Oncogenes and Tumor Suppressor Genes: Therapeutic Implications

Sanford A. Stass and A. James Mixson
University of Maryland School of Medicine, Baltimore, Maryland 21201

Abstract

Genetic instability is a hallmark of cancer. Alterations in DNA through mutations, deletions, and translocations affect genes that are fundamental to normal cell growth differentiation and programmed cell death. Here, we discuss these alterations as they relate to oncogenes and tumor suppressor genes. In addition, we describe the implications the changes in oncogenes and tumor suppressor genes have on designing new therapeutic strategies for the treatment of cancer.

Introduction

Cancer is a genetic disease. It is characterized by genetic instability and long-term uncontrolled growth. Normal cellular function requires control of growth, differentiation, and programmed cell death. These are complex processes involving a cascade of cellular events and proteins encoded by DNA. There are checks and balances in the normal (wild-type) cell DNA structure that translate normal cell function. Alterations in the DNA through mutations, deletions, or translocations result in aberrations that have been well documented to affect cell growth, differentiation, and programmed cell death. These disruptions in DNA (genetic changes) and their resulting abnormally functioning proteins manifest in an autonomous and clonal proliferation of cells that we recognize as cancer.

We now have vast information regarding these genetic changes. Unfortunately, merely identifying the defects may not sufficiently translate into therapeutic efficacy. First, one may not be able to approach all the abnormalities simultaneously. Second, there are likely interactive effects that we may not totally understand or be able to reverse. A corollary to this is that the identification of an abnormality or abnormalities may deflect our attention from an approachable end point. Third, even if the genetic defect has been specifically identified, there may not be an effective therapeutic strategy to reverse it. A case in point is sickle cell disease. Thus, as we outline the genetic alterations that follow, we will discuss how therapeutic intervention might target these molecular aberrations. However, none of the therapeutic strategies have been proven to be effective long-term.

Mutations in tumor suppressor genes, such as p53, as in the Li-Fraumeni syndrome, can be injected with germ-line p53 mutations or can occur sporadically in tumors. Mutations in oncogenes are also seen in a variety of tumors. Genes coding for DNA repair proteins can also be mutated (hereditary non-polyposis colon cancer) or be abnormally expressed (i.e., reduced activity of DNA ligase I in Bloom’s syndrome). A defect in the DNA repair process could provide the mechanisms that predispose to genetic loss and mutations. A review of the varied genetic changes, involving oncogenes and tumor suppressor genes, seen in cancer suggests that there is, indeed, an underlying defect that allows critical abnormalities in DNA to persist un repaired (1).

At the nucleotide level, genetic mutations that are associated with malignancy can be substitutions, additions, or deletions. A second group of molecular genetic changes comprises the translocations. In this instance, all or part of a gene recombines with a gene at a different chromosomal site. One of the most thoroughly studied translocations is the t(9;22) (q34;q11) or Philadelphia chromosome, which occurs in 95% of CML. The cellular ABL gene on chromosome 9 translocates to chromosome 22, where it combines with the BCR gene in a head-to-tail configuration, resulting in a hybrid BCR-c-ABL protein (p210), with tyrosine phosphokinase activity that can transform hematopoietic cells (2). A second form of translocation, resulting in aberrant expression, is the example of an oncogene translocated to a site regulated by different transcriptional activators or enhancers. This is seen in Burkitt’s lymphomas, in which c-myc is translocated in juxtaposition to the immunoglobulin heavy chain locus t(8;14) (q24;132), resulting in the deregulation of c-myc, as a result of its proximity to cis-activating elements within the IgH locus.

An additional form of genetic alteration is the amplification of the gene itself, which can result in overexpression. These amplified genes can be present in homogenous staining regions in a particular chromosome or present in double minute chromosomes outside the normal chromosomal structure. Examples of overexpressed oncogenes include neu, amplified in breast and ovarian carcinoma, and n-myc, in neuroblastoma. In addition, alterations in gene expression might be due to alterations in DNA-binding proteins, which up-regulate or do not down-regulate (i.e., activated jun, fos, ErbA, ets, myb, myc, and so on), a mutation of the protein binding that affects binding of regulator proteins or increased mRNA presence due to altered DNA, RNA, or ribosomal protein structure, which hinders or prevents degradation. In addition to the genetic defects already discussed, a loss of expression could occur with gene deletion. We know that large, chromosomal regions or whole chromosomes may be lost (i.e., −5 and/or −7 in myelodysplasia, or loss of Rb gene in retinoblastoma).

