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Chromosomes and Cancer: Activation of Oncogenes

A large number of hematological (blood cell) and solid tumors of various types show consistent chromosome abnormalities, and there is overwhelming evidence that the chromosome changes are essential for the malignant phenotype. Almost every chromosome band is involved, indicating the large number of genes that can play a role in oncogenesis (Mitelman et al., 1997). Many of these rearrangements lead to cancer by activating a cellular oncogene (*protooncogene*). Protooncogenes are normal genes present in all metazoan cells. Genes homologous to protooncogenes are found in the retroviruses known to cause cancer in various animal species. They transform cells, either by being inserted into the host genome or by being present in multiple copies in the host cell (Bishop, 1983). The retroviruses originally acquired these oncogenes from the metazoan cells they infected. The oncogene of the Rous sarcoma virus is called *v-src*, and its homologue in the normal human genome is *c-SRC*, or *SRC*. More than 80

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human protooncogenes have been localized to a specific chromosome or chromosome band. A normal cell can be transformed by activating one or more oncogenes in it. This most often occurs through chromosomal mechanisms such as translocation or amplification. In leukemias and lymphomas, these are mostly balanced reciprocal translocations; in solid tumors, deletions and trisomies are also common (Cobaleda et al., 1998; Helm and Mitelman, 1995).

Mechanisms of Oncogene Action

The normal functions of cellular oncogenes are quite important. Many play a role in the cell cycle and its checkpoints. Many stimulate cell proliferation through their role as growth factors, growth factor receptors, signal transducers from cytoplasm to nucleus, or activators of transcription or replication (Table 27.1). Other oncogenes enhance metastasis. Activated cellular oncogenes are dominant in their effects (a single copy is oncogenic), as shown by transfection experiments. Purified DNA fragments from a variety of cancers, when transferred to the DNA of recipient cells, a process called *transfection* (Chapter 23), transform nonneoplastic cells with high efficiency. DNA fragments from normal cells also accomplish transformation, although with a very low frequency, presumably by activating a cellular oncogene.

Another mechanism by which oncogenes act is blocking of cell differentiation. Overexpression of the MDM2 cell cycle regulator occurs in nearly 30% of sarcomas. It inhibits Myo-D and p53, thus blocking muscle cell differentiation as well as inducing aneuploidy. Thus, when overproduced, MDM2 becomes an

Table 27.1. Some Cellular Oncogene Products That Foster Proliferation by Acting in Signal Transduction Pathways

Location in pathway	Oncogene products
Extracellular growth factor (ligand)	INT-2, SIS, HST
Receptor at cell membrane	ERBB, FMS, MAS
Membrane-associated G-protein	H-RAS, N-RAS, K-RAS
Membrane/cytoplasmic protein tyrosine kinase	ABL/BCR, SRC, CRK
Cytoplasmic protein serine kinase	MOS, RAF, ERBA
Nuclear transcription factor	FOS, MYC

oncoprotein. An isochromosome 3q is seen in a number of sarcomas. This duplication of the *ATR* (ataxia telangiectasia and rad-3-related) gene in 3q also blocks p53 and Myo-D function (Smith et al., 1998). Acute myelogenous leukemia (AML) is frequently associated with a t(15;17) translocation. This is oncogenic because it fuses the retinoic acid receptor α (*RARA*) gene with the promyelocytic leukemia (*PML*) locus to form a chimeric receptor that activates histone deacetylase and blocks cell differentiation (Lin et al., 1998).

Some oncogenes enhance carcinogenesis by delaying programmed cell death (*apoptosis*). The t(14;18)(q32;q21) translocation present in 85% of follicular lymphomas fuses the *BCL2* gene at 18q21 with an active immunoglobulin heavy chain (*Ig*) locus and leads to overproduction of the *BCL2* gene product, a mitochondrial inner membrane protein that blocks apoptosis by preventing the creation of reactive oxygen species. Some oncogene proteins, such as Myb and Ras, induce *BCL2* expression (Adams and Corey, 1998). A second event is necessary for malignant transformation; this is usually the activation of *MYC*. The BCR-ABL fusion proteins 210 and 190 inhibit apoptosis by inducing *BCL2* expression, thus allowing more time for other oncogenic processes to act on the susceptible population of cells and produce chronic myelogenous leukemia (CML) and acute lymphatic leukemia (ALL), respectively (Sánchez-García and Grütz, 1995).

