakly oncogenic or non-transforming retroviruses. The primary differences between these two classes of retrovirus reflect the amount of time necessary for induction of tumors after infection of cells and the genomic content of their proviral DNA. Acutely transforming retroviruses have oncogenes incorporated into their genome while non-transforming retroviruses do not. Thus, the transformation potential of weakly oncogenic and non-transforming retroviruses depend on insertion adjacent to a cellular proto-oncogene. Although retroviruses insert randomly, in independently derived tumors retroviral sequences were found incorporated into similar chromosomal locations in the host genomic DNA. The site of insertion then became the focus of attention, and

cellular homologs of known retroviral oncogenes and their surrounding sequences were studied intensely. Finally, Hayward and Astrin demonstrated that non-acutely transforming retroviruses insert adjacent to and cause activation of the cellular proto-oncogene c-*myc* [21]. Insertional mutagenesis is based on the ability of proviral DNA to insert into host genomic DNA and cause either activation or inactivation of host genes, independent of expression of retroviral genes (as in the case of acutely transforming viruses). In the case of insertional activation of cellular genes, the proviral DNA may provide a promoter or enhancer for the cellular gene, resulting in an alteration in the normal regulation and expression pattern of the affected gene.

In the early 1980s data on c-*myc* activation by non-acutely transforming retroviruses in chicken lymphomas merged with data accumulating on translocations in Burkitt's lymphoma, a high grade B lymphocyte neoplasm. It was reasoned that if proviral sequences were capable of altering host cellular gene expression to cause tumors, chromosomal alterations that juxtapose promoter or enhancing sequences and cellular proto-oncogenes (through chromosomal translocation) were likely to promote neoplastic transformation. The best studied translocations at the time involved those of Burkitt's lymphoma, in which a portion of the long arm of chromosome 8 is consistently translocated to either chromosome 14, 2, or 22, adjacent to the loci for immunoglobulin heavy chain, k light chain, and λ light chain, respectively. The immunoglobulin loci on chromosomes 14, 2, and 22 were postulated to be good partner candidates to be coupled with and cause activation of a proto-oncogene that was suspected to reside on chromosome 8. Tumor DNA was directly probed for c-*myc* sequences. The gene was detected on chromosome 8, and found to be translocated to chromosomes 14, 2, and 22 in Burkitt's lymphoma and in some plasmacytomas. In plasmacytomas, a form of the c-*myc* proto-oncogene lacking the untranslated exon 1 is involved in the chromosomal translocation (Fig. 7.2). The breakpoints within the c-*myc* gene are more varied in Burkitt's lymphoma. Nonetheless, the translocation results in abnormal (constitutive) expression of a c-*myc* coding sequence identical to its normal allele in both types of tumor. This observation strongly suggested the chromosome translocation-mediated activation of the c-*myc* proto-oncogene as a causal event in human tumorigenesis [4, 22–25].

7.2.3.2 Proto-oncogene Activation Through Gene Amplification

Activation of cellular proto-oncogenes can occur as a consequence of DNA amplification resulting in overexpression of the amplified proto-oncogene, which confers a proliferative advantage to affected cells. Amplified gene segments can be discerned cytogenetically as double minute chromosomes (DMs) and homogeneously staining regions (HSRs). Proto-oncogenes (and other genetic loci) are amplified by repeated DNA replication events that can result in an abnormal homogeneous staining pattern in a karyotypic analysis of affected cells, rather than the familiar chromosomal staining pattern in R-banded or G-banded chromosome spreads. Instead, homogeneously staining regions appear as abnormally extended R-bands or G-bands (Fig. 7.3). The tandem arrays of amplified DNA forming homogeneously staining regions may be excised from the chromosome to form double minutes, which are small chromosomal structures lacking centromeres that do not replicate during cell division. Double minutes may integrate into other chromosomes to create additional stable HSRs able to propagate upon cell division [26, 27].

In the same way that investigation of the c-*myc* proto-oncogene formed the underpinnings of our current understanding of proto-oncogene activation by means of chromosomal translocation, studies of the c-*myc* proto-oncogene led to the unraveling of proto-oncogene activation through gene amplification in human neoplasms [28]. DNA amplification represents

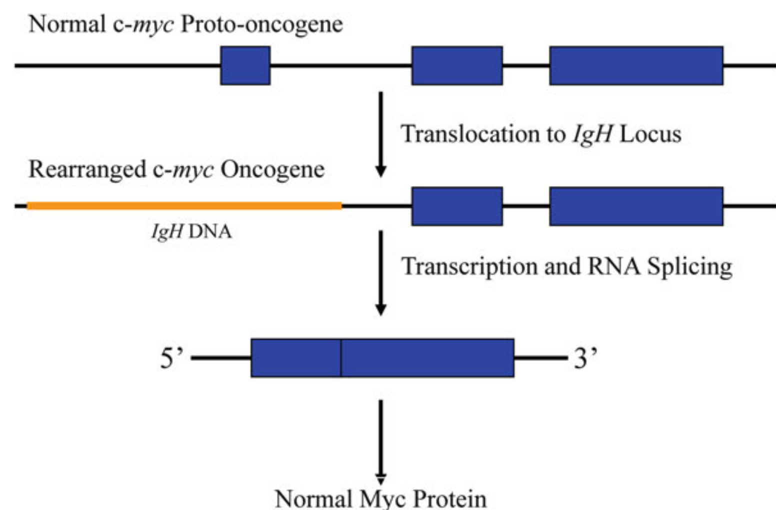

Fig. 7.2 Consequences of the t(8;14) chromosomal translocation. The c-*myc* gene of chromosome 8 is normally not expressed in differentiated B cells. After translocation, it comes under the control of either a cryptic promoter in intron 1 or an enhancer from the immunoglobulin locus of chromosome 14 (*IgH*), leading to constitutive expression of the normal c-myc protein (exon 1 is noncoding). *IgH* gene sequences are depicted in *orange* and c-*myc* gene sequences are depicted in *blue*.

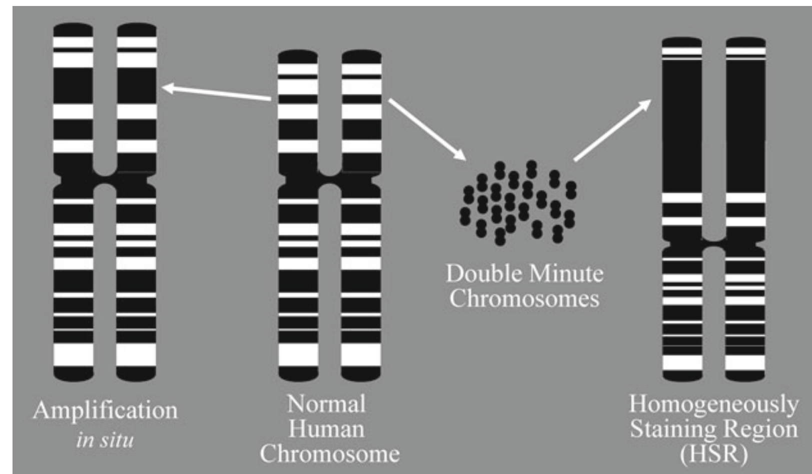

Fig. 7.3 DNA amplification. When DNA is amplified by repeated DNA replication events, the results can sometimes be seen cytogenetically as homogeneously staining regions (HSRs) or double minutes. Double minutes represent the extrachromosomal manifestation of HSRs. Double minutes can insert into any chromosome (the one they are derived form or a different chromosome).

one mechanism leading to drug resistance in mammalian cells [29]. Through direct probing for c-*myc* it was discovered that double minutes and homogeneously staining regions contained amplified copies of the oncogene in human colon carcinoma cells [30]. The c-*myc* gene has been shown to be amplified and overexpressed in a number of human neoplasms supporting the role of DNA amplification as a major mechanism for cellular proto-oncogene activation in neoplastic transformation.

