

Oncogenes and cancer: clinical applications

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■ Oncogenes are aberrant forms of proto-oncogenes, which are normal cellular genes that participate in cell growth and development; proto-oncogenes contribute to tumor formation when mutations or chromosomal translocation cause them to escape normal controls. Anti-oncogenes, also involved in neoplasm development, normally participate in inhibition of cell growth and proliferation; they become tumorigenic when mutations alter their function. Oncogene or anti-oncogene abnormalities have been characterized for a variety of tumors, with resulting clinical applications. In some forms of leukemia, for example, determining the presence or absence of the *bcr-abl* gene rearrangement has both diagnostic and prognostic value. The best-studied anti-oncogene is that found in retinoblastoma. Molecular techniques can differentiate the hereditary from the nonhereditary form of this disease and, with hereditary retinoblastoma, predict disease likelihood in family members.

□ INDEX TERM: ONCOGENES □ CLEVE CLIN J MED 1990; 57:721-726

RECENT advances in molecular biology have perhaps had their greatest impact on the field of oncology. Investigators have begun to identify genes that are at least in part responsible for the development of neoplasms. These cancer-related genes fall into two categories: cancer-causing genes, called oncogenes, and tumor-suppressing genes, called anti-oncogenes.

Oncogenes are functionally or structurally aberrant forms of normal cellular genes termed proto-oncogenes.¹⁻³ Proto-oncogenes participate in normal cell growth and proliferation, encoding for a variety of proteins that may act, for example, as growth factors, growth factor receptors, regulators of DNA synthesis, or modifiers of protein function by phosphorylation. Normally, proto-oncogenes are strictly regulated by other

genes that either promote or inhibit their transcription. When they escape these controls, proto-oncogenes become oncogenic because their protein products stimulate the cell in an unimpeded fashion.

■ See Table 1 for glossary of unfamiliar terms (p 722).

Any mutation that alters the control or function of a proto-oncogene has tumorigenic potential. A mutation may affect the proto-oncogene structurally and result in a gene that is resistant to downregulation or in a gene product that has enhanced activity. A mutation may affect a regulatory gene, which could leave the proto-oncogene structurally normal, but cause its expression to be uncontrolled. If a mutation causes chromosomal translocation, this could remove the proto-oncogene from its normal control and possibly bring it under the control of a stronger promoter gene.

Anti-oncogenes are thought to play a role in the pathways that restrain normal cellular proliferation.^{1,3,4} Loss of their function through mutation can result in the