Reciprocal Translocations and Oncogene Activation

The *MYC* oncogene is consistently activated by a chromosomal translocation in the highly malignant Burkitt lymphoma. The most common chromosome finding in malignant cells of patients with this disease is t(8;14)(q24;q32.3) (Fig. 27.1). The *MYC* gene has been mapped to 8q24, and the immunoglobulin heavy chain (*IgH*) locus to 14q32.3. The new location of the *MYC* gene places it next to and just 3′ to (downstream of) the promoter of the broken *IgH* gene; this upregulates *MYC* expression in lymphoid cells in which the immunoglobulin genes are expressed. In a minority of cases, the end of 8q is translocated to 2p11 or to 22q11, placing *MYC* next to the strong promoter of the gene coding for the Ig kappa or lambda light chain, respectively. The *MYC* oncogene is also activated in the same manner in other lymphomas (Fig. 27.2 a and b).

The first chromosome aberration consistently seen in a malignancy was the Philadelphia chromosome, found in 1960 by Nowell and Hungerford in CML;

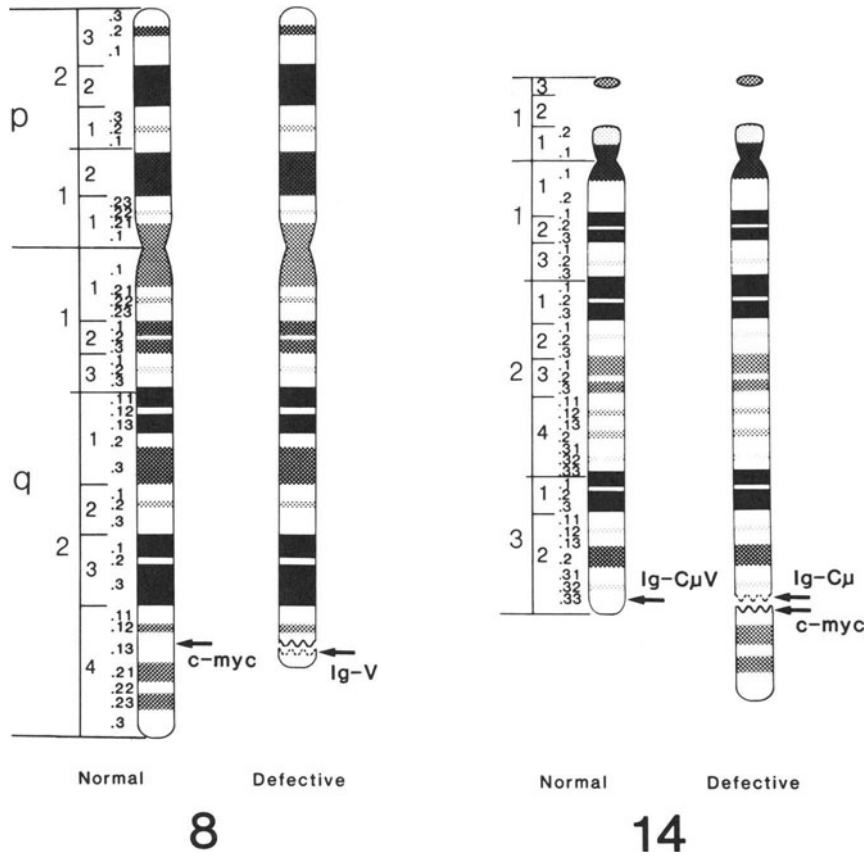

Figure 27.1. Locations (arrows) of c-*MYC* oncogene and heavy-chain immunoglobulin (Ig) variable (*V*) and constant (Cμ) genes on normal and rearranged chromosomes 8 and 14 in Burkitt lymphoma, represented at the 1200-G-band stage. Broken ends indicate breakpoints (Yunis JJ. The chromosomal basis of human neoplasia. Science 221:227–236, copyright 1983, by the AAAS).

Rowley (1973) showed this was a reciprocal translocation between 9q and 22q. With prophase banding, the breakpoints were defined as 9q34.1 and 22q11.2 (Fig. 27.1c). Molecular cytogenetic techniques have shown that the translocation activates the oncogene *ABL* at 9q34.1 by placing it next to the strong promoter of the *BCR* (breakpoint cluster region) gene at 22q11.2, which is strongly expressed in lymphocytes. The result is a novel fusion gene. Since the breakpoints are in introns of the two genes, there is no frame shift in the coding sequence of the exons, so a functional fusion protein is produced. Even when