Although the precise mechanism for gene amplification has not been entirely determined, the role amplification plays in cellular transformation in human malignancies is clear, particularly from studies of neuroblastomas and studies involving neoplastic transformation of cells in vitro [31]. Neuroblastoma is one of the most common childhood extracranial solid tumors accounting for approximately 15 % of all childhood cancer deaths [32]. Neuroblastomas exhibit DMs and HSRs that hybridize with probes to the c-*myc* gene. The hybridizing sequences were determined to be related to but distinct from c-*myc* and was designated N-*myc* [33]. The N-*myc* gene is transcribed at higher levels in neuroblastomas that demonstrate gene amplification. N-*myc* amplification is now a major prognostic determinant in neuroblastomas, with high levels of transcription from either a single copy or more commonly from increased gene copy number in the form of DMs or HSRs correlating with poor patient survival [34]. The demonstration of the link between high N-*myc* expression and poor clinical prognosis, and its demonstrated ability to cause neoplastic transformation in cell culture provides strong evidence for the importance of gene amplification in the activation of cellular proto-oncogenes. Table 7.3 lists other cellular proto-oncogenes that have been shown to be amplified in human neoplasms.

Table 7.3 Oncogene amplification in human tumors

Oncogene	Neoplasm
c-*myc* family	
c-*myc*	Leukemias, breast, stomach, lung, and colon carcinomas, neuroblastomas and glioblastomas
N-*myc*	Neuroblastomas, rhabomyosarcomas, retinoblastomas, lung carcinomas
L-*myc*	Lung carcinomas
c-*erb*B family	
c-*erb*B1	Glioblastomas, medulloblastomas, renal cell, squamous cell, breast, gastric and esophageal carcinomas
c-*erb*B2	Breast, salivary gland, gastric esophageal, lung, colon, and ovarian carcinomas
c-*ras* Family	
c-H-*ras*	Bladder carcinoma
c-K-*ras*	Lung, ovarian, breast, ovarian, and bladder carcinomas
c-N-*ras*	Breast, lung, and head and neck carcinomas
Other proto-oncogenes	
*int*2	Breast and squamous cell carcinomas
hst	Breast and squamous cell carcinomas
PRAD-1	Breast and squamous cell carcinomas
c-*abl*	K562 chronic myelogenous leukemia cell line
c-*myb*	Colon and breast carcinomas, leukemias
*ets*1	Lymphoma, breast cancers
gli	Glioblastomas
K-*sam*	Stomach carcinomas
*mdm*2	Sarcomas
11q13 locus	Breast, gastric, esophageal, squamous, ovarian, bladder carcinomas, and melanoma

7.2.3.3 Proto-oncogene Activation Through Point Mutation

Several cellular proto-oncogenes have been shown to be activated through point mutation. However, the c-*ras* family of proto-oncogenes represent the most important subset of proto-oncogenes that are activated through this mechanism. The c-*ras* genes were the first human proto-oncogenes identified using gene transfer assays [9, 10]. This family includes the cellular homologs of the Harvey-*ras* (H-*ras*) and Kirsten-*ras* (K-*ras*) retroviral oncogenes, both of which had previously been shown to induce sarcomas in rats [11]. DNA extracted from various human tumor cell lines have been

shown to induce transformation of mouse fibroblast cell lines in vitro, and the most commonly isolated sequences responsible for neoplastic transformation are members of the c-*ras* family of proto-oncogenes [35, 36]. The activated form of c-*ras* (oncogenic) exhibits markedly different transforming properties from that of the normal c-*ras* proto-oncogene. The activated form consistently and efficiently induces neoplastic transformation in cultured cells, whereas the normal proto-oncogene does not. The critical molecular difference between the two forms of c-*ras* was found in the nucleic acid sequence: the activated form of c-*ras* harbors a point mutation in codon 12 of exon 1, which results in a glycine to valine amino acid substitution [37–39]. Up to 30 % of all human neoplasms are now known to harbor c-*ras* mutations, and mutations in c-H-*ras*, c-K-*ras*, and N-*ras* reflect specific alterations affecting only codon 12 (most mutations), codon 13, or codon 61. An additional mutation in an intron of c-H-*ras* has been shown to upregulate production of the structurally normal gene product, resulting in increased transforming activity [40]. A common theme of c-*ras* mutations is that a single point mutation is capable of drastically altering the biological activity of a normal protein product into one with efficient transforming properties. Mutations of c-*ras* are found in a large number of human tumor types, including thyroid [41–43], gastrointestinal tract [44–48], uterus [49–53], lung [54–58], myelodysplastic syndromes [59], and leukemias [60–63]. The incidence of c-K-*ras* gene mutations is highest for exocrine pancreas and bile duct carcinomas, which has led to the development of ancillary diagnostic techniques for the detection of pancreatic and bile duct carcinomas [64–66].

7.2.4 Protein Products of Oncogenes

Proto-oncogene protein products regulate cell proliferation and differentiation. Oncogene protein products often closely resemble their proto-oncogene protein products, but differ in that they act independently of normal regulatory elements. Events that occur as a part of normal cell growth and differentiation can often be simplified into a series of four steps, all of which involve proto-oncogenes normally, and each of which is subject to disruption during neoplastic transformation: (1) an extracellular growth factor binds to a specific receptor on the plasma membrane, (2) the growth factor receptor is transiently activated, leading to a cascade of signaling cellular events, many of which involve signal-transducing proteins on the plasma membrane, (3) the signal/message is transmitted from the plasma membrane to the nucleus via secondary messenger molecules, and (4) the nuclear regulatory machinery is induced/activated to initiate cell replication and transcription. Within these pathways, there are three major biochemical mechanisms through which these oncoproteins function [67, 68]. The first of these mechanisms involves the phosphorylation of target proteins at serine, tyrosine, and threonine amino acid residues. The second mechanism involves intracellular signal transmission through proteins with GTPase activity. The last of these involves the transcriptional regulation of structural genes in the nucleus.

7.2.4.1 The Fibroblast Growth Factor Family

The discovery of growth factors in the early 1960s led to the isolation of a diverse group of factors affecting all cells. Growth factors are grouped into families that share significant sequence homology and cell surface receptors. One example is the epidermal growth factor (EGF) family which includes among others, EGF and the transforming growth factor TGFα [69, 70]. EGF, one of the earlier growth factors discovered, was shown to be a polypeptide of 53 amino acids that stimulated proliferation of a variety of different cell types. Growth factors were not only capable of promoting growth, but some also concurrently promoted differentiation [71]. In normal cells, growth factors induce cells to exit the resting or G_0 phase and enter the cell cycle, or they may stimulate cells already cycling. It follows that the biochemical and physiologic effect of aberrant expression of growth factors leads to constitutive stimulation of cell growth, potentiating the process of cell transformation.