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TABLE 1
GLOSSARY OF TERMS

anti-oncogene (tumor-suppressor gene): gene whose product normally suppresses the activity of certain genes involved in cellular growth and proliferation	gene amplification: increased copy number per cell of any individual gene; in the context of neoplasia, gene amplification typically refers to increased copy number of a proto-oncogene
bcl-2 gene: putative proto-oncogene that is commonly translocated in follicular lymphoma	gene locus: site on a chromosome that contains all the components of an individual gene
bcr-abl fusion gene: term used to describe the joining of the bcr and c-abl genes after the translocation of chromosomes 9 and 22 that is seen in almost all cases of chronic myelogenous leukemia	heterozygosity: two different forms of the same gene in a cell
bcr-abl mRNA: mRNA translation product of the fused bcr-abl gene that results from the translocation of chromosomes 9 and 22; the mRNA molecule contains sequences encoded by the BCR gene as well as by most of the c-abl gene	N-myc proto-oncogene: proto-oncogene that is structurally similar to the c-myc proto-oncogene; like c-myc, N-myc appears to code for a nuclear-associated DNA-binding protein that affects DNA replication and transcription; N-myc proto-oncogene abnormalities have been implicated in a variety of neoplasms
BCR gene: gene on chromosome 22 that normally encodes a 130- or 160-kd protein of uncertain function; the breakpoint cluster region (bcr) is a segment of the BCR gene	oncogenes: functionally or structurally aberrant forms of proto-oncogenes; proto-oncogenes may become oncogenes when they escape their normal controls, either by DNA mutation or chromosome translocation, or by mutations that alter their protein products in such a way that they have enhanced and possibly uncontrolled function
bcr-negative (bcr-): indicates the absence of a detectable translocation involving bcr as determined by Southern blot analysis	Philadelphia chromosome (Ph): translocation between chromosomes 9 and 22 found in most patients with chronic myelogenous leukemia
bcr-positive (bcr+): indicates the presence of a detectable translocation involving bcr; this gene rearrangement is typically detected by Southern blot analysis	promoter gene: a gene whose product enhances the transcription rates of a second gene
breakpoint cluster region (bcr): the 5.8-kilobase region within the BCR gene that is the site for the translocation between chromosomes 9 and 22 in almost all cases of chronic myelogenous leukemia	polymorphism: normal DNA sequence variation between individuals
c-abl proto-oncogene: a proto-oncogene that normally produces a protein product with tyrosine phosphokinase activity; this protein plays a role in the control of cellular growth and proliferation	proto-oncogenes (cellular oncogenes): genes that encode for a variety of proteins involved in normal cell growth and proliferation including growth factors, growth factor receptors, regulators of DNA synthesis, and modifiers of protein function by phosphorylation
c-erb B-2 proto-oncogene: proto-oncogene whose protein product is structurally similar to the epidermal growth factor receptor	Rb gene: classic anti-oncogene whose protein product normally has DNA-binding activity and serves in some capacity as a suppressor of other genes that stimulate cellular growth and proliferation; the absence of a functional product from either Rb gene in a single retinal cell results in the development of a retinoblastoma
chromosome translocation: the exchange of genetic material between structurally different chromosomes	Southern blot analysis: technique used to examine DNA for, as an example, the presence or absence of gene rearrangements; a radiolabeled DNA probe is used to identify a particular gene segment
c-myc proto-oncogene: proto-oncogene that normally encodes for a protein that affects DNA replication and transcription; mutations and translocations involving the c-myc proto-oncogene have been implicated in a variety of neoplasms	transcription: converting the DNA code into a complementary mRNA segment
DNA probe: short segment of DNA whose sequence is specifically complementary to a particular gene segment that is to be examined by Southern blot analysis	translation: converting an mRNA sequence into a protein product
downregulation: turning off the transcription of a gene	

unbridled cell growth encountered in cancer.

Oncogene or anti-oncogene abnormalities have been characterized for a variety of tumors. The ability to detect these abnormalities has diagnostic, prognostic, and potentially therapeutic applications.

ONCOGENES

Leukemia

Perhaps the best-characterized association between proto-oncogene dysfunction and neoplasia is the genetic abnormality encountered in chronic myelogenous leukemia (CML).⁵ It has long been recognized that 90% to 95% of patients with this disorder have the translocation between chromosomes 9 and 22 that results in the Philadelphia chromosome (Ph). We now understand that this translocation consistently involves the transfer

of portions of the c-abl proto-oncogene from chromosome 9 to a narrow segment of the BCR gene on chromosome 22. This narrow segment is termed the breakpoint cluster region, or bcr (Figure 1).^{5,6} The transfer creates a fusion gene that is transcribed into an 8.5-kilobase bcr-abl mRNA molecule whose translation product is an abnormal 210-kilodalton (kd) protein. This protein has greater tyrosine phosphokinase activity than the normally encountered 145-kd c-abl gene product. Recent gene transfer studies in mice suggest that expression of the 210-kd bcr-abl protein is sufficient to induce CML.⁷

A different aberrant protein is encountered in approximately half of patients with Ph-positive acute lymphocytic leukemia (ALL) and in some patients with acute myelogenous leukemia (AML).^{3,5,8,9} This 190-kd protein is the product of a c-abl translocation to a dif-

ferent portion of the BCR gene (Figure 2). It too has enhanced tyrosine phosphokinase activity. The remaining Ph-positive ALL (and AML) cases demonstrate the *bcr* rearrangement typical of CML.

The ability to recognize subsets among the leukemias based on the presence or absence of the Philadelphia chromosome and the *bcr-abl* gene may lead to refinements in our diagnostic and prognostic capabilities. For example, Southern blot analysis using DNA probes for *bcr* is able to detect the *bcr-abl* gene rearrangement in virtually all cases of CML, in contrast to cytogenetic analysis which may yield false-negative results in certain cases (eg, complicated multi-chromosome translocations). Cases considered Ph-negative CML in the past can now, in most instances, be classified as true CML (*bcr*-positive) or as another myeloproliferative/myelodysplastic disorder (*bcr*-negative) such as chronic myelomonocytic leukemia (Table 2).^{5,10,11} The impression that Ph-negative CML has a worse prognosis than Ph-positive CML may be due to the inclusion of *bcr*-negative cases in the former group.