Although we know intricate details of these molecular genetic defects in cancer, this has not translated into directed

2 To whom requests for reprints should be addressed, at University of Maryland, Department of Pathology and Greenbaum Cancer Center, 22 South Greene Street, Baltimore, MD 21201. Phone: (410) 328-1237; Fax: (410) 328-1813.
3 The abbreviations used are: CML, chronic myelocytic leukemia; G-protein, guanine-binding protein; GAP, GTPase-activating protein; CDK, cyclin-dependent kinase.
therapeutic strategies that result in substantial cures. This is analogous to sickle cell disease. Clearly, we have known the genetic defect in sickle cell disease for many years. Unfortunately, this has not translated into a therapeutic approach that can cure the disease, and long-term effective therapy has, so far, not been possible. Therefore, we should not assume that further therapeutic advances will result from additional knowledge of genetic mechanisms. Recently, Dr. Robert Weinberg said that “for a number of genetic diseases, knowing the genes might not help the patient one whit” (3). On the other hand, it would seem that we have not taken full advantage of our knowledge. We need to refocus our investigations on developing therapeutic strategies using our knowledge of molecular genetic changes, with the resultant effects on cell biology, to target our therapeutic efforts. Here, we will focus on an overview of oncogenes and tumor suppressor genes and how our knowledge in this area could be directed to therapeutic interventions.

Oncogenes

The homology of viral oncogenes found in tumor-producing RNA viruses (retroviruses) to cellular oncogenes was established in 1976 by H. E. Varmus, M. Bishop, and colleagues with their work on Rous sarcoma virus and the src gene. It is a mutation or altered expression of these cellular proto-oncogenes that is associated with cellular transformation. These proto-oncogenes are classified according to their cellular function, as follows.

One group is composed of growth factors and includes c-sis, responsible for producing platelet-derived growth factor, and Kst-1/K-fgf, encoding angiogenesis growth factor. Their involvement in oncogenesis is hypothesized to be a direct mitogenic effect (platelet-derived growth factor) or constitutive growth stimulation through an autocrine mechanism.

Growth factor receptors are a second group of proto-oncogenes. They include erb-B1, erb-B2, c-neu, met, c-fms, kit, trk, ret, and sea. The structures of these genes include ligand-binding, transmembrane, and cytoplasmic catalytic domains, which, in the majority of the proto-oncogenes, are tyrosine kinase domains. Oncogenic activation results in a constitutive activation of the receptor in the absence of ligand.

A third group of proto-oncogenes is signal transducers, composed of tyrosine protein kinases (src, ras, mos, pim-1, fes, fgr, lck, and yes). Most of the kinases are tyrosine kinases that have homology in their catalytic domain; however, some are serine and threonine kinases. Oncogenic activation appears to affect the negative regulatory domains, allowing these enzymes to constitutively phosphorylate their substrates.

A fourth group is composed of the GTP-binding oncogenes (H-, k-, and N-ras, gip2, and gsp). These are G-proteins that are responsible for transmitting signals from cell surface ligands, i.e., growth factors and hormones, and neurotransmitting to effectors, including adenylyl cyclase and phospholipase C. This transduction, mediated by GTP, to GDP involves binding a GAP. G-proteins, such as the RAS family of oncogenes, are activated by point mutations or amplification, resulting in altered GTP-binding or GTPase activity, so that there is extended stimulation of effector enzymes.

The fifth group of proto-oncogenes is composed of transcriptional regulators, including erbA-1, erbB-2, ets-1, ets-2, fos, jun, myb, c-myc, L-myc, N-myc, rel, ski, Hox11, lyl-10, lyl-1, Tal-1, E2A, PBX-1, Tig-1, and rhom2/ Tig-2. These genes contain functional domains that mediate DNA binding and protein-protein interactions. Some of the genes encode proteins that interact with each other to form heteroduplexes that control transcription of a target gene. Expression of these genes is precisely regulated to respond rapidly to proliferation and differentiation signals. In some instances (i.e., c-myc), activation is through deregulated expression, preventing the cell from terminating its proliferation phase and entering its differentiation phase.

bcl-2 is the only known oncogene that is identified as a regulator of programmed cell death. p53 is also involved in programmed cell death and is discussed later. The BCL-2 protein functions on the inner membrane of the mitochondria and prevents programmed cell death. In lymphoid cells, BCL-2 is thought to maintain the long lifespan of memory B cells and plasma cells. BCL-2 is located on chromosome 18 and becomes activated by deregulated expression, resulting from translocation into the IgH locus in the t(14;18) (q32;q21) abnormality in lymphomas. Although increased survival may not result in malignant transformation, the extended lifespan provides lymphoid expansion, accumulation, and proto-oncogene activation.

Therapeutic Interventions

ras. Activated ras oncogene is the acutely transforming component of Harvey sarcoma virus 15. There are three cellular homologues: H-ras, k-ras, and N-ras. The ras superfamily is part of the large family of G-proteins and is a component of the growth-promoting signal transduction pathway (4). Therefore, activating ras mutations in cancer have been extensively studied. ras mutation in codons 12, 13, and 61 of H-ras, k-ras, and N-ras are mutational hot spots in a wide variety of premalignant and malignant lesions. For example, k-ras is mutated in nearly 90% of pancreatic carcinomas, 50% of adenomas and adenocarcinomas of the colon, 30% of adenosarcoma of the lung, 20–30% of male germ-cell tumors (including N-ras), and 30% of myelodysplasias and acute myeloid leukemias (including N-ras), and 60% of CMLs (including N-ras). This diverse involvement of the ras mutation in cancer makes it a likely target for therapeutic intervention.