Platelet derived growth factor (PDGF) is another growth factor shown to have transforming potential, and has an important history in that it provided the first link between two originally disparate tracks of research: (1) biochemical studies of the regulation of cell proliferation (growth factors), and (2) molecular analysis of neoplastic transformation (oncogenes) [72]. In the early 1980s, two groups working independently reported PDGF was the protein product of an oncogene [4, 73, 74]. Each group determined a partial amino acid sequence of PDGF, and with a computer search of a protein sequence database, amino acid sequence homology was demonstrated with the predicted sequence of v-*sis*, the simian sarcoma virus. From this initial work, oncogenes activated by mechanisms described above frequently have been shown to encode growth factors that participate in mitogenic signaling and cell transformation [69].

Further understanding the mechanism of action of transforming growth factors came from a hypothesis forwarded in 1977 by George Todaro [75]. He suggested that because transformed cells are capable of producing growth factors, autocrine stimulation of cell growth could be, at least in part, responsible for transformation [75]. An individual cell abnormally overexpressing a growth factor to which it responds would result in continuous cell proliferation. This hypothesis gained credence with the discovery of the homology between PDGF and the protein product of v-*sis* as well as from work on the EGF family of growth factors (EGF and TGFα, among others). Several human tumors are known to

overexpress TGFα as well as its receptor the epidermal growth factor receptor (EGFR) which substantiated the autocrine mechanism [76, 77]. The transfection of TGFα genes into cultured cells could induce transformation [78, 79]. Finally, the link between mitogenic signaling and cell transformation properties of growth factors and oncogenes was strengthened by data showing TGFα overexpression in transgenic mice results in the development of tumors [4, 80, 81]. Additional growth factors with oncogenic potential are listed in Table 7.4.

7.2.4.2 The EGFR Family of Growth Factor Receptors

The EGFR family is one of many growth factor families capable of promoting neoplastic transformation. The EGFR family includes c-*erb*B1, c-*erb*B2 (also known as *neu*), and c-*erb*B3, and are structurally related to other transmembrane tyrosine kinase proteins with an external ligand binding domain, a transmembrane domain, and an internal tyrosine kinase domain (Fig. 7.4) [82]. These receptors are activated by binding of a ligand which is followed by transduction of a signal into the cell through the kinase activity of the intracellular domain of the receptor protein. Kinase enzymes regulate protein function by phosphorylation of tyrosine, serine, or threonine amino acid residues. Examples of these receptor tyrosine kinase proteins are listed in Table 7.5. The EGFR was implicated as playing a central role as a regulator of normal cellular growth and differentiation primarily because the kinase activity of the EGFR is stimulated by EGF or TGFα binding [83, 84]. Also, the human *EGFR* gene was linked via significant sequence homology to a known avian erythroblastosis virus oncogene, v-*erb*B. Eventually it was determined that the v-*erb*B gene product was a truncated protein derived from the *EGFR* gene. The v-*erb*B gene product lacks the extracellular ligand binding domain (the amino-terminal half

Table 7.4 Growth factors with oncogenic potential

PDGF family	A chain
	B chain (c-*sis*)
FGF family	Acidic FGF (aFGF)
	Basic FGF (bFGF)
	Int-2
	hst (KS3)
	Fgf-5
EGF family	EGF
	TGFα
Wnt family	Wnt-1
	Wnt-3
Neurotrophins	NGF
	BDNF
	NT-3
Hematopoietic growth factors	Interleukin-2
	Interleukin-3
	M-CSF
	GM-CSF

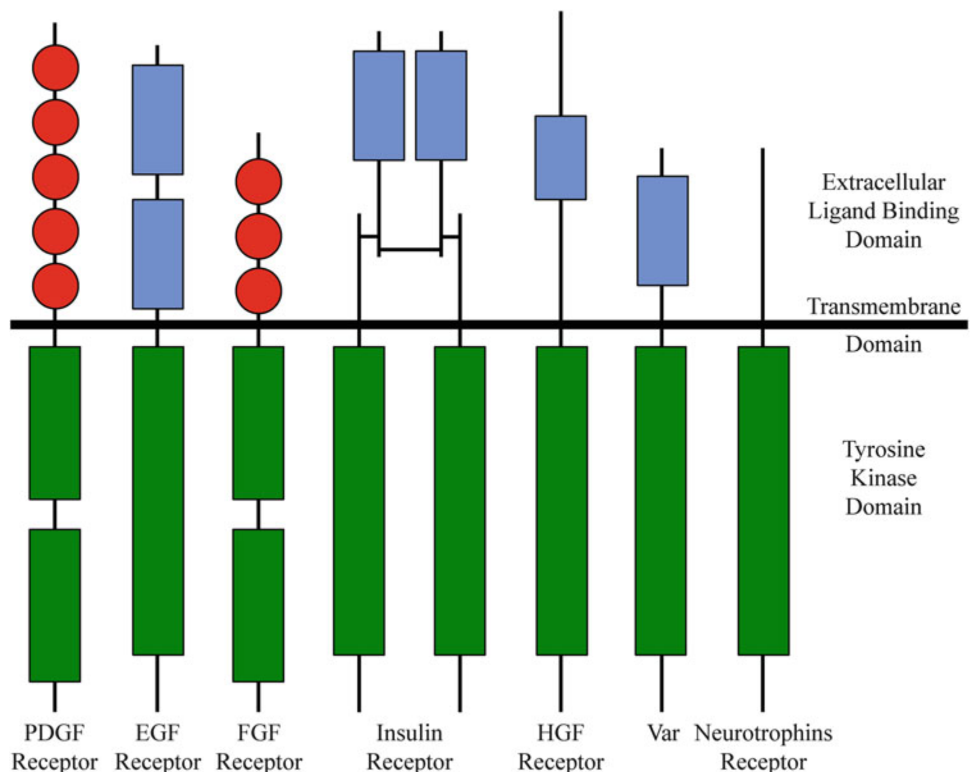

Fig. 7.4 Transmembrane tyrosine kinases. Growth factor receptors with tyrosine kinase activity share similar overall structure with extracellular binding domains, transmembrane domains, and cytoplasmic tyrosine kinase domains. Each receptor is designated according to its prototype ligand. *Red circles* illustrate immunoglobulin-like repeats. *Blue boxes* denote cytosine-rich domains. *Green boxes* represent conserved tyrosine kinase domains. The receptor labeled "Var" depicts the structure for receptors bound by ret, ros, axl, alk, eph, and eck (possibly others).