In addition, Ph-positive ALL and AML can be divided into two subgroups based on the presence of *bcr* (Table 2).^{3,5,12} While *bcr*-positive ALL or AML probably arises from a silent CML phase, *bcr*-negative disease is postulated to arise *de novo*. Recognition of this distinction may generate separate treatment protocols for each subgroup.

Finally, although conflicting data have been published, several reports suggest that the precise location of the fusion of *c-abl* with *bcr* may predict the duration of the chronic phase in CML.¹³⁻¹⁵ If true, gene fusion location may provide the marker needed to identify those patients who are more likely to progress rapidly to an accelerated phase and who, therefore, are candidates for

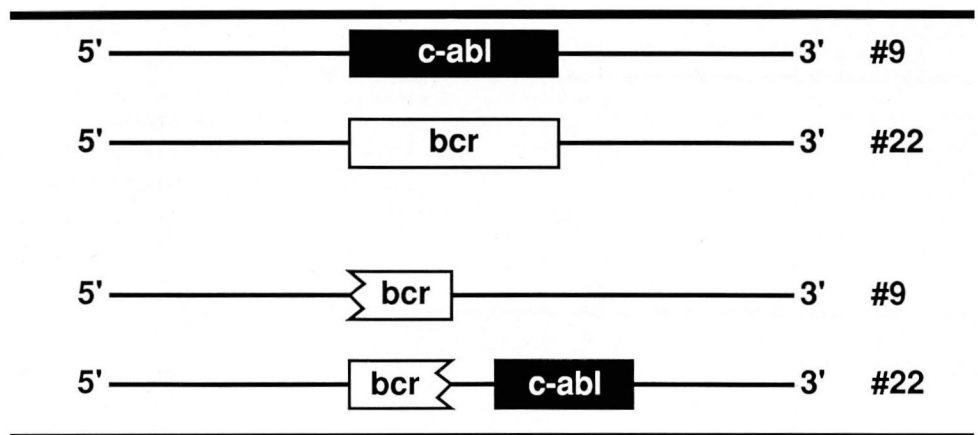


FIGURE 1. Diagram of the chromosomal translocation that results in the Philadelphia chromosome. This abnormality is encountered in 90% to 95% of patients who have chronic myelogenous leukemia.

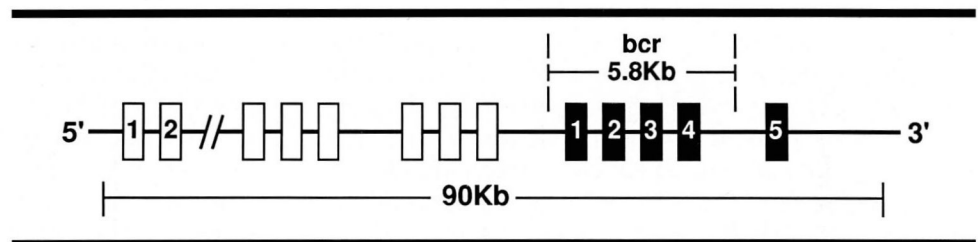


FIGURE 2. Diagram of BCR gene. Philadelphia chromosome translocation that is characteristic of chronic myelogenous leukemia as well as some cases of acute lymphocytic leukemia (ALL) and acute myelogenous leukemia (AML) involves the segment labeled *bcr*. In cases of ALL and AML associated with production of the 190-kd *bcr-abl* protein, translocation involves that portion of the BCR gene adjacent to exon 1 (open box 1).

allogeneic bone marrow transplantation early in the disease course.

Non-Hodgkin's lymphoma

Chromosome translocation also underlies the oncogene abnormalities frequently found in association with non-Hodgkin's lymphomas; however, the molecular mechanisms appear different from those encountered in CML. In Burkitt's lymphoma, a translocation between chromosomes 8 and 14 causes the transfer of the *c-myc* proto-oncogene to the immunoglobulin heavy chain gene locus.³ This transfer brings the intact *c-myc* gene under the control of a new promoter gene. The result is an overproduction of a structurally normal *c-myc* gene product.