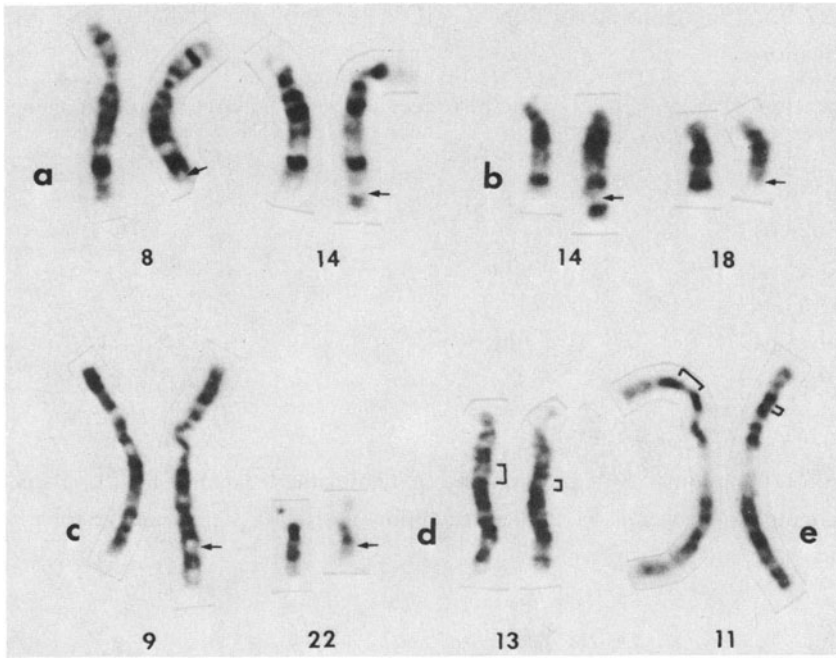

Figure 27.2. High-resolution (850- and 1200-band) G-banded chromosomes from (a) non-Burkitt lymphoma with t(8;14), (b) follicular cell lymphoma with t(14;18), (c) chronic myelogenous leukemia with t(9;22), (d) retinoblastoma with del(13)(q14), and (e) Wilms tumor with del(11)(p13). Arrows indicate breakpoints in the translocations, and brackets indicate deletions (Yunis JJ. The chromosomal basis of human neoplasia. Science 221:227–236, copyright 1983, by the AAAS).

the breakpoints are in the same band at a cytological level, they may be at different molecular locations. This accounts for the fact that some t(9;22)(q34;q11) translocations lead to CML and some to acute lymphoblastic leukemia (ALL); a BCR-ABL fusion protein 210 kDa in size leads to CML and one 190 kDa in size leads to ALL.

How Do Translocations Activate Cellular Oncogenes?

There are two general mechanisms by which translocations activate oncogenes. The first is to place the protooncogene under the control of a strong active pro-

Table 27.2. Oncogene Activation by V(D)J Recombinase-mediated Translocations

Translocation	Type of cancer	Activator, oncogene
t(8;14)(q24;q32)	BL, B-ALL	*IgH*, *MYC*
t(2;8)(p12;q24)	BL, B-ALL	*IgL-κ*, *MYC*
t(8;22)(q24;q11)	BL, B-ALL	*IgL-λ*, *MYC*
t(11;14)(q13;q32)	B-CLL	*IgH*, *BCL1*
t(14;18)(q32;q21)	FL	*IgH*, *BCL2*
t(8;14)(q24;q11)	T-ALL	*TCR-α*, *MYC*
t(7;19)(q35;p13)	ALL	*TCR-β*, *LYL1*
t(7;10)(q35;q24)	T-ALL	*TCR-β*, *HOX11*

BL, Burkitt lymphoma; B-ALL, B-cell acute lymphatic leukemia; B-CLL, B-cell chronic lymphatic leukemia; FL, follicular lymphoma; T-ALL, T-cell acute lymphatic leukemia

moter, as just described for the *MYC* gene. In hematopoietic malignancies, such as lymphomas, leukemias, and myelomas, one breakpoint is usually in an *IgH*, *Igκ*, *Igλ*, or T-cell receptor (*TCR*) gene. This suggests that the translocations in hematopoietic cells usually arise as a result of aberrant action of the enzymes normally involved in the DNA double-strand breakage and repair that lead to the V(D)J recombination responsible for antibody and histocompatibility receptor diversity. There are many examples of this; a few are shown in Table 27.2. This is supported by precise molecular evidence in many cases. An intriguing example is the t(4;14) translocation seen in some multiple myelomas. This fuses the *IgH* gene with the Wolf–Hirschhorn critical region gene *WHSC1*, which is normally expressed in early development (Stec et al., 1998).