Table 7.5 Receptor protein-tyrosine kinases

EGF	erbB1 (c-*erb*B)
	erbB2 (*neu*)
	erbB3
	erbB4
FGF	FGF receptor-1 (*fig*)
	FGF receptor-2 (K-*sam*)
	FGF receptor-3
	FGF receptor-4
PDGF	PDGF α-receptor
	PDGF β-receptor
	CSF-1 receptor (c-*fms*)
	SLF receptor (c-*kit*)
Insulin	Insulin receptor (α, β)
	IGF-1 receptor (c-*ros*)
Hepatocyte growth factor	HGF receptor (*met*) (α, β)
	c-*sea* (ligand unknown) (α, β)
Neurotrophin	NGF receptor (*trk*)
	BDNF and NT4 receptor (*trk*-B)
	NT3 receptor (*trk*-C)
Ligands unknown	*eph*/*elk*
	VEGF Receptor
	eck
	c-*ret*
	axl

Table 7.6 Proto-oncogenes which encode for cytoplasmic serine/threonine kinases and non-receptor tyrosine kinases with oncogenic potential

Serine–threonine kinases
c-*raf* Family
raf-1
A-*raf*
B-*raf*
Protein kinase C family
PKC-β1
PKC-γ
PKC-ε
PKC-ζ
Other serine–threonine kinases
mos
pim-1
akt
cot
tpl-2
Non-receptor tyrosine kinases
yes
fgr
fyn
lck
abl
fps/fes

of the normal protein) that is present in the normal EGFR protein. This structural aberration results in a constitutively activated protein with tyrosine kinase activity. The constitutive cell signaling activity of the truncated receptor drives signal transduction and cell proliferation in the absence of growth factor stimulation. Thus, an oncogene was shown to correspond to a known growth factor receptor, which established a direct link between the two [4, 85–89].

Although structural aberrations play an important role in EGFR-mediated neoplastic transformation, a more common mechanism is overexpression of the normal proto-oncogene product, as is seen in breast cancers. Overexpression occurs not only as a result of gene amplification, but also in the absence of gene amplification, suggesting another as yet undetermined mechanism. Overexpression of EGFR has been found to have prognostic significance in several human tumors [77, 90].

7.2.4.3 Proteins Involved in Signal Transduction

c-abl. Once a cell receives a signal via plasma membrane bound receptors, it is transmitted to the cell nucleus by a cascade of messenger molecules. Because abnormal growth factor receptors can function as oncoproteins by stimulating cell proliferation, it follows that protein messengers coupled to receptors, or even proteins involved in signal transduction that are not associated with receptors, can act as equally potent oncoproteins. In fact, many such oncoproteins have been identified, and they have been shown to mimic the normal function of signal transducing proteins. The signal transducing proteins can be widely grouped into two categories (Table 7.6): (1) protein kinases (non-receptor associated tyrosine kinases, such as the c-*abl* protein product, and cytoplasmic serine/threonine kinases) and (2) receptor-associated GTP-binding proteins (which include the c-*ras* proteins). The c-*abl* protein product is present on the inner surface of the plasma membrane. However, its tyrosine kinase activity is not dependent on coupling with a plasma membrane bound receptor. Rather, negative regulatory domains are lost when c-*abl* of chromosome 9 is translocated to the breakpoint cluster region of chromosome 22. The hybrid protein product has increased enzymatic activity responsible for phosphorylating downstream substrates. This constitutive activity drives cells to proliferate, contributing to neoplastic growth. This form of molecular aberration characterizes chronic myelogenous leukemia (CML) and some forms of acute lymphoblastic leukemia (ALL).

c-ras. The larger category of signal transducing proteins is associated with membrane bound receptors, such as the GTP-binding proteins (G proteins), which include the c-*ras* family of proteins. There are many similarities between the latter two sets of proteins. G proteins are located on the inner face of the cell membrane, where they couple signals received

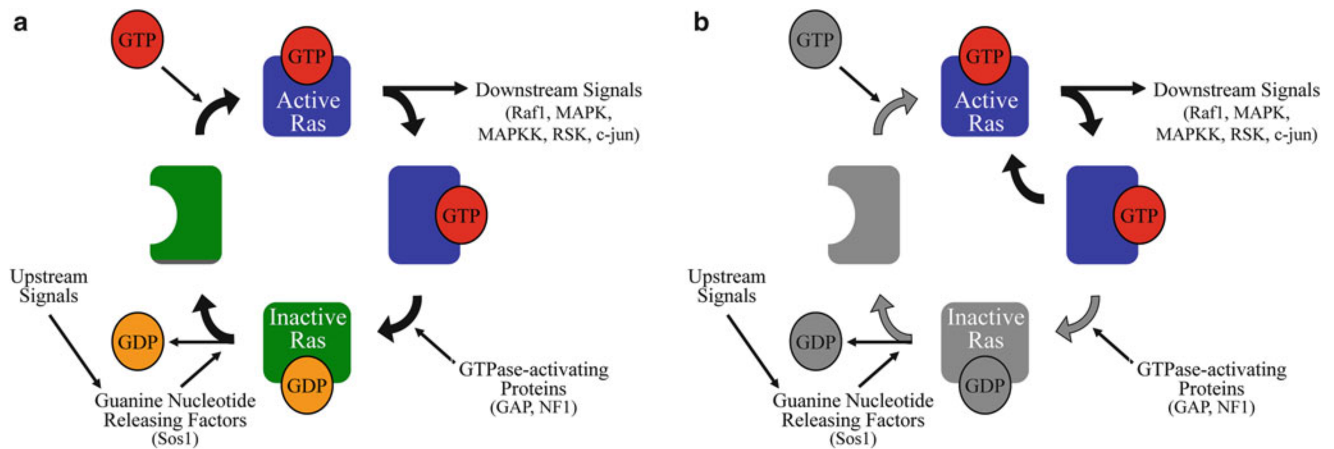

Fig. 7.5 c-ras mechanism of action. The c-ras protein is active when complexed with GTP, and this interaction is facilitated by GEFs in response to growth factor stimulation. Although c-ras has its own GTPase activity, its inactivation by GTP hydrolysis is stimulated by GAPs, such as neurofibromin. Mutated c-ras protein has a decreased ability to hydrolyze GTP, or an increased rate of exchange of bound GDP for free GTP. By either mechanism, the result is increased activated c-ras. (**a**) Depicts normal c-ras regulation (activation and inactivation). (**b**) Depicts aberrant c-ras constitutive activation when mutated.

from activated plasma membrane receptors to other, mainly cytoplasmic, second messengers in a cascade ultimately culminating in the cell nucleus. Cell signaling through G proteins requires GTP binding. Hydrolysis of the bound GTP terminates signaling through a specific G protein molecule.

The c-*ras* proto-oncogenes are activated by point mutations. As would be predicted, the point mutations alter the function of the GTPase activity of its protein product. The mutated protein has a decreased ability to hydrolyze GTP, or has an increased rate of exchange of bound GDP for free GTP (Fig. 7.5). The decreased ability to hydrolyze GTP results from interactions between c-*ras* and GTPase activating proteins (GAPs), an example of which is neurofibromin, the gene product of the tumor suppressor gene neurofibromatosis 1 (NF1). GAPs inactivate normal c-*ras* protein by augmenting the conversion of GTP-*ras* (active form) to GDP-*ras* (inactive form). The activated c-ras oncoproteins bind GAP, but their GTPase activity is stunted, leading to upregulation of active GTP-*ras*. Given the central role of GAPs in c-*ras* regulation, it is not surprising that the loss of activity of the GAP neurofibromin also results in upregulation of *ras*-GTP in affected cells. In response to growth factor stimulation c-*ras* is activated by guanine nucleotide releasing or exchange factors (GEFs). They are responsible for the exchange of GDP for GTP (converting inactive ras-GDP to active ras-GTP). The increased exchange of GDP for GTP produces the same result: constitutively active c-ras protein bound to GTP. Interestingly, a GEF domain is present in the *bcr* protein product as well as in the *bcr-abl* fusion protein product created in the t(9;22) translocation, suggesting a possible role in c-*ras* regulation [4, 35, 91–95].