Several therapeutic approaches are possible. These approaches need not necessarily be limited to ras because similar approaches could be used in other activated genes (mutated genes) to block transformation.

The approach of ras antisense takes advantage of a technique that has been used in basic molecular biology to study the effects of blocking expressed genes at the mRNA level. The principle is based on the ability of complementary nucleic acids to bind to each other. The mRNA transcribed from the gene encoded in DNA is oriented as sense in mRNA. Therefore, the antisense molecules, which are constructed to be complementary to a specific sense mRNA, bind to it and prevent its processing and translocation. An alternative action is thought to be mediated by RNase H, an endonuclease that cleaves the RNA nucleotides of RNA-DNA duplexes. Antisense therapy is designed to be specific for the target. Because we know the
molecular genetic abnormality or abnormalities in many tumors, cancer is an excellent model for its use. Furthermore, \textit{ras} mutation is an excellent target because of its involvement in so many tumors.

However, there are many disadvantages. First, one has to assume that targeting a single molecular genetic event is sufficient to totally inhibit malignant proliferation. Cancer is a complex process, and so one can not assume that, if mutated \textit{ras} mRNA and its protein were totally inhibited, the malignant cell would be permanently inhibited. Certainly, evidence indicates that close to 100\% of mRNA can be inhibited, with resultant inhibition of growth. However, cells have alternate mechanisms for growth, and a bypass process is possible, depending on the “total” initiating factors. Thus, the inhibition of \textit{ras} mRNA may ultimately prove to be ineffective. One form of antisense used is the oligonucleotide. Oligonucleotides are usually designed as small stretches of DNA, from 8 to 40 or 50 nucleotides long, that inhibit pre-mRNA and mRNA. The protein translation start site, exon-intron borders, and the region adjacent to the mRNA cap site appear to be the most effective sites to target, suggesting an inhibition of RNA interaction with cell function that is necessary for translocation of mRNA into protein. Unfortunately, antisense oligonucleotides are degraded rapidly by endonucleases, and stability needs to be improved for effective use. Furthermore, the affinity and permeability of antisense oligonucleotides represent potential obstacles. As a result, large and repeated doses of oligonucleotides are necessary. However, this does not necessarily result in delivery of the oligonucleotides to all the tumor cells. Recently, improved oligonucleotides, other than phosphorthioates, have been developed to circumvent degradation and delivery difficulties.

The introduction of sequences that can be transcribed to produce an antisense molecule inside cells is an alternate approach. The advantage of this antisense RNA approach is the continuous generation of antisense sequences in the cell as a result of stable transduction, using vectors (such as retroviruses) expressing antisense RNA. The disadvantages are inefficient transduction into the cell, lack of an ideal vector that is relevant to the target cell, resulting in 100\% of the cells being transduced, and failure to produce long-term expression of the transduced antisense due to a number of unknown factors, which are currently under investigation. Retroviral vectors, such as Moloney leukemia virus backbone, are commonly used because they transduce dividing cells. However, high viral titers are needed because it may be that as few as 1 in 100 viruses infect the cell. Furthermore, there is essentially no information on the effects of retroviral transduction on the target cell. Insertional mutagenesis, induction of other genes, and effects on immune response are only a few of possible effects. We are currently studying these potential effects in our laboratory.

Retrovirus-mediated introduction of antisense RNA has been studied in a human lung cancer model using K-\textit{ras}. Using H460 cells (human lung adenocarcinoma with a point mutation at codon G1 of K-\textit{ras}), a 95\% decrease in K-\textit{ras} production was seen. The H460 cells expressing the antisense in culture showed a 3-fold decrease in cell growth and did not produce tumors in nude mice. Using a retroviral vector (instead of electroporation), we targeted H460 cells after intratracheal injection in nude mice. Only 10–14\% of mice treated developed tumors, in contrast to 73–100\% of controls (5). A Phase I trial is now underway.

Using antisense ribozymes is another potential approach. Ribozymes are RNA molecules that are capable of catalyzing nucleic acid hydrolysis at specific residues. There are groups of self-cleaving ribozymes, called “hammerhead” and hairpin, that have a consensus secondary structure. Both the hammerhead and hairpin have catalytic motifs that can be incorporated into an antisense RNA, creating an enzymatic antisense RNA. The antisense portion of this hybrid ribozyme gives target specificity and the ability to cleave multiple substrates derived from the catalytic component. \textit{In vitro} studies have shown some success with ribozymes. Ribozymes have been shown to target \textit{H-ras} and N-\textit{ras} RNA substrate but not a wild-type RNA substrate. This has also resulted in altered growth. In a study in nude mice, mice with bladder carcinoma administered intravesically lived longer when ribozyme was transfected into their tumors. However, the use of ribozymes has similar problems to the use of oligonucleotides, including delivery to the target, stability within the cell, and a high turnover rate (6, 7).

