Conferences and Reviews

Molecular Mechanisms of Cancer

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Cancer is caused by specific DNA damage. Several common mechanisms that cause DNA damage result in specific malignant disorders: First, proto-oncogenes can be activated by translocations. For example, translocation of the c-*myc* proto-oncogene from chromosome 8 to one of the immunoglobulin loci on chromosomes 2, 14, or 22 results in Burkitt's lymphomas. Translocation of the c-*abl* proto-oncogene from chromosome 9 to the *BCR* gene located on chromosome 22 produces a hybrid BCR/ABL protein resulting in chronic myelogenous leukemia. Second, proto-oncogenes can be activated by point mutations. For example, point mutations of genes coding for guanosine triphosphate-binding proteins, such as H-, K-, or N-*ras* or G proteins , can be oncogenic as noted in a large variety of malignant neoplasms. Proteins from these mutated genes are constitutively active rather than being faithful second messengers of periodic extracellular signals. Third, mutations that inactivate a gene can result in tumors if the product of the gene normally constrains cellular proliferation. Functional loss of these "tumor suppressor genes" is found in many tumors such as colon and lung cancers. The diagnosis, classification, and treatment of cancers will be greatly enhanced by understanding their abnormalities at the molecular level.

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H. PHILLIP KOEFFLER, MD*: Malignancy results from DNA damage that modifies the normal checks and balances that regulate cellular proliferation and differentiation. Genetic alterations that change proto-oncogenes into oncogenes activate and dysregulate normal genes and provide the cell with a growth advantage, usually in a dominant manner. Oncogenes can be created through translocations, point mutations, or amplification of genes. A loss of genetic material may also be oncogenic by the removal of a growth regulator that normally constrains cellular proliferation. Loss of these so-called tumor suppressor genes (recessive oncogenes or anti-oncogenes) is usually recessive—that is, the activity of both alleles must be missing or markedly reduced for oncogenesis to occur.

Chromosomal Structure and Neoplastic Transformation: Specific Chromosomal Abnormalities Are Linked to Certain Tumors

CHRISTOPHER DENNY, MD†: More than 95% of all tumor specimens exhibit karyotypic abnormalities[1]: duplications or deletions of genetic material or rearrangements between genetic loci on the same (inversion) or different (translocation) chromosomes. Any one tumor specimen can contain several different chromosomal abnormalities. Though many rearrangements appear to be nonspecific, specific chromosomal abnormalities are often strongly correlated with specific tumor types. In a few tumor-specific chromosomal translocations, the chromosomal rearrangements and the genetic loci adjacent to the break points have been defined at the molecular level. These rearrangements usually occur in only one allele, leaving the remaining allele karyotypically and molecularly intact, a finding consistent with a dominant effect.

Translocation could either affect the regulation of adjacent genes, resulting in structurally normal protein products being expressed at inappropriate levels, or alter coding nucleic acid sequences, creating structurally altered protein products that behave aberrantly. We examine two rearrangements, those found in Burkitt's lymphoma and in Philadelphia chromosome-positive acute lymphocytic leukemia (ALL) and chronic myelogenous leukemia (CML), to elucidate these two general mechanisms of genetic disruption brought about by genomic recombination.

Burkitt's Lymphoma—A Model for Transcriptional Deregulation

Burkitt's lymphomas contain one of three characteristic chromosomal translocations—that is, the juxtaposition of the c-*myc* proto-oncogene normally found on chromosome 8 with one of the immunoglobulin loci on chromosomes 2, 14, or 22.[2-4] The t(8;14) translocation, which occurs in 75% to 80% of patients, places immunoglobulin heavy-chain sequences upstream of c-*myc*. The variant t(8;22) and t(2;8)

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ABBREVIATIONS USED IN TEXT

ADP = adenosine diphosphate
ALL = acute lymphocytic leukemia
cAMP = adenosine 3′:5′-cyclic phosphate
CML = chronic myelogenous leukemia
EF = elongation factor
GAP = GTPase [guanosine triphosphatase]-activating protein
GDP = guanosine diphosphate
GTP = guanosine triphosphate
PCR = polymerase chain reaction

rearrangements involve immunoglobulin λ and κ light chains and have frequencies of 15% to 20% and 5%, respectively, in Burkitt's lymphoma.

Chromosomal break points differ depending on which type of rearrangement occurs in the c-*myc* locus (Figure 1).[5,6] The major protein product of c-*myc* seems to be encoded exclusively in the last two of its three exons, although first-exon sequences may be included in a minor protein species.[7] Break points of the t(8;14) translocations occur within the first intron, the first exon, or 5′ to the c-*myc* gene (Figure 1).[8] The variant t(8;22) and t(2;8) break points are found exclusively 3′ to the last c-*myc* exon. Transcription is initiated at the normal c-*myc* promoters in exon one or at cryptic promoters in the first intron if the first exon has been deleted by the translocation. Structurally abnormal c-*myc* proteins seem not to occur in Burkitt's lymphoma.

The translocation of c-*myc* into immunoglobulin loci presumably participates in, rather than results from, tumor formation. In transgenic mice containing the Burkitt's translocation, B-cell lymphoid tumors develop at high frequency.[9,10] It is postulated that the cellular transforming effect of the Burkitt's translocation is mediated by a deregulation of c-*myc*. In Burkitt's lymphoma, c-*myc* is transcribed exclusively from the translocated locus,[11,12] suggesting that c-*myc* transcription should normally be suppressed and that, as a consequence of chromosomal rearrangement, it is not.

What is the mechanism by which c-*myc* is deregulated in Burkitt's lymphoma? Initially investigators postulated that the alteration or removal of c-*myc* first-exon sequences, which frequently occur with the translocation (Figure 1), results in the loss of important inhibitory regulatory elements.[13,14] Although regulatory elements have been identified in this region,[15] these mutations, though frequent, are not invariably present in Burkitt's tumors. Alternatively, deregulation might follow the juxtaposition of immunoglobulin enhancer elements near c-*myc*. The known enhancer elements, however, are more frequently present on the translocated allele not containing c-*myc*. Nevertheless, c-*myc* deregulation seems to be associated with the B-cell phenotype. A genomic immunoglobulin–c-*myc* construct is transcribed in a deregulated manner when placed in a Burkitt's lymphoma cell line but is normally expressed in a fibroblast cell line.[16] This suggests that c-*myc* deregulation in Burkitt's lymphoma could depend on transacting factors or higher order chromatin configurations that are B-cell specific.