In many follicular and some diffuse large cell lymphomas, a translocation is found between chromosomes 14 and 18. Here, the mutation involves the transposi-

TABLE 2
MOLECULAR SUBCLASSIFICATION OF CML AND ALL

	Ph and bcr	Gene product
Normal	Ph-, bcr-	145 kd
Chronic myelogenous leukemia	Ph+, bcr+	210 kd
	Ph-, bcr+	210 kd
Acute lymphocytic leukemia	Ph+, bcr+	210 kd
	Ph+, bcr-	190 kd

Ph, Philadelphia chromosome; bcr, breakpoint cluster region of BCR gene; -, absent; +, present

tion of most of the putative proto-oncogene, *bcl-2*, to a site within the immunoglobulin heavy chain gene locus. As a result, *bcl-2* is deregulated with consequent production of increased amounts of the normal *bcl-2* protein.

Similar to gene rearrangement in CML, *bcl-2* rearrangement typically involves only a limited region, in this case, one of two narrow segments of DNA within the *bcl-2* gene of chromosome 18.^{3,16} Southern blot analysis using probes for these regions has identified *bcl-2* rearrangement in most cases of follicular lymphomas as well as in a subset (20% to 40%) of diffuse large cell lymphomas.¹⁷⁻¹⁹ Since this rearrangement has not been demonstrated in reactive lymphoid proliferations, it may serve as a diagnostic marker. In addition, the subset of diffuse large cell lymphomas with *bcl-2* rearrangement appears to behave clinically like follicular lymphoma in that disease-free survival is decreased but survival with residual disease is extended.¹⁸ Further study of these critical clinical correlations is needed to determine whether *bcl-2* gene status should play a role in selecting therapy for malignant lymphoma.

Neuroblastoma and breast cancer

Quantitative abnormalities of other oncogenes may provide important prognostic information, although they may have less diagnostic value than the detectable defects in the *bcr* and *bcl-2* gene systems. A well-studied example is the abnormality of the *N-myc* proto-oncogene found in association with some neuroblastomas.²⁰⁻²² In some patients, the neoplastic cells from this tumor contain an increase in *N-myc* gene copy number, an abnormality referred to as gene amplification. Such patients have their tumor diagnosed at a later stage than those without gene amplification and have a poor prognosis with rapid tumor progression.²⁰⁻²² In contrast, stage 1 and 2 neuroblastomas usually fail to demonstrate *N-myc* gene amplification. The few early-

stage tumors that do possess increased *N-myc* gene copy number appear to have greater metastatic potential. Interestingly, those patients who have limited metastatic disease, classified as stage IV-S, typically have tumors with normal *N-myc* copy number and clinically have a good prognosis.

Gene amplification also may play a role in breast cancer.²³⁻²⁵ Some studies have demonstrated that amplification of the *c-erb B-2* proto-oncogene with increased production of the *c-erb B-2* protein appears to correlate with disease progression and poor prognosis. Confirmation of these clinical correlations between gene amplification and tumor aggression may well result in altered therapeutic approaches based upon the presence or absence of gene amplification.

ANTI-ONCOGENES

Unlike oncogenes, which stimulate cell growth, tumor-suppressor genes or anti-oncogenes are thought to function normally as inhibitors of cell growth and proliferation, probably as a balance to proto-oncogene function. As might be expected, loss of tumor-suppressor gene function is potentially oncogenic.

The best-studied anti-oncogene is the retinoblastoma (*Rb*) gene.^{3,26,27} Located on chromosome 13, band q14, the *Rb* gene is 200 kilobases in length and codes for a 110-kd nuclear phosphoprotein that has DNA-binding activity. Retinoblastoma develops when both *Rb* genes in a cell are made nonfunctional by mutation or deletion. In the hereditary form of the disease, all cells in the body contain one mutated *Rb* gene and one normal *Rb* gene. Any subsequent mutation or loss of the normal *Rb* gene in a retinal cell results in loss of *Rb* gene function and development of a tumor from that cell clone. Since all retinal cells inherit a single defective *Rb* gene, multiple independent tumors may arise in one or both eyes.

In contrast, in the random, nonhereditary form of retinoblastoma, all cells within the body including the retinal cells contain two normal *Rb* genes. For retinoblastoma to develop, two mutations must occur in a single cell clone. Since such an occurrence is of low likelihood, it is not surprising that nonhereditary retinoblastoma is an uncommon tumor that is uniformly single and unilateral.