Several additional approaches to inhibiting the effects of \textit{ras} are possible. One approach would be to target amino acids 32–40 of p21\textit{ras}. This is the binding effector domain that is involved in binding GAP, which results in a cascade of signals downstream. Potentially, the injection of an inactive form of \textit{ras} with a mutation at this site competes for downstream effectors. Obviously, this strategy has the same difficulties as mentioned above.

One therapeutic approach that is under active investigation by major pharmaceutical companies is inhibition at several potential sites of the cholesterol biosynthetic pathway. It has been shown that both the normal and mutated \textit{ras} oncogene must be attached to the inner surface of the plasma membrane to function. This occurs through a farnesyl group attaching to the cysteine of the COOH-terminal sequence CAAX (A represents any aliphatic acid, and X is any residue) by farnesyl transferase. Following attachment of the farnesyl group, —AAAX is cleaved. This is a common mechanism for proteins to attach to membranes.

Inhibition of the farnesylation of \textit{ras} will inhibit function. However, this is not a selective approach because both wild-type and mutated \textit{ras} will be inhibited. Posttranslational modification of \textit{ras}, because it is not preferential for mutated \textit{ras}, could block other essential proteins. Still, this may be an attractive approach because cellular dependence on oncogenic \textit{ras} might result in tumor cells being more sensitive than normal cells to the action of a farnesyl transferase inhibitor. Inhibitors of CAAX farnesyl transferase have been identified, including compounds from \textit{Streptomyces} related to \(\alpha\)-hydroxy-farnesyl, tetrapeptides, and their analogues, such as L-731,734, developed by Merck, and benzodiazepine peptidomimetics (8). Another approach is the use of inhibitors of the production of farnesyl diphosphate, which is a substitute for farnesyl transferase and is synthesized in the cholesterol biosynthetic path from mevalonic acid. It is hypothesized that the interference with farnesyl production will translate into decreased \textit{ras} activity. A drug used to lower cholesterol, such as lovastatin, inhibits hydroxymethyl glutaryl coenzyme A reductase, which is the initial and rate-limiting step of cholesterol biosynthesis. Lovastatin, \textit{in vitro}, has been shown...
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The HER-2/neu Gene

This gene represents an oncogene that has been found to be amplified or overexpressed in a wide variety of human primary tumors, especially adenocarcinomas (10). This gene was originally isolated as an amplified V-erb-B-related sequence in human breast cancer, salivary gland adenocarcinoma, and gastric cancer. It has been most frequently studied in breast cancer. The HER-2/neu gene has been found to be amplified or rearranged in breast cancer. There is a 2–20-fold amplification in about 30% of primary human breast cancer. There is a significant correlation between level of HER-2/neu gene amplification and time to relapse and overall survival (11). HER-2/neu can be coamplified with other proto-oncogenes, i.e., erbB. Other tumors reported to have HER-2/neu amplification include ovarian carcinoma, gastric carcinoma, colon cancer, lung cancer, and cervical cancer, further supporting the role of HER-2/neu in human carcinogenesis.

In terms of therapeutic implications, it seems that breast tumor with HER-2/neu overexpression may be less responsive to adjuvant therapy. Non-small cell lung cancer HER-2/neu-overexpressing tumors may have intrinsic chemo resistance. This is an area for investigation. Targeting p185 neu on the surface of HER-2/neu overexpressing tumor cells is an attractive therapeutic strategy. One approach would be the use of monoclonal antibodies that bind to the extracellular domain of the p185 protein, which has been shown to inhibit tumor formation and tumor metastasis in animal models (12). This approach may also increase chemosensitivity to drugs such as cisplatin and Taxol. Gene therapy also represents a possible approach. The adenovirus E1A gene product has been identified as a viral suppression gene for ras-induced metastasis, and it has been demonstrated that E1A represses neu expression via a CIA DNA element in the neu promoter (13). It was shown that introduction of the E1A gene into neu-transformed 3T3 cells reduced the formation of experimental metastatic tumors (14). However, E1A may also suppress transformation features of human cancer that do not overexpress HER-2/neu. Animal studies have shown that liposome-mediated E1A gene therapy may serve as a powerful therapeutic reagent for HER-2/neu-overexpressing human ovarian cancers by targeting the overexpressed HER-2/neu oncogene (15). This approach has also been used to produce a liposomal-p53 complex to suppress primary and metastatic human breast tumors in a nude mouse model (see below).