Many of the translocations seen in specific types of lymphoid malignancies and virtually all solid tumors do *not* involve *Ig* or *TCR* genes. How do they arise? A precise molecular answer is not available for most of them. However, the *BCR-ABL* translocations seen in CML arise preferentially within the 5-kb breakpoint cluster region (*BCR*) by recombination involving Alu repeat elements (Jeffs et al., 1998). In most of these cases, and in many types of solid tumors, the specific translocation associated with the tumor leads to a novel fusion gene that

Table 27.3. Oncogene Activation or Fusion Neooncogene Formation by Translocations

Translocation	Type of cancer	Fusion genes
t(9;22)(q34;q11)	CML, B-ALL	*BCR, ABL*
t(9;12)(q34;p13)	AML	*TEL, ABL*
t(11;22)(q24;q12)	EWS, neuroblastoma	*EWS, FL1*
t(12;22)(q13;q12)	Melanoma	*EWS, ATF1*
t(2;13)(q37;q14)	Rhabdomyosarcoma	*FKHR, PAX3*
t(15;17)(q21;q11–q22)	AML, PML	*PML, RARA*
t(12;21)(p13;q22)	ALL	*TEL, AML1*

CML, chronic myelogenous leukemia; B-ALL, B cell acute lymphatic leukemia; AML, acute myelogenous leukemia; EWS, Ewing sarcoma; PML, promyelocytic leukemia

acts as a neooncogene. The *BCR-ABL* fusion gene seen in CML is the best-known example, but there are many others, some listed in Table 27.3.

Amplification and Oncogene Activation

Gene amplification is an important chromosomal mechanism of carcinogenesis (Chapter 25). Oncogenes are frequently activated or the steady-state level of their gene products increased by amplification (Table 27.4). Homogeneously stained regions (HSRs) and double minutes (DMs) have been observed in chromosomes of many primary tumors and cultured malignant cells. In colon carcinoma, the HSRs and DMs reflect the amplification of the *MYC* gene. The gene for the cell cycle regulator, cyclin D1, in the 11q13 region, is amplified in many types of cancer. This includes cancer of the esophagus, which is closely associated with environmental factors, such as alcohol consumption, tobacco smoking, and nitrosamine ingestion (Jiang et al., 1992). Tumor promoters, too, may act by promoting gene amplification. Growth of cells for several generations in the phorbol ester TPA (12-O-tetradecanoyl-phorbol-13-acetate), one of the most potent tumor promoters, enhances 3- to 16-fold the amplification of the *DHFR* (dihydrofolate reductase) gene under methotrexate selection and presumably has

Table 27.4. Oncogenes Amplified in Yarious Types of Cancer

Oncogene	Map position
ERBB1/EGFR	7p12.3–p12.1
CDK6	7q21–q22
MYC	8q24.12–q24.13
FGF4	11q13.3
INT2	11q13
HST1	11q13
Cyclin D1	11q13
Cyclin D2	12p13
CDK4	12q13
MDM2	12q13–q14
SAS	12q13–q14
HER2/NEU	17q12–q21
Cyclin E	19q12–q13

the same effect on various oncogenes. Cyclin D2, a pituitary gonadotrophic hormone (FSH)-responsive gene involved in gonadal cell proliferation, is amplified in many ovarian and testicular cancers (Sicinski et al., 1996). Amplification and overexpression of cyclin E leads to the formation of modified cyclin E/CDK2 kinase complexes that remain active throughout the cell cycle, bypassing the usual p16/RB repression (Gray-Bablin et al., 1996).

The cyclin-dependent kinase 6 (*CDK6*) gene, which maps to 7q21–q22, is amplified up to 50-fold in many gliomas, as shown by restriction landmark genome scanning by two-dimensional pulsed-field gel electrophoretic (PFGE) separation of very large *Not*I restriction enzyme fragments of DNA (Costello et al., 1997). Multiple genes in 12q13–q14 are often amplified in malignant gliomas. One is the cell cycle gene *CDK4*. Another is the cell cycle regulator gene *MDM2*, which is also amplified in other tumors, such as sarcomas (Reifenberger et al., 1994). The MDM2 protein binds the RB (retinoblastoma) protein, causing it to release the E2F transcription factor and thus stimulate cell division (Xiao et al., 1995). MDM2 also represses the transcription of the *TP53* gene and thus prevents activation of cell cycle checkpoints by DNA damage or spindle assembly defects (Levine, 1997).

Relaxation of Imprinting and Oncogene Activation

Hypomethylation of many genes is observed in cancer (Laird and Jaenisch, 1996). When this involves an imprinted gene, the imprint is relaxed (lost) and the gene may be expressed, leading to an increased dosage of its product. Such a gene may function as an oncogene. The *H19* and *IGF2* (insulin-like growth factor 2) genes are imprinted (Chapter 21), showing monoallelic expression of the paternal allele. Loss of imprinting of these genes has been observed in lung, renal, cervical, ovarian, testicular, and gastric cancers. It is often seen in benign tumors, suggesting that it is an early event in multistep tumorigenesis (Kim et al., 1998).

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