Philadelphia Chromosome-Positive Leukemia— A Model of Protein Structure Alteration

The Philadelphia chromosome (Ph), the result of a characteristic translocation between band q34 of chromosome 9 and band q11.2 of chromosome 22,[17] is present in greater than 90% of patients with chronic myelogenous leukemias

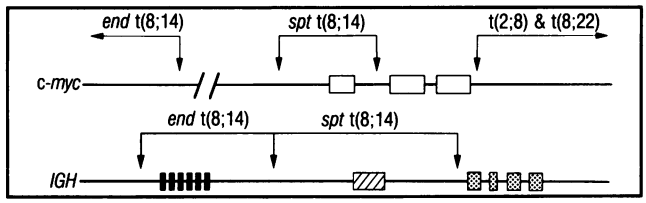

Figure 1.—The genomic representation shows the proto-oncogene c-*myc* (upper schematic) and immunoglobulin heavy chain *(IGH)* (lower schematic) translocation break-point locations in Burkitt's lymphoma. Endemic African *(end)* and spontaneous American *(spt)* Burkitt's lymphoma rearrangements can be distinguished on a molecular level by their tendencies to break at different locations in both c-*myc* and *IGH* loci (McLaughlin et al[6]). Variant Burkitt translocations occur uniformly downstream of c-*myc*. Intervening sequences are indicated by lines and exon sequences by boxes. □=c-*myc*, ■=*IGH* joining segments, ▨=*IGH* switch region, ▩=*IGH* constant segment

and 4% to 5% of pediatric patients with acute lymphoblastic leukemias.[18,19] These rearrangements in ALL and CML are karyotypically indistinguishable, but their molecular structures differ. In both instances, this translocation juxtaposes upstream regions of the *BCR* gene normally located on chromosome 22 to a distal portion of the c-*abl* proto-oncogene on chromosome 9. This results in the formation of hybrid *BCR/ABL* transcripts and proteins.

The translocation break points within the *BCR* gene have been molecularly defined. In CML, this rearrangement of the *BCR* gene (Figure 2) results in a 210-kilodalton (kd) hybrid protein (p210).[20-29] The *BCR* rearrangement in Ph-positive ALL in most cases occurs upstream of the CML break-point cluster within the 70-kilobase first intron[30-33] and produces a shorter *BCR/ABL* fusion protein of 185 kd (p185).[34-36] The messenger RNA (mRNA) coding for the p210 and p185 proteins differs only in the presence of mid-*BCR* exons. The same c-*abl* sequences are included in both molecules, which suggests that *ABL* rearrangements in Ph-positive ALL and CML are similar. A precise mapping of c-*abl* break points has been hampered by its large genomic size. The exons of c-*abl* are dispersed over at least 250 kilobases, and both ALL and CML rearrangements occur upstream of the second exon.[37,38]

The proto-oncogene c-*abl* is the normal cellular counterpart of v-*abl*, a transforming gene isolated from the Abelson murine leukemia virus. Both genes code for known tyrosine kinases, although the normal substrates that they phosphorylate are not known. The proto-oncogene v-*abl* consists of a c-*abl* complementary DNA (cDNA) that has been truncated at both 5′ and 3′ ends with viral *gag* sequences fused to the 5′ end.[39] Normal murine and human c-*abl* genes have similar

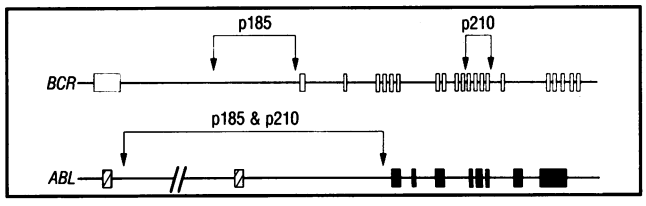

Figure 2.—The schematic shows *BCR* (upper) and c-*abl* (lower) break-point locations in Ph-positive acute lymphocytic leukemia and chronic myelogenous leukemia. Sites of *BCR* rearrangements denoted by arrows are limited to 2 regions: the distal portion of the first intron that gives rise to p185 and intron sequences of a 5-kilobase region that result in p210 formation. Recombination in c-*abl* encompasses a large, imprecisely defined region 5′ to the second exon. Schematics are not drawn to scale relative to each other. Intervening sequences are shown as lines; individual or clusters of exons are denoted as boxes. □=*BCR*, ▨=c-*abl* first exons, ■=c-*abl* common exons

genomic structures. Both use several upstream first exons to initiate transcription.[40-42] These exons account for the 26 to 45 *N*-terminal amino acids. These sequences are deleted along with the next 88 common amino acids in the *GAG/ABL* fusion of v-*abl*. The loss of these c-*abl* sequences may be important for creating the high transforming activity of v-*abl*.[43,44] In contrast to this, those regions that code for kinase domains are preserved in v-*abl* as in c-*abl*.[44,45] Ablation of kinase activity uniformly results in a total loss of transformation potential.

The *BCR/ABL* moieties may act in a similar fashion to *GAG/ABL*. As with v-*abl*, the c-*abl* first-exon sequences are lost and are replaced with varying amounts of *BCR*. Neither p185 nor p210 has the transforming capacity of v-*abl*, however. Nevertheless, retroviral constructs containing both *BCR/ABL* moieties confer a distinct growth advantage to murine long-term bone marrow cultures and clonal rodent cell lines.[46,47] Transformed clonal outgrowths are observed, but only after a more prolonged latency period than that of v-*abl*. This suggests that *BCR/ABL* alone may not suffice to transform and that subsequent events are required. In contrast to viral *GAG* sequences in v-*abl*, the incorporated *BCR* sequences may modulate the transforming activity of *BCR/ABL*.[46] Although they differ only in the amount of incorporated *BCR*, p185 has greater transformation and kinase activities than does p210. It is unclear to what extent p185 is responsible for the more aggressive clinical course of Ph-positive ALL relative to that of CML, which expresses p210.