7.2.4.4 The c-myc Family of Nuclear Regulatory Proteins

The final steps in the mitogenic signaling pathways involve signal entry into and the subsequent events which occur in the nucleus. The protein products of many proto-oncogenes (and tumor suppressor genes) are localized to the nucleus, and function to control the transcription of growth-related genes through interaction with specific regulatory DNA sequences. Regulation of transcription is a key mechanism through which proto-oncogenes (and tumor suppressor genes) exert control over cell proliferation. Nuclear proteins involved in these processes generally bind upstream to a specific gene and function as a transcription factor. Once bound to a specific DNA sequence, they act to increase expression of the target gene by interacting with other proteins involved in transcription. In order to be able to span the interaction between DNA and other proteins, most transcription factors have two functional domains: (1) a DNA binding domain, and (2) a protein binding domain. The DNA binding domain is often either a cysteine rich region, the secondary structure of which binds zinc and forms looped structures (called zinc fingers), or a stretch of basic amino acids proximal to leucine zipper motifs (ZIP) or basic helix-loop-helix (bHLH) domains. The leucine zipper is composed of a stretch of hydrophobic leucine residues and functions in protein dimerization. The bHLH motif is composed of two amphipathic α-helical regions separated by a loop and also functions in protein dimerization [5].

Because the c-*myc* oncogene is commonly involved in human tumorigenesis, there has been tremendous research activity into its mechanism of action. The c-*myc* proto-oncogene is expressed in nearly all eukaryotic cells and its

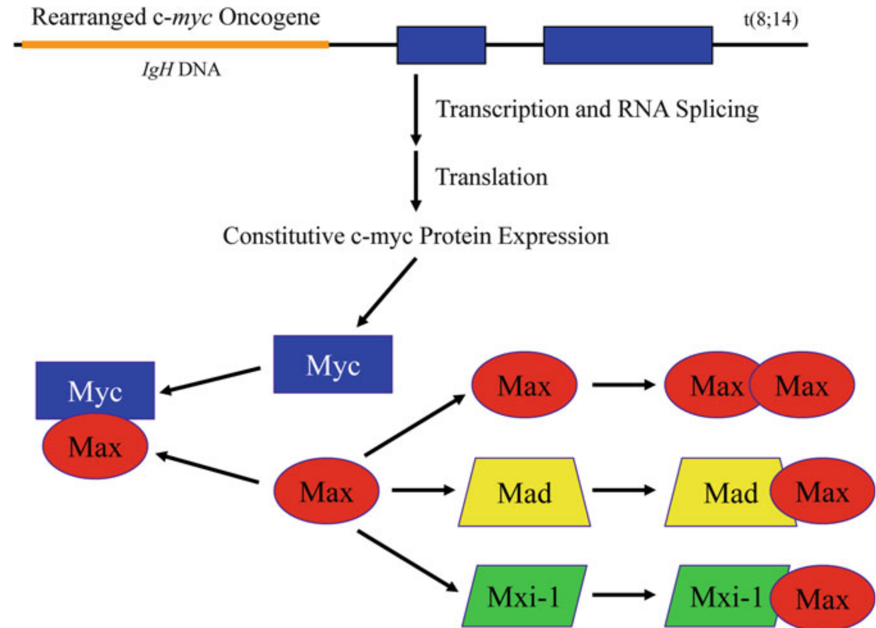

Fig. 7.6 The myc/max/mad/mxi-1 transcription network in Burkitt's lymphoma. The c-myc protein is expressed constitutively as a result of a chromosomal translocation: t(8;14), t(8;22), or t(2;8). The t(8;14) translocation is depicted in the schematic. The Myc protein dimerizes with Max to activate transcription. The Max protein can homodimerize, or form complexes with Mad or Mxi-1. Each of these complexes inhibits transcription. Constitutive expression of c-myc leads to a shift in the equilibrium in favor of transcriptional activation.

mRNA synthesis is rapidly induced when quiescent cells receive a signal to divide. The C-terminal region of c-myc proteins has one basic DNA binding domain followed by bHLH and ZIP domains. The c-myc protein was suggested to function as a transcription factor based upon the pattern of transient expression following cell stimulation by growth factors and sequence similarities with other DNA binding transcription factors. However, it was found that the c-myc protein by itself does not bind DNA well. Rather, c-*myc* binds to DNA with greater affinity when dimerized with another protein possessing c-*myc*-like bHLH and ZIP domains, called max (Fig. 7.6). Max is a small protein that forms homodimers, interacts with all members of the c-myc family, and forms heterodimers with other proteins called mad and mxi1. Although c-*myc*, max, and mad share sequence domains, they differ in that c-*myc* has a transcriptional activation domain at its amino terminus. Thus, the *myc*-max heterodimer represents the functional form of c-*myc*, and upon binding to specific CACGTG DNA sequences, stimulates expression of genes involved in cell proliferation.

Max-max homodimers and heterodimers composed of max-mad and max-mxi1 also binds DNA efficiently. These other max-containing complexes compete with *myc*-max heterodimers for DNA binding. However, the proteins that compose these complexes lack a transcriptional activation domain. Therefore, DNA binding by any of these other complexes results in repression of transcription. The control over cell proliferation is influenced by the balance between transcriptional activation by *myc*-max heterodimers and transcriptional repression by max-max, max-mad, and max-mxi1 complexes. A common theme of c-*myc* activation by chromosome translocation, insertional mutagenesis, or amplification, is overexpression of the c-myc protein. Overexpression of the c-myc protein leads to a shift in equilibrium toward *myc*-max dimers, activating transcription, promoting cell proliferation, and thereby contributing to neoplastic transformation [4, 22, 96–103].

7.3 Tumor Suppressor Genes

While proto-oncogenes are normal cellular genes that act in a positive fashion to promote physiologic cell growth and differentiation, tumor suppressor genes act as the cellular braking mechanism, regulating cell growth in a negative fashion. Normal tumor suppressor proteins exhibit diverse functions and are found in all subcellular compartments. As alterations of tumor suppressor protein function contribute to the development of cancer, their principle normal function is likely the control of cellular proliferation and differentiation. The specific types of mutations in these genes invariably lead to the inability of the encoded protein to perform its normal function. In general, neoplastic transformation requires loss of tumor suppressor protein function, and this requires mutational inactivation or loss (deletion) of both alleles of the tumor suppressor gene. Thus, tumor suppressor genes are termed recessive and alterations of these genes are typically considered loss of function mutations. A list of known or putative tumor suppressor genes is given in Table 7.7.