Chromosomal Translocations

As previously mentioned, chromosomal translocations, many of which have been especially well characterized in leukemias, result in translocated DNA regions, which produce hybrid messages. This may play a role by in transformation by affecting cell growth and/or differentiation. Many translocations have been identified and characterized. These translocations and the genes involved are reviewed in several publications (for review see Ref. 16). Some common translocations and/or translocations of particular biologic interest with affected genes are listed in Table 1. Clearly, the therapeutic approach to translocations is potentially similar to that discussed with ras. This approach includes antisense, ribozymes, and retroviral constructs to inhibit fusion transcripts. Unfortunately, one cannot be sure that, in the hybrid, transcripts are fully responsible for leukemogenesis. Therefore, blocking them may not result in inhibition of leukemic growth. Furthermore, the translocation abnormality affects cellular development and other complex cellular functions (such as transcription) that are not easily reversible. Finally, even if blocking the transcript was therapeutically effective, doing so in all cells is virtually impossible unless the appropriate hematopoietic stem cell (committed earlier) is targeted. This approach might be feasible for bone marrow purging through organs, although targeting all abnormal cells is a major obstacle. Currently, our laboratories, we are focusing on identifying the appropriate hematopoietic stem cell and delivery system for genetic modification that will result in long-term hematopoietic reconstitution (in the transplant setting) and long-term gene expression.

Other approaches to targeting hybrid transcripts could be considered. With CML as a model, the use of tyrosine kinase inhibitors might be of value. There are several candidate tyrosine kinase inhibitors, including tyrphostin, herbimycin A, erbstatin, and geristein (17). However, because tyrosine kinase is important in growth and development of normal cells, these inhibitors would affect normal tissue and, thus, have the potential for significant toxicity.

Tumor Suppressor Genes

The tumor suppressor genes that have been most widely studied are the retinoblastoma gene (Rb) and p53. We will focus on these two tumor suppressor genes as models for therapeutic intervention. Other tumor suppressor or potential tumor suppressor genes have been reviewed, including the Wilms’ tumor gene (WT1), the APC and DCC genes in familial adenomatous pol-

4 S. A. Stass, E. J. Freireich, and C. Platsoucas, unpublished data.
**Table 1** Molecular characteristics of neoplastic rearrangements in acute leukemia*

<table>
<thead>
<tr>
<th>Type of oncogene activation</th>
<th>Cytogenetic rearrangements</th>
<th>Disease</th>
<th>Genes involved/functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juxtaposition to immunoglobulin regulatory element</td>
<td>t(8;14) (q24;q32), t(2;8) (p11;q24), t(8;22) (q24;q11) breakpoints are scattered over 350 kbp</td>
<td>Burkitt's lymphoma, ALL-L3 (B-ALL)</td>
<td>MYC-nuclear HLH protein</td>
</tr>
<tr>
<td>Juxtaposition to T-cell receptor regulatory element</td>
<td>t(5;14) (q31;q32), t(11;14) (p15;q11)</td>
<td>Pre B-ALL</td>
<td>Interleukin-3 growth factor</td>
</tr>
<tr>
<td></td>
<td>t(11:14) (p13;q11) breakpoints are clustered within 2 kbp; variant translocation (7:11) (q35:p13)</td>
<td>T-ALL</td>
<td>TIG-1/homobolin-1 Lim domain protein, a transcription factor</td>
</tr>
<tr>
<td></td>
<td>t(10:14) (q24;q11) breakpoints are clustered; variant translocations (7:10) (q34;q24)</td>
<td>T-ALL</td>
<td>TIG-2/homobolin-1 Lim domain protein, a transcription factor</td>
</tr>
<tr>
<td></td>
<td>t(1:14) (p32;q11) clustered in 3' untranslated region or 5' untranslated region; variant translocation (1:7) (p32;q34)</td>
<td>T-ALL</td>
<td><strong>TCL5/SCLL/TAI</strong></td>
</tr>
<tr>
<td></td>
<td>t(7;9) (q34;q34), t(1:7) (p34;q34)</td>
<td>T-ALL</td>
<td><strong>TAL2-HLH</strong> protein</td>
</tr>
<tr>
<td></td>
<td>t(7;9) (q34;q34.3)</td>
<td>T-ALL</td>
<td><strong>LCK-p56</strong> member of Src family; tyrosine kinase</td>
</tr>
<tr>
<td></td>
<td>t(7;19) (q35;p13)</td>
<td>T-ALL</td>
<td><strong>LYL1-HLH</strong> protein</td>
</tr>
<tr>
<td></td>
<td>t(8;14) (q24;q11)</td>
<td>T-ALL</td>
<td><strong>MYC-HLH</strong> protein</td>
</tr>
<tr>
<td></td>
<td>(8;12) (q24;q22) del(1) (p32)</td>
<td>B-CLL</td>
<td><strong>BTG-1</strong> deregulated MYC; <strong>SL</strong> deregulates **TAL-1; <strong>TAL-1-HLH</strong> protein</td>
</tr>
<tr>
<td>Juxtaposition to other than immunoglobulin/T-cell receptor loci regulatory element</td>
<td>t(9;22) (q34;q11); most of them are clustered</td>
<td>T-ALL</td>
<td><strong>AML1-ETO</strong> fusion protein</td>
</tr>
<tr>
<td>Fusion genes</td>
<td>t(1:19) (q23;p13); most of them are clustered</td>
<td>Pre-B-ALL</td>
<td><strong>E2A-PBX</strong></td>
</tr>
<tr>
<td></td>
<td>t(15;17) (q22;q11–12); clustered breakpoints</td>
<td>APL</td>
<td><strong>E2A</strong> is an HLH; <strong>PBX</strong> is a homeodomain protein</td>
</tr>
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<td></td>
<td>t(8;21) (q22;q22); clustered breakpoints</td>
<td>AML-M2</td>
<td><strong>PML-RARA</strong> and <strong>RARA-PML</strong>, a zinc finger protein; <strong>RARA-Zn</strong>, finger-retinoid acid receptor, fusion proteins</td>
</tr>
<tr>
<td></td>
<td>t(6;9) (p23;q34); clustered breakpoints</td>
<td>AML</td>
<td><strong>AML-M2</strong></td>
</tr>
<tr>
<td></td>
<td>inv(16) (p13.1;q22), t(16;16) (p13.1;q22), t(9;11) (q22;q23), t(11;19) (q23;p13), t(11;19) (q23;p13), 5q−</td>
<td>AML-M4</td>
<td><strong>AML-M5</strong></td>
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<tr>
<td></td>
<td>inv(16) (p13.1;q22), t(16;16) (p13.1;q22), t(9;11) (q22;q23), t(11;19) (q23;p13), t(11;19) (q23;p13), 5q−</td>
<td>AML-M4</td>
<td><strong>MLL (ALL)</strong>, homeobox regulator, <em>trithorax</em> homology, fusion proteins</td>
</tr>
</tbody>
</table>

*CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; HLH, helix-loop-helix; APL, acute promyelocytic leukemia.

yposis, VHL gene in Von-Hippel-Lindau, and NF1 and NF2 genes in neurofibromatosis (1).

The tumor suppressor genes represent the antipathy of oncogenes. They normally function to suppress and regulate cell growth. Usually, a loss of both alleles of a tumor suppressor gene is necessary for failure to suppress and control cell growth. Mutations that occur in one allele are recessive and can be transmitted to the next generation. Individuals with a mutation in one allele are obviously at a greater risk to develop a malignancy because the molecular genetic abnormality can adversely affect the normal tumor suppressor gene allele resulting in transformation. In Rb, loss of normal function of one Rb gene appears to be adequate to result in malignant transformation. This is not always the case because, in some instances, other events may be necessary for malignant transformation. The report that fusion of nontumorigenic human cells with tumorigenic human cells resulted in hybrid cells that could no longer form tumors supported the concept that there was genetic information in normal cells that could suppress cancer cells. This would indicate that malignancy could be caused by abnormalities of genes (mutation or deletion) capable of controlling and suppressing cell growth (18). Karyotypic analysis (approximately 5% of Rb tumors have a 13q14 deletion), restriction fragment length polymorphism analysis in tumors and families, examina-
tion of variable numbers of tandem repeats in paternal and maternal alleles, and sophisticated chromosome transfer techniques (microcells), in which smaller chromosomal portions are transferred into tumor cells, resulting in inhibition of tumor growth, have enabled the chromosomal localization and, ultimately, identification of tumor suppressor genes.

The retinoblastoma gene Rb1 was cloned using specific probes and was later further characterized (19, 20). The product of the Rb1 gene is a 928-amino acid protein, which is phosphorylated at multiple sites with three functional domains (oligomerization, pocket, and DNA-binding/transcription factor). The pocket domain can bind to viral oncoproteins. The Rb protein is phosphorylated at several sites by cdc2 kinase. Phosphorylation of Rb is critical to its activity, and its biologic activity is regulated by phosphorylation. Inactivation of Rb leads to tumor formation in various cell types. Thus, Rb protein is involved in controlling cell growth. Tumor cells lacking Rb can demonstrate control of growth by introducing normal Rb alleles. Furthermore, phosphorylation of Rb is essential to cell growth suppression. The data that show that viral oncoproteins (large T antigen of SV40, E1A protein of human adenovirus type 5, E7 protein of human papillomavirus types 16 and 18, and EBNA-5 protein of EBV) bind to hypophosphorylated Rb and that Rb is hypophosphorylated through early G1, with hyperphosphorylation in S, G2, and M, suggest that the hypophosphorylated form of Rb has activity that suppresses cell division, which can be reversed in late G1 by phosphorylation or oncogene binding. Purified unphosphorylated Rb injected into cells results in G1 arrest, further supporting this concept. Furthermore, the data support that cyclin-cdk complexes are responsible for hyperphosphorylation of Rb, with resultant release of the cell from growth control. In addition, binding of oncoproteins to Rb protein can inactivate Rb function, which results in loss of controlled cell growth and malignant transformation. Rb can bind E2F, a cellular transcription factor. E2F binds to the adenovirus EZ promoter and activates E2 expression and also binds to genes activated in mid to late S phase (27, 28). Evidence that is consistent with the model that inhibition of CDK activity is thought to block the release of the transcription factor E2F and related transcription factors from the retinoblastoma protein Rb, with consequent failure to activate transcription of genes required for S-phase entry (28, 29). Evidence that is consistent with the model that pRB is a downstream effector of p53-induced G1 arrest has recently been reported (30).