Future Progress

Thus far, the tumor-associated translocations that have been molecularly characterized have been almost exclusively of lymphoid origin, involving either immunoglobulin or T-cell receptor genes. These loci normally undergo genomic rearrangement. This skewed population also reflects the limitations of the techniques applied to analyze these rearrangements. Only those rearrangements that involve a gene that has already been cloned are detectable at a molecular level. Most tumor-specific translocations, however, remain unexplored. Recent developments of pulse-field gel electrophoresis and yeast artificial cloning techniques permit the manipulation and isolation of large genomic fragments. These and other techniques hold considerable promise for the near future. The molecular elucidation of this wider population of rearrangements is likely to reveal other mechanisms capable of mediating cellular transformation through chromosomal recombination.

Guanosine Triphosphate-Binding Proteins as Oncogenes in Human Cancers

FRANK MCCORMICK, PHD*: The guanosine triphosphate (GTP)-binding proteins are involved in an astonishing variety of cellular processes, including signal transduction, protein synthesis, and protein secretion. At least three classes of GTP-binding proteins are known: the heterotrimeric G proteins, the *ras*-like small GTP proteins ("smg"s), and elongation or initiation factors. Proteins in each of these classes share structural similarities that are largely confined to the regions involved indirectly in guanine nucleotide binding. The nucleotide binding sites of human *ras* p21 protein and bacterial elongation factor (EF) Tu, however, are virtually identical, as determined by x-ray crystallographic analysis.[48] The structure of the GTP-binding subunit of heterotrimeric G proteins (the α-subunit) is expected to be similar to that of *ras* p21 and EF-Tu, but this has yet to be verified.

All of these proteins are regulated by guanine nucleotides; GTP-bound forms are active, whereas guanosine diphosphate (GDP)-bound forms are inactive. Hydrolysis of bound GTP to GDP occurs in all cases, although different mechanisms are used. For *ras* p21 proteins (and probably all smg proteins), hydrolysis needs other cellular proteins, referred to as guanosine triphosphatase (GTPase)-activating proteins (GAPs).[49] Elongation factors and initiation factors apparently need other proteins for this reaction. For EF-Tu, these are ribosomal proteins that ensure that EF-Tu GTP is only converted to the GDP-bound form when aminoacyl transfer RNA is correctly positioned in the ribosome. For heterotrimeric G proteins, the hydrolysis mechanism is built in, so that these proteins hydrolyze GTP at rates determined by the intrinsic properties of the protein rather than by rates determined by an interaction with other cellular molecules.

These molecular mechanisms for deactivation are a potential liability to the cell: if mutations occur that prevent the hydrolysis of bound GTP to GDP, then deactivation cannot occur and the protein from the mutated gene is constitutively active. This seems to be the method by which *ras* and G proteins become activated to become oncogenes in human cancers, as discussed later. Bacterial toxins have exploited the same Achilles heel: adenosine diphosphate (ADP)-ribosylation by cholera toxin of the G protein, known as Gs, inhibits its GTPase activity, which jams Gs in the active state. As a result, the pathway regulated by Gs (production of adenosine 3′:5′-cyclic phosphate [cAMP] by activation of adenylyl cyclase) is permanently activated.

Activation of ras *Mutations in Human Cancer*

Activated *ras* genes were the first oncogenes detected in human cancers.[50] Three *ras* genes in the human genome encode similar *ras* proteins, referred to as H-*ras*, K-*ras*, and N-*ras* p21 proteins. Mutations at codons 12, 13, and 61 occur most frequently. Originally these mutations were detected by transfecting genomic DNA from human tumors into NIH3T3 cells; foci indicated the presence of transforming genes, usually of the *ras* family. Subsequent cloning and sequence analysis, or, in some cases, altered sensitivity to restriction endonucleases showed the frequency of mutations at codons 12, 13, and 61.

The invention of the polymerase chain reaction (PCR) has made it possible to analyze many tumors for the presence of *ras* oncogenes with relatively little effort or technical expertise.[51] Briefly, DNA is isolated from biopsy tissue (which can be in the form of paraffin-embedded slices or blocks, or frozen tissue, for example), and regions of the three *ras* genes around codons 12, 13, and 61 are amplified by PCR. The PCR products are then hybridized with oligonucleotides specific for wild-type or mutant sequences. With this technique, it is possible to detect tumor cells containing point mutations in *ras* genes when the tumor DNA consists of more than about 10% of the total DNA. This imposes a limitation on the technique because it is only possible to detect mutations in cells that have undergone substantial clonal expansion. Another limitation is that mutations that are not expected (that is, not in codons 12, 13, or 61) will not be detected. Experi-

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TABLE 1.—*Activation of the Oncogene* ras *in Human Cancers*

Type of Cancer	*Incidence, %*	*Mutated ras Gene*
Pancreatic carcinoma	90	K-*ras*
Colon (adenoma; adenocarcinoma)	50	K-*ras*
Thyroid (follicular)	50	N-, K-, H-*ras*
Lung (adenocarcinoma)	40	K-*ras*
Acute myelogenous leukemia	30	N-*ras*
Myelodysplastic syndromes	30	N-*ras*
Kidney carcinoma, melanoma	10-20	N-*ras*
CML, ALL, CLL, non-Hodgkin's lymphoma	<5	
Breast, ovarian, cervical, bladder, stomach	<5	

ALL = acute lymphocytic leukemia, CLL = chronic lymphocytic leukemia, CML = chronic myelogenous leukemia

ence in animal and in vitro systems suggests that mutations at other positions are unlikely to occur.

Table 1 summarizes the results of some PCR analyses of *ras* mutations in human cancers. Several issues are raised by these results: first, it is not at all clear why a particular type of *ras* gene (N-*ras* versus K-*ras* and H-*ras*, for example) should predominate in a particular type of cancer. This does not seem to reflect the differential expression of a particular *ras* species in a particular type of tumor progenitor cell, as all three *ras* genes are expressed ubiquitously. The biologic properties of activated *ras* genes in vitro seem to be similar: activated K-*ras* genes are functionally interchangeable with H-*ras* genes in transforming NIH3T3 cells, for example.