In the mid and late 1960s theories about the genesis of malignant tumors were enormously influenced by studies of virus and gene transfer experiments. Introduction of a virus

Table 7.7 Putative and cloned tumor suppressor genes

Gene	Chromosomal location	Inherited cancer	Sporadic cancer
Rb1	13q14	Retinoblastoma	Retinoblastoma, sarcomas, bladder, breast, esophageal, and lung carcinomas
p53	17p13	Li–Fraumeni cancer family syndrome	Bladder, breast, colorectal, esophageal, liver, lung, and ovarian carcinomas, brain tumors, sarcomas, lymphomas, and leukemias
DCC	18q21	–	Colorecetal carcinomas
MCC	5q21	–	Colorectal carcinomas
APC	5q21	Familial adenomatous polyposis	Colorectal, stomach, and pancreatic carcinomas
WT1	11p13	Wilms tumor	Wilms tumor
WT2	11p15	Weidemann–Beckwith syndrome	Renal rhabdoid tumors, embryonal rhabdomyosarcoma
WT3	16q	Wilms tumor	–
NF1	17q11	Neurofibromatosis type 1	Colon carcinoma and astrocytoma
NF2	22q12	Neurofibromatosis type 2	Schwannoma and meningioma
VHL	3p25	von Hippel–Lindau syndrome	Renal cell carcinomas
MEN1	11q23	Multiple endocrine neoplasia type 1 (MEN1)	Endocrine tumors such as pancreatic adenomas
nm23	17q21	–	Melanoma, breast, colorectal, prostate, meningioma, others
MTS1	9p21	Melanoma	Melanoma, brain tumors, leukemias, sarcomas, bladder, breast, kidney, lung, and ovarian carcinomas

into cultured cells added new genetic information that led to transformation. Most investigators believed at the time that neoplastic transformation resulted from a simple gain of genetic information, rather than from a loss of some cellular gene. In Mendelian terms, malignancy was thought to be a dominant characteristic. Henry Harris of the University of Oxford in collaboration with George Klein of Stockholm forwarded another approach [104]. Cells from mouse tumor cell lines were fused with non-malignant cells, and the resulting hybrids were evaluated for their tumorigenic potential in appropriate hybrid animals. The hybrid cells produced very few tumors compared with the malignant parent cells. These results were interpreted to mean that normal cells contain one or more genes that act as negative regulators of the neoplastic phenotype. They postulated that malignancy was determined by a loss and not a gain of genetic information. At the time, these results were vigorously challenged [105].

Additional cell hybrid studies strengthened the concept that normal cellular genes can function to suppress the tumorigenic potential of neoplastically transformed cells. A cytogenetic analysis of cell hybrids that reexpressed tumorigenic potential established the chromosomal location of one of the normal genes in mouse that suppressed the malignant phenotype [106, 107]. Similar studies in human hybrid cells derived from the fusion of normal fibroblasts and HeLa cells (cervical carcinoma cell line) showed that reversion to the tumorigenic phenotype followed the loss of chromosome 11. Introduction of the wild-type allele by fusion with a normal cell once again suppressed malignancy, suggesting the presence of a tumor suppressor gene on this chromosome [4, 108, 109].

Some generalizations about tumor suppressor genes can be made (Table 7.8), and are further illustrated with specific examples below. Tumor suppressor gene mutations or deletions are often found as germ-line mutations associated with hereditary syndromes that predispose to the development of specific tumors. Mutations or deletions in the same genes involved in cancers arising in the setting of these hereditary syndromes are also found in sporadic tumors (tumors that arise in individuals known not to have germ-line mutations). Commonly, these somatic mutations can be found in tumors not related to those associated with hereditary syndromes. The latter findings in sporadic tumors suggest a broader role for these genes in tumorigenesis. In many but not all cases, tumor suppressor activity can be demonstrated in gene transfer assays. Tumor suppressor gene products are integral components of cell signaling pathways, in addition to having

Table 7.8 Characteristics of oncogenes and tumor suppressor genes

Characteristic	Oncogenes	Tumor suppressor gene
Number of mutational events required to contribute to cancer development	One	Two
Function of the mutant allele	Dominant (gain of function)	Recessive (loss of function)
Activity demonstrated in gene transfer assays	Yes	Yes
Associated with hereditary syndromes (inheritance of germ line mutations)	Seldom (c-*ret* proto-oncogene)	Often
Somatic mutations contribute to cancer development	Yes	Yes
Tissue specificity of mutational event	Some	In inherited cases, there is often a tissue preference

roles in cell–cell and cell–matrix interactions. Their role in the development of cancers has been demonstrated to be as significant as the role played by proto-oncogenes.

7.3.1 Mechanism of Tumor Suppressor Gene Action

The mechanism of action for the products of tumor suppressor genes is diverse and not fully understood. Conceptually, the products of tumor suppressor genes can be thought of as functioning to receive and process growth inhibitory signals from their surroundings. When a cell loses components of this signaling network, or loses responsiveness to extracellular growth inhibitory signals, the cellular consequences are the same as for unchecked stimulation of cell growth, neoplasia. The tumor suppressor gene products function in parallel with the protein products of proto-oncogenes, but work instead to suppress cell proliferation through the regulation of signal transduction and nuclear transcription. Not all tumor suppressor gene products conform to the growth inhibitory concept. Cell surface and cell matrix molecules are responsible for normal cell morphology, cell–cell interactions, and cell–extracellular matrix interactions. Tumor suppressor genes encode for such proteins, and mutations in these genes lead to altered cellular morphology, loss of normal intracellular signaling pathways, and loss of normal intercellular interactions, all of which are features of neoplastic cells.

7.3.1.1 Tumor Suppressor Gene Products That Regulate Signal Transduction

An example of a tumor suppressor gene whose product regulates signal transduction is the gene product of neurofibromatosis 1 (NF1), which is responsible for the clinical syndrome neurofibromatosis or von Recklinghausen's disease. Neurofibromatosis is one of the more common autosomal dominant disorders in humans and is clinically associated with café-au-lait spots (brown skin macules), benign neurofibromas, and other abnormalities. Patients with neurofibromatosis have a higher incidence of malignant tumors, including neurofibrosarcomas, pheochromocytomas, optic nerve gliomas, and malignant myeloid diseases [110].

The product of the *NF1* gene (neurofibromin) encodes for a GTPase activating protein (GAP). GAP proteins interact with c-ras proteins, which in normal cells are transiently activated upon exchange of bound GDP for bound GTP (Fig. 7.5). The c-ras proteins have intrinsic GTPase activity which is significantly increased upon binding with GAP proteins. Mutations in c-*ras* alter the GTPase activity of its protein product. The ras-GTP oncoprotein is significantly less responsive to GAP augmented hydrolysis [4, 35, 91–95]. It follows that mutations in genes that encode for GAP proteins, such as *NF1*, should be similarly deleterious to affected cells. In fact, *NF1* mutations are associated with and may contribute to the development of not only those tumors found in neurofibromatosis 1, but also adenocarcinomas of the colon, anaplastic astrocytomas, and myeloid malignancies, among others [110].

7.3.1.2 Tumor Suppressor Gene Products That Regulate Transcription

Examples of tumor suppressor genes whose products regulate transcription include *Rb1* and *p53*, the latter representing the gene most frequently involved in human cancers [111–113].