Understanding the genetics of retinoblastoma has led to the development of tests that predict the likelihood of disease inheritance in many cases. Within a family, certain normal DNA sequence variations, or polymorphisms, are co-inherited with the mutated gene. By identifying these polymorphisms, we can

determine whether a child has inherited the chromosome that, in his or her family, contains the defective Rb gene and, thus, predict that child's risk of retinoblastoma (Figure 3).^{28,29} This technique is applicable, however, only if at least two family members have the predictive polymorphism pattern. Alternatively, more sophisticated molecular techniques can be used to sequence segments of the Rb gene directly to detect specific mutations responsible for the defective gene in any individual.³⁰

This ability to identify genetic predisposition to the development of retinoblastoma has important clinical implications. When diagnosed and treated early, retinoblastoma is curable. Yet, the repeated ophthalmologic examinations under anesthesia required for early detection in children are traumatic. By characterizing the hereditary versus nonhereditary nature of the disease in an affected child, we can now readily predict whether the patient's siblings or future children are at risk for this disease. If the tumor is found to be hereditary, we can then determine, even prenatally, whether other family members have inherited the defective gene. In this way, repeated ophthalmologic examinations can be limited to those at risk of disease development.

Determining whether a given case of retinoblastoma is hereditary is of added clinical importance because of the high incidence of second neoplasms, most commonly osteosarcoma, in patients with the hereditary form. Presumably, acquired mutations in addition to the Rb gene defect are a prerequisite to osteosarcoma development in these patients, thereby explaining its later age of onset.

Defects in tumor-suppressor genes have been implicated in several other acquired and hereditary tumors, including Wilms' tumor, familial adenomatous polyposis, neurofibromatosis, small cell lung cancer, and breast cancer.^{3,4,31-35} The mutations responsible for these disorders have not been as well characterized as that for retinoblastoma. In some of these tumors, however, loss of specific chromosome sites (termed loss of heterozygosity) has been noted.

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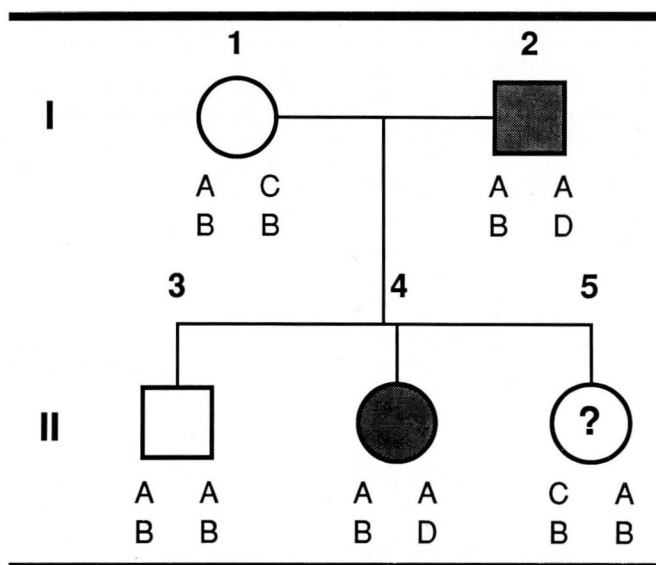


FIGURE 3. Predictive pattern of retinoblastoma gene inheritance. The letters A through D indicate different normal DNA polymorphisms of the Rb gene. Individual I-2 has transmitted a defective Rb gene to his daughter II-4. The abnormal gene, therefore, must be on the chromosome that contains the A and D polymorphisms; thus, infant II-5 is not at risk of retinoblastoma development.

COMMENT

Research in molecular biology has generated an explosion of information that has significantly expanded our understanding of carcinogenesis. In addition, for a few tumors, genetic abnormalities have been characterized well enough that molecular analysis may help in patient management. Undoubtedly, we will encounter an increasing number of molecular genetic discoveries that have diagnostic, prognostic, and therapeutic implications for an expanding list of neoplasms. Moreover, perhaps a broadened understanding of proto-oncogene and tumor-suppressor gene function will lead to the development of therapeutic interventions aimed at reversing the biologic alterations that result from molecular mutation.

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