The exact role of *ras* mutations in human cancers is not yet known, but *ras* activation can clearly be either an early event (possibly an initiating event) or a later event contributing to the progression to more malignant forms of the tumor. A model has been derived to explain how point mutations activate *ras* gene function (Figure 3): the active form of the *ras* p21 protein (the GTP-bound form) is converted to the inactive form by the action of a cellular protein called GAP.[49] This protein is now known to have several interesting properties beyond its ability to interact with *ras* p21: it associates with activated tyrosine kinase receptors—platelet-derived growth-factor receptor, epidermal growth factor receptor, M-central nervous system receptor—and seems to be part of the process of signal transduction from these receptors.[52] In normal cells, GAP keeps p21 in its inactive GDP-bound state through activating the GTPase of p21. In *ras*-transformed cells, however, GAP binds to the GTP-bound *ras* mutant but fails to convert it to the inactive state. This permits a persistent activation of the pathways that *ras* p21 is presumed to control, and transformation is the end result. The protein GAP itself is involved in this pathway.[49]

Heterotrimeric G Proteins

Heterotrimeric G proteins play a crucial role in transmitting signals from hormone receptors to internal second messengers. The heterotrimeric G-protein, Gs, for example, stimulates adenylyl cyclase in response to the activation of β-adrenergic receptors (Figure 3). As with *ras* proteins, the GTP-bound state of the G protein is the active state. Unlike *ras* proteins, intrinsic GTPase (rather than GAP-mediated GTPase) converts the GTP-bound state to the inactive, GDP-bound state. The exposure of cells to cholera toxin inhibits this intrinsic GTPase so that the G protein is jammed in its active state constitutively. This causes an uncontrolled accumulation of cAMP. For most cells, the overproduction of cAMP is not mitogenic. For cells of pituitary and thyroid origin, however, exposure to cAMP stimulates proliferation. This finding, together with the discovery that some pituitary tumors have high levels of cAMP constitutively, led to the speculation that these tumors contain defective G proteins that activate adenylyl cyclase constitutively and thus cause uncontrolled growth.[53] Sequence analysis of Gs-α cDNA has shown that point mutations in codons 201 and 227 occur in these tumors. Arginine 201 is a major site of ADP ribosylation by cholera toxin. Mutations at this position (like cholera toxin modification) inhibit GTPase activity. Mutations at codon 227, the Gs-α equivalent of codon 61 in *ras* p21, also inhibit Gs-α GTPase. Thus, both types of mutation resemble activating mutations in *ras* p21 and permit the G protein to exist in its active GTP-bound state constitutively.

To extend these findings, we have used PCR to examine the frequency of codon 201 and 227 mutations in 300 human tumor biopsy specimens. Of all the specimens analyzed, mutations were detected only in tumors of pituitary and (less frequently) thyroid origin.

For 16 tumors, the cyclase levels were elevated in 8 and normal in 8. All 8 high-cyclase tumors had mutations at either codon 201 or (less frequently) 227; none of the tumors with normal cyclase were found to have mutant Gs-α alleles. It thus seems that codons 201 and 227 are the most frequent sites of activation. The tumors with high cyclase levels were few, however, and it is possible that other codons are activated in Gs-α in other tumors.

We then applied the same PCR strategy to examine possible point mutations in another G protein, Gi-2, that inhibits cyclase activity (hence the name) but is also thought to be involved in other signaling pathways. It might be anticipated that mutations at equivalent positions in Gi-2 would occur in some human tumors. We therefore screened the same panel of tumors for mutations in codons 179 and 205. Of all the specimens analyzed, mutations were found only in tumors of the adrenal cortex and ovarian origin.[54] We do not yet know which pathways are turned in on these tumors to stimulate constitutive growth; clearly this is of great interest to the understanding of the development of these kinds of cancer.

Summary

Mutations of the *ras* and heterotrimeric G proteins can persistently activate the pathways that these proteins control. In some cases, these pathways seem to be involved in the control of cell growth, and their achromic activation is thought to cause uncontrolled cell division. This seems to be a major mechanism for the development of tumors in humans and will be the focus of attempts in the future to improve diagnostic and therapeutic methods.

Tumor Suppressor Genes

DR KOEFFLER *and* CARL W. MILLER, PHD*:

Somatic Cell Hybrids

Original evidence for tumor suppressor genes was indirect. The fusion of a normal and a tumor cell results in somatic hybrids that usually have the phenotype of the normal cell and are nontumorigenic.[55-59] These somatic hybrids lose chromosomes, and, with this loss, the hybrids occasionally revert to a tumorigenic phenotype. This reversion per-

