Biological and clinical importance of the p53 tumor suppressor gene

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The p53 tumor suppressor gene controls cellular growth after DNA damage through mechanisms involving growth arrest and apoptosis. Mutations that inactivate p53 occur commonly in virtually all human malignancies and can be detected by sequencing of the p53 gene, immunohistochemical staining of tumor tissue with anti-p53 antibodies, single-strand conformation polymorphisms, or other biological assays. Identification of p53 mutation in the germ line is diagnostic of the cancer-prone Li-Fraumeni syndrome. Alterations of the p53 gene result in defective cellular responses after DNA damage and predispose cells to dysregulated growth, tumor formation and progression, and potential resistance (of tumor cells) to certain chemotherapeutic agents or ionizing radiation. A variety of tumors involving mutant p53 have a worse prognosis than tumors of the same type containing no p53 mutations. New diagnostic and therapeutic strategies are evolving as the p53 pathways of cell-cycle arrest and apoptosis become elucidated.

INDEXING TERMS: DNA damage • mutation • Li-Fraumeni syndrome • cancer • heritable disorders • cell cycle • apoptosis • oncogenes • p21^{WAF1/CIP1} • SDII • MDM2 • GADD45 • bax • cyclin • cyclin-dependent kinases • chemoresistance • radioresistance • immunohistochemistry

p53 has become the subject of intense investigation for several reasons: p53 is the most commonly altered gene yet discovered in human cancer [1, 2]; it suppresses the growth of tumor cells containing multiple genetic changes [3]; germ line mutation of p53 has been linked to an inherited predisposition to cancer (Li-Fraumeni syndrome) [4, 5]; increased amounts of cellular p53 protein after DNA damage have been associated with cell-cycle arrest [6] and programmed cell death (apoptosis) [7]; and mutations or losses of p53 have been associated with gene amplification [8, 9] and polyploidy [10]. Given its ability to monitor DNA damage and promote damaged cells’ responses (by repair and continued survival, or death) [11], p53 has been dubbed "the guardian of the genome" [12].

Discoveries about the biological properties of p53, and the molecular mechanisms of its functions, have given p53 much attention among tumor suppressor genes. p53 was chosen as "molecule of the year" in 1993 by Science [13], and between 1991 and 1995 >4000 manuscripts relating to p53 were published. A major transcriptional target of p53, p21^{WAF1/CIP1} [14–16], also known as SDII [17], CAP20 [18], or MDA6 [19], has been discovered and found to regulate a molecular switch that arrests the progression of cells through various cell-cycle checkpoints.

In this review, we will summarize the history of p53 research, the molecular epidemiology of p53 mutations, and the biological and biochemical properties of the p53 protein, including recent advances that suggest a model for p53-mediated cell-cycle arrest. In addition, we will explore the diagnostic and prognostic value of detecting p53 mutations, as well as prospects for therapeutic intervention in the treatment of tumors containing dysfunctional p53.

Historical Perspective

p53 was first identified in 1979 by virtue of its association with simian virus 40 (SV40) large T-antigen and by its apparently high expression in chemically induced tumors or spontaneously transformed cells [20–22]. For the next decade, p53 was thought to be a transforming oncogene whose expression resulted in increased DNA synthesis, a necessary feature of cellular proliferation. Early studies demonstrated that cloned p53 cDNA could immortalize cells of limited lifespan [23] or could cooperate with ras in transforming cells [24, 25]. Absence of p53 expression in the human promyelocytic cell line HL-60 was attributed to p53 gene deletion, and it was hypothesized that other oncogenes might substitute for p53 in transforming these cells [26]. As early as 1984 Maltzman and Czyzyk observed that exposure of nontransformed cells to ultraviolet radiation stimulated p53 production, on the basis of a posttranslational stabilization [27], and by 1987 p53 associated with cellular heat shock protein had been observed in transformed cells [28].

By 1989 several lines of evidence were beginning to suggest
that the wild-type p53 gene was actually a tumor suppressor gene instead of an oncogene. For example, only mutant p53 could cooperate with ras in cellular transformation [29], and wild-type p53 could inhibit transformation by either adenoviral E1A plus ras or mutant p53 plus ras [30]. Moreover, colorectal carcinomas that lost one chromosome 17p allele were demonstrated to harbor mutations of the remaining allele within the coding region of the p53 gene [31], a theoretical hallmark of tumor suppressor genes [32]. These results were generalized to other common malignancies, including carcinomas of the lung, breast, and brain [33]. Additionally, introduction of wild-type but not mutant p53 genes resulted in suppression of the growth of human colorectal carcinoma cells [3].

Over the past 5 years, studies have begun to clarify the molecular basis for tumor suppression by p53, as well as provide insight into the role of p53 in normal growth control. Several p53-associated proteins as well as p53-regulated genes have been identified. Scores of studies have cataloged mutations of p53 in human tumors. The value of early detection of p53 gene alterations and their utility in assessing prognosis are currently being explored. Understanding the molecular details of the p53 pathway may provide new diagnostic and prognostic markers as well as targets for therapeutic intervention.