Rb1 gene. The discovery of the retinoblastoma gene (*Rb1*) gene resulted in an intense research effort to understand the mechanism of action of its gene product. In studies of cell cycle regulation, the *Rb1* tumor suppressor gene product was found to be active in a hypophosphorylated state, and inactive in a hyperphosphorylated state. Further, the active hypophosphorylated gene product was present in abundance in the G_0/G_1 stage of the cell cycle as compared to the finding of abundant inactive hyperphosphorylated pRb protein in late G_1, S, G_2, and M, suggesting a role for pRb as a suppressor of cell proliferation between the G_0/G_1 and S phase of the cell cycle. This was found to be true. The pRb protein binds transcription factors, and in particular, the E2F family of transcription factors as well as the product of the c-*myc* oncogene [114, 115]. Cyclin-dependent kinases are responsible for phosphorylating pRb (resulting in inactivation) when cells are stimulated to divide by exogenous growth factors or other mitogenic signals. The pRb protein dissociates from sequestered/bound transcription factors, allowing the cell to progress from G_0/G_1 to the S phase. After mitosis, a phosphatase returns pRb to its active, hypophosphorylated form. Unlike other regulators of transcription, pRb does not directly interact with DNA [116–120].

The inability of pRb to bind and negatively regulate the function of certain transcription factors leads to unregulated cell proliferation, and this can result from deletion or mutation of the *Rb1* gene, or functional inactivation of the pRb protein. In general, mutation of the *Rb1* gene results in truncated and unstable proteins [111]. As might be expected, the significant mutations or deletions in the *Rb1* gene occur in the transcription factor binding domain, also known as the pRb pocket. Functional inactivation of pRb was first recognized in studies of viral oncogenes. DNA viral oncogene products from animals (SV40 large T antigen) and humans (human papilloma virus E7, adenovirus E1A) inhibit pRb function by binding and occupying the pRb pocket. Gene mutations or occupation of the pRb pocket/transcription binding domain have, as a common result, the liberation of activating transcription factors with subsequent uncontrolled cell proliferation. The interplay between the products of these viral oncoproteins and pRb is an illustration of their mutual cooperation, which serves as a paradigm of the multistep

nature of oncogenesis. Inhibition of tumor suppressor genes represents an important way in which oncogenes exert their neoplastic potential [111, 121].

Mechanism of Rb1 Inactivation in Retinoblastoma. Although by the mid-1970s cell hybrid studies clearly established that malignancy is at least in part due to loss of function of critical regulatory genes in malignant cells, the identification of tumor suppressor genes at the molecular level did not occur until more than a decade later. As the prototypic tumor suppressor gene, the mechanism of inactivation and loss of function associated with the *Rb1* are illustrative of the whole class of tumor suppressor genes. The inactivation of both alleles of the *Rb1* gene is required for development of retinoblastoma, an eye malignancy that usually occurs at a very young age. Until the end of the nineteenth century, this tumor was uniformly fatal. During the twentieth century, more of these tumors were recognized and diagnosed at an earlier stage, permitting a surgical cure. It was noted that the offspring of retinoblastoma patients cured by surgery developed the disease at a very high frequency. Pedigree analysis of these families suggested a dominant pattern of Mendelian inheritance [122, 123].

The suggestion that a specific gene was responsible for the disease stemmed not from molecular or cell hybrid analyses, but rather from epidemiological data first reported by Alfred Knudson [123]. He noted that 40 % of the cases of retinoblastoma were bilateral and occurred in young infants (mean age 14 months), who if cured went on to develop secondary tumors (often osteosarcomas). In these patients, there was often a relevant family history of retinoblastoma. In contrast, the remaining 60 % of cases were unilateral and occurred in older children (mean age 30 months), who if cured did not develop secondary malignancies. These patients generally lacked a relevant family history. Knudson proposed that the first group inherited a mutant allele (germline mutation) which conferred a dominant predisposition to cancer to this group of patients. In these patients, a second somatic mutation in retinal cells resulted in retinoblastoma. The second nonfamilial, later onset, form was very rare (occurring in 1 in 30,000 people). Knudson suggested that these patients did not inherit a mutant gene, but rather two independent somatic mutations in retinal cells occurred to give rise to retinoblastoma.

The two-hit model proposed by Knudson did not address the mechanism of action of the gene(s) involved. There were at least two possible explanations. The first is that a dominant mutation of a single proto-oncogene allele is insufficient for the development of neoplasia, or that a second mutation, perhaps in a second proto-oncogene, is required. The second possibility, which proved to be correct, is that two mutations are required for development of retinoblastoma and that these mutations are inactivating mutations. Thus, the loss of both functional copies of the *Rb1* gene is necessary for neoplastic transformation and tumor formation. This conclusion is based upon numerous studies involving cytogenetic, linkage, and restriction fragment length polymorphism (RFLP) analyses of constitutional and tumor DNAs from affected individuals.

Cytogenetic studies demonstrated a loss of the long arm (13q14 region) of chromosome 13 in retinoblastoma tumors, and in the germ line of patients with a hereditary predisposition to retinoblastoma development [124]. Esterase D, a gene present on chromosome 13q14, was used in linkage analysis studies. Assuming that a mutant *Rb1* is closely linked to one of the two esterase D alleles which can be traced back to an affected parent, one can detect offspring who have inherited the mutant *Rb1* allele. Tumors arising in these individuals were shown to be homozygous for one form of esterase D, and by extension, homozygous for the mutant *Rb1* gene [125]. Similar conclusions were drawn from RFLP analysis of retinoblastomas arising in patients with familial predisposition, by comparing tumor DNA with germ-line DNA. Paralleling the findings using esterase D, homozygosity was found in these tumors as well [125–127]. Shortly thereafter, probes from the 13q14 region were used to screen retinoblastomas, and several demonstrated homozygous deletions for at least 25 kb of DNA.

Molecular cloning of the gene was accomplished using probes to human 13q14 to isolate genomic DNA clones corresponding to flanking DNA. The genomic DNA clones from the tumor suppressor gene region were then used as probes against RNA to compare the pattern of mRNA expression between retinoblastoma and normal retinal cells. The retinoblastoma gene was found to have 27 exons extending over approximately 200 kb of DNA and encoding a 928 amino acid protein [127–129]. Once the *Rb1* gene was isolated, mechanisms of inactivation were found and included deletions and inactivating point mutations [130, 131]. Importantly, tumor cell lines into which a cloned normal *Rb1* gene was introduced lost their malignant phenotype, confirming the tumor suppressing action of *Rb1* [132]. These studies definitively established the role of deletion and/or loss of function as a major genetic mechanism involved with neoplastic transformation, and solidified the existence of a new class of genes, the tumor suppressor genes.