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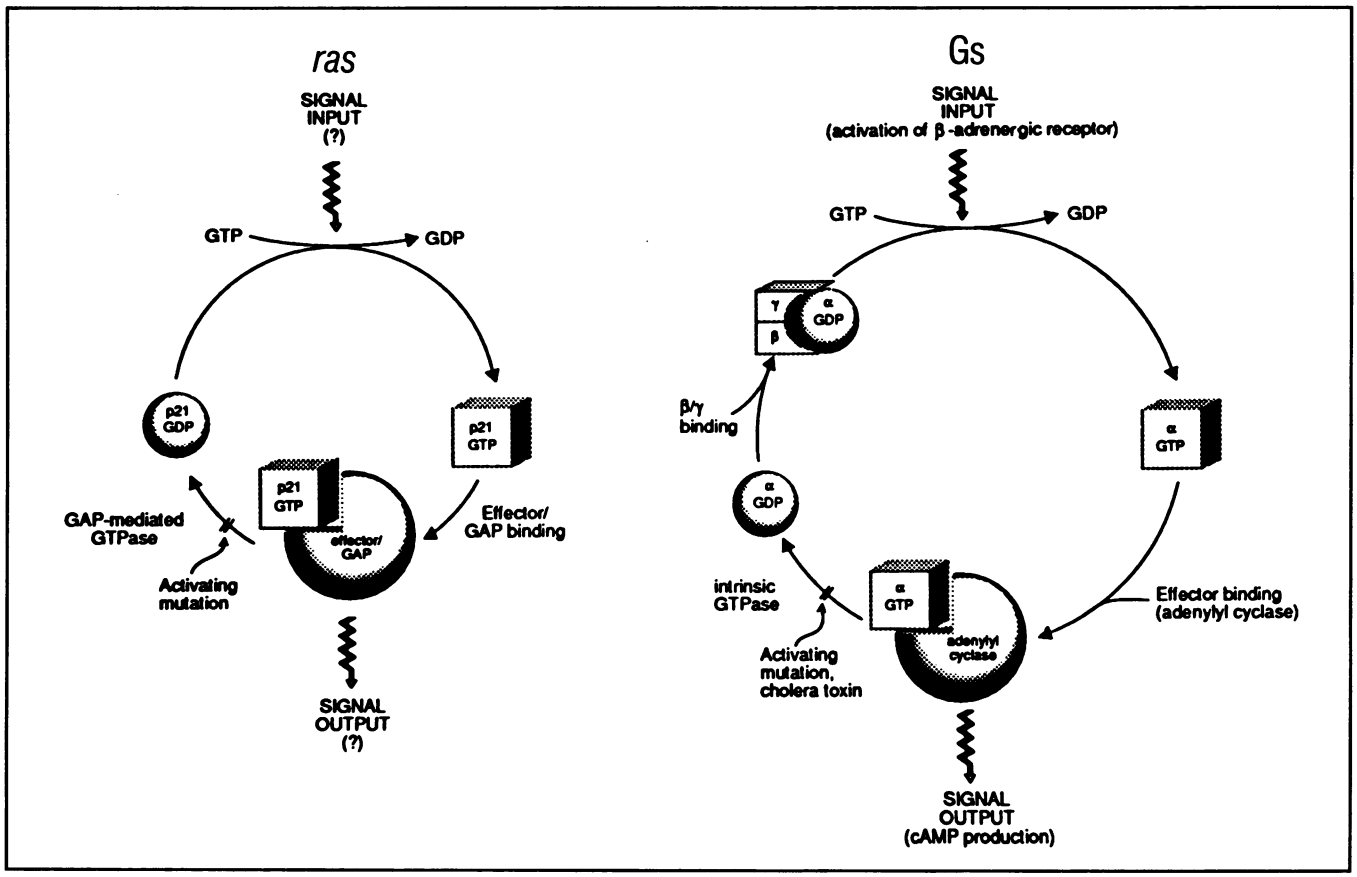

Figure 3.—The regulation of *ras* p21 and Gs proteins by the guanine nucleotides guanosine diphosphate (GDP) and guanosine triphosphate (GTP) is schematically depicted. GAP=guanosine triphosphatase (GTPase)-activating protein

mitted the identification of chromosomes associated with the normal phenotype, which, when lost, resulted in a return to the malignant phenotype. This technique has become more sophisticated: single normal chromosomes added to malignant cells permit the identification of normal chromosomes, which can cause a reversion of specific malignant cells to a nonmalignant phenotype (Figure 4). For example, the insertion of normal chromosome 11 can cause cells from cervical carcinomas, Wilms' tumors, and rhabdomyosarcomas to revert towards a normal phenotype.

Karyotypic Analysis

Specific chromosomal deletions are associated with malignant tumors. Leukemia cells, because of their availability and ease of growth in liquid culture, provided one of the earliest paradigms that specific subtypes of leukemia were associated with specific, nonrandom losses of chromosomal material. For example, a loss of the long arm of chromosome 5 (5q) or 7 (7q) is found in a recognizable group of patients with either preleukemia or acute myelogenous leukemia, who previously had exposure to a mutagen such as alkylating chemotherapy. Karyotypes of solid tumors have also identified nonrandom losses of chromosomal material. Chromosomal deletions at 3p, 11p13, 13q14, and 22 frequently occur in patients with lung cancer, Wilms' tumor, retinoblastoma, and acoustic neuroma, respectively.

Loss of Heterozygosity

The use of restriction-fragment-length polymorphism to identify allelic loss is a powerful method to associate specific tumors with specific chromosomal deletions. These deleted areas often represent regions where a normal allele has been lost and a mutated allele has been duplicated by either nondisjunction or mitotic recombination. Thus, a reduction to homozygosity unmasks the recessive, defective allele. A loss of heterozygosity of various chromosomal regions is the tombstone that identifies genes whose homozygous functional loss triggers tumor formation. Many probes are now available to identify restriction-fragment-length polymorphisms. Probes that identify varying numbers of tandem repeats of DNA sequences are particularly useful because each can distinguish corresponding parental alleles in a high proportion of normal DNA specimens.[60] Table 2 provides a partial list of chromosomal locations that are associated with a loss of heterozygosity in human tumors.[61-82]

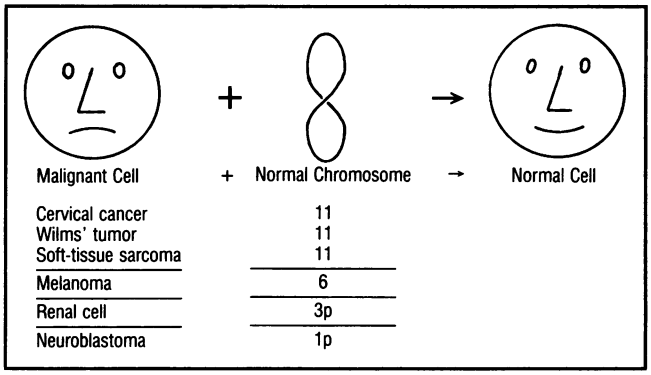

Figure 4.—Examples are shown of specific normal chromosomes that, when placed into a cell, can cause a malignant cell (symbolized by a frowning face) to revert to a nonmalignant cell (symbolized by a smiling face).