In conclusion, our knowledge regarding the molecular genetics of oncogenes and tumor suppressor genes in cancer is now vast. The challenge is to now effectively translate this information into therapeutic strategies that result in short-term and long-term benefits to the patient.

**p53.** In 1979, Arnold Levine of Princeton University and David Lane of the University of Dundee independently discovered the p53 protein. However, the p53 protein initially appeared to act only as an oncogene and not as a tumor suppressor. This was due to scientists inadvertently working with a mutant form of p53 gene. It was not until 1989 that Levine and Vogelstein discovered that p53 did, indeed, suppress cancer. Approximately, 60% of tumors are known to have a p53 mutation, including those of breast, lung, liver, skin, prostate, bladder, cervix, and colon. More than 90% of p53 mutations are localized between codons 120 and 290 in the DNA-binding domain of the p53 protein (24). Furthermore, the vast majority of p53 mutations are missense mutations (85.6%). Although mutations of the p53 gene are most frequently acquired, they can also be inherited through the Li-Fraumeni syndrome. In these families, one mutant p53 allele is inherited, and the second allele acquires a mutation. In this syndrome, members of these families frequently developed breast cancer, sarcomas, and colon and lung cancer. Similarly, mice harboring a p53 allele have a higher incidence of cancer than do normal mice with wild-type p53 genes. p53 is not only important because it mutates frequently in a number of malignancies but also because it plays an increasingly important role in the diagnosis. The presence of mutations in the tumor suppressor genes, particularly p53, have been associated with increased tumorigenicity, metastases, and mortality in human breast cancer (25, 26) and many other cancers. We will first focus on the function of p53 and using p53 for the therapy of malignancies.

It is now known that p53 plays a critical role in the cell cycle. p53 coordinates multiple responses to DNA damage. DNA damage results in an increase in the level of the p53 protein. Following DNA damage, an important function of wild-type p53 function is to control the progression of cells from G1 to S phase. Recently, several groups have found that p53 transcriptionally activated a p21 kd protein (also known as WAF1, Cip1, Sdi1, p20CAP, and Pic1), an inhibitor of CDKs (27, 28). Inhibition of CDK activity is thought to block the release of the transcription factor E2F and related transcription factors from the retinoblastoma protein Rb, with consequent failure to activate transcription of genes required for S-phase entry (28, 29). Evidence that is consistent with the model that pRB is a downstream effector of p53-induced G1 arrest has recently been reported (30).

In addition to cell cycle arrest, p53 also induces apoptosis. The molecular details of this form of cell death are not fully understood, but conserved features include activation of proteases of the ICE/Ced-3 class and regulation by members of the
Bcl-2/Ced-9 family (31, 32). p53 has been shown to induce expression of BAX, a gene encoding a positive regulator of this pathway (33). A second way in which p53 may induce apoptosis is through the repression of transcription. This function is mediated via TATA box elements when sites for p53 binding are absent in the promoter of the target gene (28, 29). For example, Bcl-2 can be repressed, perhaps by p53 binding to the TATA-binding protein and inhibiting its function as a basal transcription factor.

Depending on the cell type, an increase in p53 can cause G1 arrest and/or apoptosis. Apoptosis is promoted by some of the Bcl-2 homologues (Bax, Bak, Bad, and BclXs). Other members of the Bcl-2 family (Bcl-2, BcXa, and Bag-1) inhibit apoptosis by forming dimers with Bcl-2 homologues that promote apoptosis (34). The various ratios of the Bcl-2 family members may result in either suppressed or accelerated apoptosis in response to accelerated stimuli. These two cellular outcomes, G1 arrest and apoptosis, are also influenced by growth factors, oncogenes, and activation of cytokine-like signal transduction pathways (35).

It has also been well established that genes induced by p53 include: MDM2, the product of which is a negative regulator of p53 (36); thrombospondin I, which inhibits angiogenesis (37); IGF-BP3, which may be an autocrine/growth regulator through its interaction with IGF1 (38); cyclin G, the function of which has yet to be determined (36); and GADD45, which may play a role in DNA repair (36, 39). Besides inducing GADD45, there is also some evidence that p53 may play a role in DNA mismatch repair by directly binding to the DNA (40, 41). Of the proteins that p53 is known to induce, only thrombospondin I and IGF-BP3 are secreted from the cell.

Because p53 is a central regulator in controlling the cell cycle, one might expect that restoration of wild-type p53 function can inactivate the proliferative effects of the mutated product. In fact, various groups have found that in vitro transfection of a tumor suppressor transgene into a variety of tumor cells decreased the tumor growth rate in ex vivo experiments (42–47).