The *Rb1* gene has since been found to be associated with many human neoplasms, usually through a genetic mechanism involving mutations or deletions. Most other tumor suppressor genes have similar mechanisms of inactivation as described for *Rb1*. Specifically, any alteration in DNA or functional inactivation of the gene protein product that results in a loss of function of both copies or alleles of the gene is required for the development of tumors. These alterations include inactivating point mutations, deletions, or

insertions. In general, all of these mechanisms have been described for many of the tumor suppressor genes, and it is possible to find in one inactivated gene of a clonal cell population a combination of two mechanisms of inactivation, a different one for each allele.

p53 gene. The *p53* gene is briefly mentioned because of its important and frequent involvement in human neoplasia [82, 112]. Like the *Rb1* gene product, p53 is a nuclear phosphoprotein and functions primarily as a regulator of transcription [113]. Specifically, when the genome sustains mutagenic damage (from radiation or a chemical insult) wild-type p53 protein accumulates in the nucleus where it binds DNA and causes cells to halt in the G_1 phase of the cell cycle, where the genetic damage is repaired. If the damage is not repaired, p53 induces apoptosis [133]. When first discovered, *p53* was thought to be a proto-oncogene because overexpression of cloned *p53* genes was shown to be related to transformation in gene transfer assays. It was later shown that the clones used were actually mutant forms of the *p53* gene. When the wild-type gene was cloned and used in similar transfection studies it did not demonstrate the ability to participate in the neoplastic transformation of normal cells. Rather, overexpression led to inhibition of neoplastic transformation, suggesting it to be a tumor suppressor gene.

Mechanism of p53 inactivation in cancer. The tumor suppressor function of p53 can be inactivated by either mutational events or through negative protein-protein interactions. The *p53* gene is composed of 11 exons spanning 20 kb of genomic DNA, encoding a protein of 393 amino acids. Mutations have been described for each exon, but appear to be most common in exons 5–9. A similar distribution of mutations is found in the Li Fraumeni syndrome, in which patients inherit a germ-line mutation in the *p53* gene. This syndrome is characterized by a familial predisposition to many tumor types, including breast (among other epithelial) carcinomas, soft tissue sarcomas, brain neoplasms, and leukemias [133]. Mutant p53 protein products lose their ability to suppress transformation, and gain the ability to inactivate wild-type p53. A cell with one mutant and one wild type copy of p53 behaves as if it has no wild-type p53 at all. This type of mutation is referred to as dominant negative because the mutant allele acts in a dominant fashion to alter the functioning of the normal allele [134–136]. The half-life of mutant species of the p53 protein tend to be increased compared to the very short half-life of the wild-type protein. This increase in protein half-life enhances the dominant negative effects of the mutant protein. Furthermore, when complexed with oncoprotein products such as from DNA tumor viruses (SV40 large T antigen, adenovirus E1B protein, and the E6 protein of human papilloma virus), the p53 protein is functionally inactivated in a manner analogous to the oncoprotein inactivation of the pRb [136].

7.3.1.3 Tumor Suppressor Gene Products That Function as Cell Surface/Cell Matrix Molecules

Properties of malignant cells include not only uncontrolled proliferation capabilities, but also changes in cell morphology, loss of contact inhibition or cell–cell interactions, and loss of cell–extracellular matrix interactions. This results in an altered phenotype, including morphologic changes which allow recognition by microscopy as malignant, inability to process inhibitory or other signals from adjacent cells, and loss of adhesion properties resulting in metastatic potential. Cell surface and cell matrix molecules thought to play a role in these processes include products from the neurofibromatosis 2 (*NF2*) gene, the adenomatous polyposis coli (*APC*) gene, and the deleted in colon cancer (*DCC*) gene, among others.

APC gene. The *APC* gene was isolated in 1991 and is responsible for the familial adenomatous polyposis (FAP) syndrome, an uncommon autosomal dominant disease affecting approximately 1 in 8000 individuals [137–140]. Patients with FAP typically develop 500–2500 adenomas of the colonic mucosa. The frequency of progression to colon adenocarcinoma approaches 100 % necessitating prophylactic colectomy by the second or third decade of life, and early surveillance of siblings and first degree relatives [140]. The *APC* gene was found to be located on the long arm of chromosome 5 (5q21), a locus known to be deleted frequently in colonic adenocarcinomas. Patients with FAP carry germ-line *APC* mutations leading to the production of truncated APC proteins, the detection of which has provided a diagnostic assay [141]. In addition to its role in the development of hereditary FAP, *APC* mutations have been found in sporadic adenomas, the majority of sporadic colorectal cancers [141, 142], and other human malignancies of the pancreas, esophagus, stomach, and lung [142–146]. Sporadic mutations also lead to the production of truncated proteins. Tumor suppressor activity has not been demonstrated in gene transfer assays using the *APC* gene.

The *APC* gene is large, extending over 8500 nucleotides, and encoding a protein of approximately 2840 amino acids. The protein interacts with catenins, which are cytoplasmic proteins thought to play a role in signal transduction because of their interactions with cadherins, a family of cell surface molecules. Cadherins have been shown to regulate cell–cell interactions and morphogenesis. Based on these observations, it has been postulated that the APC-catenin complex plays a role in cell adherence, and possibly signal transduction such as contact inhibition of cell growth [146]. In FAP, mutations in the 5′-portion of the gene have been shown to correlate with an attenuated form of the disease [147], while mutations at codon 1309 are associated with an early onset of colon cancer in FAP families [148].

DCC gene. The *DCC* gene was discovered using polymorphic DNA markers that showed a loss of heterozygosity (LOH) of the long arm of chromosome 18 in colorectal tumors [46]. The gene was subsequently isolated and shown to be composed of more than 29 exons spanning more than 1×10^6 nucleotides [149, 150]. The *DCC* gene encodes for a transmembrane molecule with unknown function which has homology to cell adhesion molecules (CAMs) involved in cell–cell or cell–extracellular matrix interactions [151]. It may play a role in transmitting negative signals, and inactivation of *DCC* function through deletion or mutation (a rare event) may lead to loss of contact inhibition and subsequent uncontrolled cellular proliferation [151]. LOH at the 18q locus was initially described in colorectal carcinomas as one of several steps involved in the sequence of events from premalignant adenomas to invasive carcinomas. Although no hereditary conditions involving germ-line alterations of the gene have been described (in contrast to most other tumor suppressor genes), *DCC* abnormalities are associated with several tumor types other than colon cancer, including stomach cancer, pancreatic cancer, and leukemias [152–155]. The *DCC* gene has not been formally classified as a tumor suppressor gene because the predicted tumor suppressor activity has not been demonstrated in gene transfer assays [4, 156, 157].

NF2 gene. NF2 is genetically and clinically distinct from NF1 discussed above. It is a rare autosomal dominant disorder in which patients develop bilateral schwannomas affecting the vestibular branch of the eighth nerve (acoustic neuromas), and other tumors of the central nervous system such as meningiomas and ependymomas (hence its designation as central neurofibromatosis). The gene is located on the long arm of chromosome 22 (22q22) and was isolated in 1993 [158, 159]. Mutations of the *NF2* gene have been found in tumors (breast carcinomas and melanomas) other than those associated with the NF2 syndrome [160], as well as in sporadic meningiomas and ependymomas [161, 162], suggesting a role in tumorigenesis extending beyond that played in NF2. Its protein product (schwannomin or merlin) shows sequence homologies to proteins which act as linkers between cytoskeletal scaffolding components and proteins in the cell membrane. On this basis schwannomin is thought to play a role in cell shape, cell–cell interactions, and cell–matrix interactions [159, 160]. Inactivation of the *NF2* gene may lead to changes in cell shape and loss of contact inhibition [110].

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