TABLE 2.—*Loss of Heterozygosity in Human Tumors*

Chromosome	Type of Cancer	Reference
1p	Breast; melanoma; neuroblastoma; pheochromocytoma	Limon et al, 1988[61]; Genuardi et al, 1989[62]; Tsutsumi et al, 1989[63]
1q	Breast	Chen et al, 1989[64]
3p	Lung; cervical; renal	Naylor et al, 1987[65]; Yokota et al, 1987[66]; Kovacs et al, 1988[67]; Yokota et al, 1989[68]
5q	Colon, myelodysplastic syndrome	Solomon et al, 1987[69]; Vogelstein et al, 1989[70]; Nagarajan et al, 1990[71]
11p	Breast; lung; bladder; Wilms' tumor; hepatoblastoma	Fisher et al, 1987[72]; Mackay et al, 1988[73]; Weston et al, 1989[74]; Mannens et al, 1990[75]; Tsai et al, 1990[76]
11q	MEN-1	Larsson et al, 1988[77]
13q	Lung; stomach; retinoblastoma; osteosarcoma	Yokota et al, 1987[66]; Motomura et al, 1988[78]
17p	Breast; lung; colon; cervical; bladder; ovary; osteosarcoma	Yokota et al, 1987[66]; Weston et al, 1989[74]; Tsai et al, 1990[76]; Monpezat et al, 1988[79]
18q	Colon	Monpezat et al, 1988[79]
22	Meningioma; acoustic neuroma; pheochromocytoma	Rouleau et al, 1987[80]; Takai et al, 1987[81]; Okazaki et al, 1988[82]

MEN-1 = multiple endocrine neoplasia type 1

DNA-Transforming Viruses

Proteins coded by DNA-transforming viruses often bind to cellular proteins. The simian virus 40 (SV40) T antigen, adenovirus E1A, protein E7 of human papillomavirus type 16, and polyoma T antigen can bind and possibly inactivate Rb protein, a putative tumor suppressor protein missing in retinoblastomas. Furthermore, SV40 T antigen, adenovirus E1B, and protein E6 of human papillomavirus type 16 can bind and perhaps inactivate the p53 protein, also a putative tumor suppressor. The viral oncoproteins may trigger abnormal proliferation by blocking key regulatory functions of Rb and p53, which are normally responsible for constraining cellular proliferation.

Other as-yet-undefined cellular proteins also bind to DNA-transforming viruses. DNA-transforming viruses may be our fishing nets to catch and identify new tumor suppressor proteins.

Tumor Suppressor Genes

Five tumor suppressor genes have been cloned: *RB* ("retinoblastoma susceptibility" gene),[83] *P53, DCC* (deleted colon cancer "gene"),[84] *MCC* (mutated colon cancer gene),[85] and *WT* (Wilms' tumor gene).[86,87]

RB. The *RB* gene, located at chromosome 13, band q14, codes for a protein that does not change in amount during the cell cycle.[88,89] The Rb protein is a 110-kd unphosphorylated nuclear protein during G_0 and G_1 stages of the cell cycle and a phosphorylated 115-kd protein during S and G_2.[90,91] Terminally differentiated normal cells, such as macrophages, have a predominance of phosphorylated Rb. The transforming protein (T antigen) of SV40 binds only the unphosphorylated form of Rb, which may constrain cell cycle progression. Release of this constraint may be by phosphorylation of Rb in normal cells after exposure to growth factors or by either mutation of *RB* or the binding of viral protein (for example, T antigen) to Rb, which releases critical constraints in the cellular regulatory circuitry. Cloning of the *RB* gene has permitted molecular genetic analysis of retinoblastoma and other malignant neoplasms that have a loss of heterozygosity of restriction-fragment-length-polymorphism at 13q14. Transfection of the *RB* gene into tumor cell lines bearing *RB* gene abnormalities inhibits proliferation.[92] Rearrangements of the *RB* gene have been found in retinoblastomas, osteosarcomas, lung cancers, and leukemias.

P53. The *P53* gene, first identified in the nucleus of 3T3 murine cells transformed by SV40,[93] binds heat-shock protein, a protein (which may be cdc-2), and single-stranded DNA.[94-96] It competes with DNA polymerase-α for binding to T antigen, suggesting *P53* may be involved in the regulation of the cell cycle. We and others have shown that *P53* can act as a transcriptional activator, presumably activating genes that regulate cell proliferation.[97-99]

Several observations define *P53* as a tumor suppressor: transfection of normal *P53* into rodent cells decreases the transformation potential of activated *ras* plus either *myc*, mutant *P53*, adenovirus E1A, or SV40 T antigen.[100] In addition, transfection of normal *P53* gene into cell lines bearing mutant or rearranged *P53* inhibits proliferation of the cells.[101,102] Mutant *P53*, on the other hand, has the ability to confer transformed phenotype on rodent primary cells in culture.[103,104] Transgenic mice carrying a highly expressed mutant *P53* have an increased incidence of several cancers.[105] Mutant *P53* in these mice may compete with normal *P53* to prevent normal suppressive interaction of *P53* with either DNA or other proteins. A loss of heterozygosity of chromosome 17p is a feature of many cancers, occurring in at least 60% of the tumors of colon, breast, lung, brain, cervix, adrenal cortex, bone (osteosarcoma), and urinary bladder. About 20% of human osteosarcomas have gross rearrangements of the *P53* gene detectable by Southern blots.[106,107] Mutations of *P53* have been detected in cancers of colon, lung, and esophagus, T-cell leukemias, chronic myelogenous leukemias, brain and breast cancers, and a variety of cell lines.[104,108] Most of these mutations occurred in four "hot spots" that represent evolutionarily conserved regions of *P53*.[109] Cellular transformation by viruses may be mediated in part by viral antigen binding of *P53*. For example, *P53* binds to SV40 T antigen and adenovirus E1B, perhaps contributing to transformation by interfering with the tumor suppressor function of *P53*.[93,110] This interaction may cause transformation by inactivating the tumor suppressor activity of *P53*. Many similarities exist between *P53* and *RB:* both bind to T antigen of SV40.[93,111] *RB* binds to E1A, and *P53* binds to E1B of adenovirus.[110,112] These viral proteins may foster tumor formation by binding and inactivating the suppressor function of *RB* and *P53*.[107,108]

DCC. The *DCC* (deleted colon cancer) gene on the long arm of chromosome 18 is frequently abnormal in colon cancer.[62] The predicted amino acid sequence of *DCC* is highly similar to a neural cell adhesion molecule. Expression of this gene is greatly reduced or absent in colorectal cancers. Most

frequently, this gene is disrupted in colorectal tumors by DNA insertions in one of its introns. Perhaps *DCC* protein plays a role in the pathogenesis of colon cancer by altering the normal cell-cell interactions controlling growth.