One might expect that restoration of the wild-type p53 gene into tumors is technically difficult. Although efficient transfer into tumors is rarely possible, various groups have reported that p53 decreases tumor growth by a bystander effect and not necessarily by efficient restoration of p53 into the tumor. In an orthotopic lung cancer model, Roth’s group reported that retrovirally transduced p53 inhibits developing lung cancer in mice, despite the fact that p53 had only been transduced into 30% of the tumor cells (46). These studies have been extended to clinical studies involving lung cancer patients in a terminal state. Of the nine patients with lung tumors treated with p53, three had tumors that did not increase in size, and three had their tumors decrease in size. In fact, six separate biopsies of one tumor after retroviral injection showed no evidence of a viable tumor.

Ionizing radiation or DNA-damaging agents, such as cisplatin, when coupled to p53, have been found to be synergistic in their antitumor activity (48). Direct injection of a p53 adenovirus construct into lung tumors, followed by i.p. administration of cisplatin, induced massive apoptotic destruction of the tumors (49). Currently, there are three gene therapy p53 clinical trials in Phase I using viral vectors.

Another novel gene therapy approach is the use of a E1B 55-kb gene attenuated adenovirus. This virus selectively replicates in and causes the lysis of p53-defective tumor cells but not cells with functional p53 (50). Replication in and cytolysis of normal cells are reduced 100–1000-fold in vitro and in vivo by this gene deletion. Injection of this mutant virus into p53-defective human cervical carcinomas grown in nude mice caused a significant reduction in tumor size and caused complete regression of 60% of the tumors. Thus, this promising result has clinical implications, but its use for clinical trials is limited to local therapy at present.

The vast majority of ex vivo and in vivo experiments have used viral vectors that are known to have a high transfection efficiency to restore p53 in the p53-deficient tumor cells. However, toxicity of these viral vectors precludes them from being given systemically to treat metastatic disease, whereas it has been demonstrated that liposome-DNA complexes are not toxic (51).

Although transfection efficiency of liposomes complexed to DNA is not thought to be highly efficient (52), we demonstrated that systemic administration of a liposome-p53 complex significantly affects tumor growth and metastases of breast cancer cells injected into nude mice (53). Surprisingly, the transfection efficiency of the tumor by the liposome-p53 complex was quite low. The mechanism by which systemic administration of liposome-p53 complex inhibits tumor growth appears to be a bystander effect (46, 53, 54). Potential mechanisms for this bystander effect are the following: inhibition of angiogenesis (37); stimulation of the IGF-BP3 protein (38); and activation of an immunological response. Although the mechanism of p53’s bystander effect is unclear, the most important aspect of this study is that it demonstrates that i.v. p53 can potentially decrease metastatic tumors.

Another approach to the treatment of malignancies is the potential of using mutant p53 for vaccinations (55). CTLs recognize processed peptide fragments of any endogenous protein, after these peptides are carried to the cell surface by MHC class I molecules. Thus, a tumor antigen does not have to be expressed as an intact protein on the cell surface to be recognizable by CTLs. Recently, CD8+ CTLs were induced to specifically target mutant p53 products. Immunizing BALB/c mice with spleen cells pulsed with a mutant p53 peptide completely inhibited the development of lung cancer with the same mutation. In contrast, the wild-type peptide, without the mutation, had no effect on the growth of the lung tumor. Vaccinations with other mutant p53 peptides have been found to be effective against tumors expressing a variety of mutant p53 proteins (56). These findings point the way toward an approach to selective immunotherapy against tumors. Alternatively, canary pox virus vectors expressing p53 as a cancer have been used (49). The protective effect of this live virus vaccine was not dependent on the p53 mutation, and wild-type and mutant p53 were both effective in reducing tumor growth.

When malignancies (lung, breast, colon, prostate, bladder, or skin) harboring a p53 mutation arise, they tend to be more aggressive, resist chemotherapy, and respond more poorly to most protocols than do cancers of similar tissues with wild-type p53. An exciting area of research is to define novel agents and/or stratagems (57) that preferentially target p53-defective cancer cells for destruction. In one study, more than 60,000
compounds are being screened for activity against a panel of 60 different cancer cell lines. The cell lines have been characterized with respect to a number of indices of p53 status, including p53 function and p53 protein expression. Most standard clinical agents are more active in p53 wild-type cells than they are in p53 mutants in this particular assay. However, several compounds have now been identified that are more active in p53-defective cells.

Therapies directed toward proteins that bind to p53 and inactivate its functions are being developed. MDM2, overexpressed in sarcomas and in some breast cancers, is a paradigm of a cellular protein that binds to the NH2-terminal region of the p53 protein. Antisense oligonucleotide to decrease the levels of the MDM2 protein or drugs (i.e., peptide) that directly interfere the binding of MDM2 to p53 are being advanced (58). Similarly, therapies are being developed to prevent the formation of the p53-E6 protein complex. E6 is a protein form of the oncopgenic papillomavirus, which has been associated with cervical and rectal carcinomas.

References
Oncogenes and tumor suppressor genes: therapeutic implications.

S A Stass and J Mixson


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