WT. A loss of heterozygosity affecting the short arm of chromosome 11 occurs in Wilms' tumors. The putative tumor suppressor gene involved at this locus is known as the *WT* gene.[86,87] The *WT* product has properties of a transcriptional activator and has DNA binding specificity for the epidermal growth factor I gene.[113] Deletions and rearrangements of the *WT* locus have been reported in Wilms' tumors.[114,115]

MCC. Allelotype analysis of colorectal cancer frequently shows a loss of heterozygosity affecting the long arm of chromosome 5. A gene frequently mutated in this region in colorectal cancer has been identified and named a mutant in colon cancer *(MCC)*. This gene has sequences suggesting that it is related to the G-protein family.[85]

Tumors

Retinoblastoma. Retinoblastoma is a malignant neoplasm of the retina that occurs in children usually younger than 4 years. It occurs in hereditary and nonhereditary forms. About 40% of patients with retinoblastoma have the hereditary disease; therefore, the predisposition of retinoblastoma is passed to their progeny as an autosomal disorder. Only 25% of these patients with retinoblastoma have a familial history of the disease; new *RB* mutations of the germ cells probably occur in the remaining 75% of those with familial retinoblastoma. These germ-line mutations of *RB* almost always occur on the paternal allele.[116]

In patients with hereditary retinoblastoma as compared with the nonhereditary form, tumors usually develop at an earlier age and are more frequently multifocal. Tumor formation requires that both *RB* alleles be either altered or inactivated. In the hereditary form, one *RB* allele is already mutated in the germ line. In the nonhereditary or "sporadic" form, two rare somatic alterations are needed within the same retinal precursor cell to eliminate the expression of normal *RB*. Several mechanisms might account for the loss of a second, normal *RB* gene. A random somatic mutation, such as a point mutation, could occur on the second allele. The frequency of this second mutation is low, about 10^{-6} per cell generation. More frequent genetic events are either mitotic recombination or gene conversion of the entire region of the chromosome carrying the mutated *RB*. This produces a doubling of the initially defective allele and loss of the hitherto normal chromosome. This reduction to homozygosity, which unmasks a recessive, defective allele, may be a frequent genetic mechanism by which other genes behave like the *RB* gene in tumorigenesis. About 20% of retinoblastomas have either a large deletion or rearrangement of the *RB* gene; most of the others have point mutations. Most frequently a point mutation of these produces a stop codon, halting translation of the *Rb* protein. Mutations at mRNA splice sites also occur often, and missense mutations occur occasionally. Most retinoblastomas do not express *Rb* protein.

Patients with hereditary retinoblastoma have an increased incidence of osteosarcomas, suggesting that a loss of the expression of *RB* is important in that tumor also. Most osteosarcomas develop after the patient has a retinoblastoma, suggesting that the former malignant tumors need mutations in addition to *RB*. Somewhat perplexing is the infrequency of reports of urinary bladder, breast, or lung cancer in patients with hereditary retinoblastomas. Each of these tumors is associated with a mutated *RB* gene. Perhaps these cancers need the accumulation of so many mutations that heterozygosity for an *RB* mutation does not provide a great liability.

Lung cancer. Tobacco smoke is the major causal agent that contains numerous chemical and physical carcinogens and clastogenic agents. These play an interactive role in the genetic changes that occur during the multistage process of carcinogenesis of lung. One or several proto-oncogenes may be activated in these tumors, including members of the *myc, ras, raf,* and *jun* multigene families.[117] The inactivation of several tumor-suppressor genes is probably also important.

The most frequent cytogenetic abnormality in lung cancer is deletion of the chromosomal region 3p14−23. Cytogenetic abnormalities in this region occur in about 70% of small-cell and 60% of non–small-cell lung cancers.[65,66,118] Analysis of restriction-fragment-length polymorphism shows a frequent loss of heterozygosity of several other chromosomal regions, suggesting that lung cancers result from the accumulation of mutations of several oncogenes.[66,70,74] The loss of heterozygosity of specific chromosomal regions varies between different histologic subtypes.[74] For example, a loss of heterozygosity on 3p and 13q (*RB* gene) occurs more frequently in small-cell lung cancer than in non–small-cell lung cancer.[66,74] More than 75% of squamous cell cancers have a loss of heterozygosity for 17p (*P53* gene); in contrast, less than 20% of adenocarcinomas have a loss of heterozygosity for this region.[66,74] A loss of heterozygosity for 3p, 13q, and two loci on chromosome 11 (11pter−p15.5; 11p13) are of equal frequency in both squamous cell carcinoma and adenocarcinoma of the lung.[74] The risk of squamous cell lung cancer is greater in heavy smokers (> 1 pack per day) than is the risk of adenocarcinoma. Taken together, this suggests that an inactivation of *P53* (17p) may be closely associated with the smoking of cigarettes and the development of cancer.

Major rearrangements of the *RB* gene occur in about a sixth of small-cell lung cancers,[119] but the methods used are insensitive for detecting point mutations. Expression of Rb mRNA was absent or barely detectable in cell lines from 75% of small-cell lung cancers and 20% of non–small-cell lung cancers. The Rb protein may be absent in nearly all small-cell lung cancers and 20% of non–small-cell lung cancers.[110] Analysis of *P53* in cell lines from 14 small-cell and 15 non–small-cell lung cancers showed that more than half of the specimens had DNA abnormalities including point mutations.[66] Our analysis of 40 lung cancers with a loss of heterozygosity of chromosome 17p showed a 30% frequency of *P53* mutations in these specimens.

Colon cancer. Allelotyping of allelic deletions using probes for every relevant autosomal arm suggests that colon cancer probably develops as a result of a multistep process of mutagenesis (Figure 5).[70] Deletion on chromosome 5q (35% incidence) and mutations of K-*ras* (45% incidence) usually occur at a relatively early stage of tumorigenesis. Allelic deletions of chromosomes 17 (region of *P53*), 18 (region of *DCC*), and 5 (region of *MCC*) often develop at a later stage of carcinogenesis. The order of these changes, however, is probably less important than their accumulation. Alterations probably continue to accumulate even after colon carcinoma

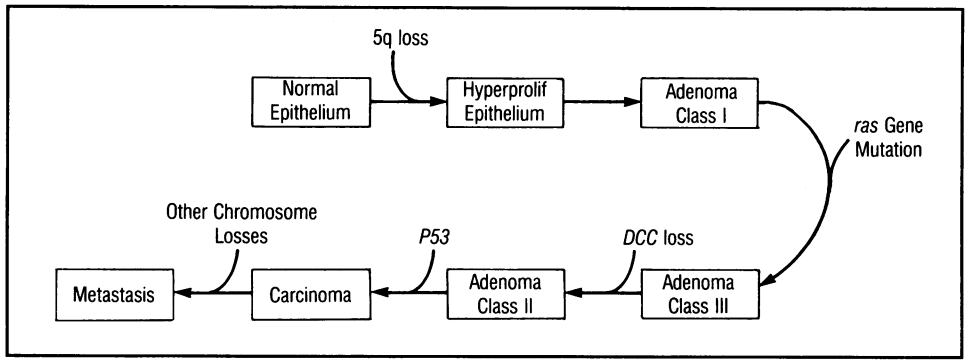

Figure 5.—The diagram shows the accumulation of mutations that can cause a normal colonic cell to become an adenocarcinoma cell. The tumor suppressor gene on the long arm of human chromosome 5 (5q) is *MCC* (Kinzler et al[85]). The drawing is adapted from studies by Vogelstein et al.[70]

develops. Most colon cancers have from three to six genetic alterations. Patients with greater than 20% allelic losses have a poor prognosis and are more likely to get a recurrence of their disease and to die of colon cancer.[121] Most of the allelic deletions are subchromosomal, rather than a loss of an entire chromosome.[121]

The *DCC* protein is not expressed in colon cancer, whereas it is expressed in most normal tissues. The *P53* gene is often mutated in colon cancer, and most of these are missense mutations.[108] More than 75% of colon tumors express apparently normal-sized *P53* mRNA, but little is known of the expression of p53 protein in these specimens.[108] A loss at 5q15−q22 seems to be specific to colorectal tumors, and the relevant gene in this region may be the *MCC* gene, which has some homologies to a G-binding protein. Patients with hereditary adenomatous polyposes coli *(APC)* have deletional mutation in this region, but the other allele seems to be normal, suggesting that a mutation of only one allele is sufficient for adenoma formation. This mutated gene, therefore, is not acting recessively. An array of linked DNA probes flanking the *APC* gene now permits the identification of persons who have inherited a mutated *APC* and have at least a 50% chance of carcinomas developing. These patients need close monitoring and prophylactic colectomy.

Urinary bladder cancer. Patients with bladder cancers often (70%) have a loss of heterozygosity of 17p (location of *P53*), 9q, and 11p. Deletions on 6p (30%) and 14q (20%) occur less frequently.[76,122] A coincidental loss of 17p and 11p in bladder cancers (50% incidence) is similar to findings in squamous cell carcinoma of the lung.[74,76] Cigarette smokers have an increased incidence of urinary bladder cancer; perhaps cigarette smoke preferentially causes mutations in genes in these regions.

Astrocytomas. Gliomas are the most common primary tumors of the human central nervous system, most of which are astrocytomas. A loss of heterozygosity of chromosomal regions in astrocytomas occurs on chromosomes 13, 17, and 22, with nearly 50% of the tumors having a loss of 17p.[123] A loss of heterozygosity on 17p occurs in high- and low-grade astrocytomas, suggesting that abnormalities of *P53* occur at an early stage of tumor formation. Patients with the Turcot syndrome have familial polyposis and either malignant astrocytoma or medulloblastoma. This is consistent with a mutated *P53* being associated with tumorigenesis of colon cancer and astrocytomas. Perhaps these patients have hereditary mutation of *P53*, as has been described in the Li-Fraumeni syndrome. In families with the Li-Fraumeni syndrome, one *P53* allele has a mutant codon between residues 240 and 260. Diverse mesenchymal and epithelial neoplasms develop at multiple sites in these patients.[124,125]

Suppressor Genes and the Heterozygous State

Tumor suppressor genes are thought to act in a recessive manner.[56,126] That is, inactivation of both parental alleles is needed if a loss of the constraining effects of a normal tumor suppressor gene is to occur. The paradigm for this concept is retinoblastoma. Recessive action may not always be the case in other cancers, however. Hereditary tumors of some patients have not lost the normal allele that corresponds to the mutated locus linked to the predisposition to cancer, such as neurofibromatosis type 1, multiple endocrine neoplasia type 2, and adenomatous polyposis coli. Here the normal alleles of chromosomes 17, 10, and 5, respectively, seem to be intact in the familial tumors.[127] Also, transfection of a mutated *P53* gene into normal fibroblasts can cause transformation; this occurs even though these cells also express normal *P53*.[128,129] The *P53* produces oligomers, and perhaps the oligomerization of normal and mutated *P53* alters the function of the normal *P53*, explaining the dominant effect of such a *P53* mutation. In brief, mutations of some tumor suppressor genes may affect growth even in the presence of a normal allele.

Future Direction

In the coming decade, we should see a rapid advancement in the clinical application of the evolving knowledge of tumor suppressor genes. We should be able to use the polymerase chain reaction to identify rapidly the loss of tumor suppressor genes by analyzing a variable number of tandem repeats using only a few tumor cells. The classification and prognosis of these cancers will be possible by rapidly identifying all mutations of tumor suppressor genes in the patients' tumor cells by analyzing the loss of heterozygosity. A targeted replacement of deleted or mutated tumor suppressor genes in either the tumor or premalignant tissue may permit a reversal of malignancy in these patients.

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