Prevalence of p53 Alterations in Human Cancers

p53 is the most commonly mutated gene in human cancer [34, 35]. Genetic alterations of p53 in human tumors include allelic losses, missense and frameshift mutations, intragenic deletions, and epigenetic changes. Unlike other tumor suppressor genes, which are frequently disrupted by nonsense mutations that result in a truncated protein product, most of the mutations involving p53 are substitution changes that render the protein incapable of binding to DNA or of activating gene transcription [35, 36]. The mutations cluster in four evolutionarily conserved hotspot domains, which encompass the DNA-binding function of the wild-type p53 protein [37]. Analysis of the spectrum of p53 alterations in different tumor types, exposure risks, or geographic locations has led to a new understanding of cancer pathogenesis based on molecular epidemiology. Thus, new risk-assessment and intervention strategies are being developed [35].

A recent compilation and analysis of screening data from 2567 cancers worldwide revealed that 37% contained mutations in the p53 gene [38], including >50% of lung and colon cancers. p53 mutations were also common in esophageal (45%), ovarian (44%), pancreatic (44%), skin (44%), stomach (41%), head and neck (37%), bladder (34%), sarcoma (31%), prostate (30%), hepatocellular (29%), brain (25%), adrenal (23%), breast (22%), endometrial (22%), mesotheliomal (22%), renal (19%), thyroid (13%), hematological (12%), carcinoid (11%), melanoma (9%), parathyroid (8%), and cervical cancers (7%). In contrast, p53 mutations were extremely rare in Wilms tumor, testicular cancer, pituitary tumors, or pheochromocytoma.

p53 mutations are inherited in Li–Fraumeni syndrome [4, 5]. Patients develop tumors by young adulthood, often multiple tumors. A compilation of data from 43 families (231 patients) revealed that the most common tumor type among these patients was breast carcinoma (60 patients), followed by soft tissue sarcoma (29), brain tumors (28), osteosarcoma (14), leukemia (14), and adrenocortical carcinoma (5)—the component tumors of Li–Fraumeni syndrome [4]. These patients also developed other tumors later in life, including lung cancer (19 patients), prostate cancer (8), pancreatic cancer (7), melanoma (3), colon cancer (8), lymphoma (6), and stomach carcinoma (4).

An analysis of p53 mutation profiles has revealed that the spectrum of DNA base changes is distinctly different in various types of cancer and that certain mutations are associated with specific carcinogens, supporting the idea that some mutagens may leave “fingerprints,” depending on the site and type of DNA damage [39] and references therein. For example, G:C→A:T transitions are common in colon cancer but less frequent in hepatocellular and lung carcinoma; G:C→T:A transversions are common in lung carcinoma but rare in colon and gastric cancers. Dipyrimidine mutations, such as CC→TT transitions, occur with an appreciable prevalence (10%) among p53 mutations in skin cancer, where ultraviolet light is a major carcinogen, but are rare in noncutaneous malignancies with p53 mutations (10 of 2400). In hepatocellular carcinoma, where hepatitis B and aflatoxin act as synergistic carcinogens, the prevalence of p53 mutation at codon 249 is much higher than in other cancers. Epidemiological and in vitro studies suggest that aflatoxin, a potent carcinogen, is probably responsible for this transversion at 249.

To test the importance of p53 disruption in tumor formation, Donehower et al. developed genetically engineered mice lacking both alleles of the p53 gene (p53−/−) [40]. These “p53 knockout” mice developed normally but were predisposed to tumorogenesis (75% of the mice developed tumors by age 6 months and all died within 2 years). The most common tumor type in these mice was malignant lymphoma (20 of 30), followed by sarcoma (9 of 30). Some mice also developed breast and ovarian cancer. Mice that were heterozygotes for p53 inactivation (p53+/−) developed fewer lymphomas (32%) and relatively more carcinomas (12%) and had a delayed onset of tumorigenesis that was accelerated by carcinogen exposure [41]. Crosses between p53−/− and retinoblastoma +/− mice (which develop pituitary tumors) produced mice that developed endocrine tumors [42]. A chemoprevention trial in p53−/− mice both delayed the onset of tumors and altered the spectrum of tumors the mice developed [43].

Biological Properties of p53

p53 Induction and Cell-Cycle Arrest or Apoptosis

The nuclear phosphoprotein p53 functions in cell-cycle arrest, programmed cell death (apoptosis), inhibition of tumor growth, and preservation of genetic stability. It performs these functions through involvement in several biochemical pathways, including transcriptional activation, transcriptional suppression, and inhibition of DNA replication.

p53 is usually found in minute quantities inside most normal cells but its amounts can be "induced" by ionizing radiation, ultraviolet radiation, or other DNA-damaging agents through posttranscriptional mechanism(s) [6, 27]. Although p53 induction appears in some cases to depend on the presence of
double-stranded DNA breaks [44], the precise mechanism of p53 stabilization remains unclear.

Overexpression of wild-type p53 was initially shown to induce growth arrest of colorectal carcinoma cells [3, 30]. Later work localized the timing of p53-specific growth arrest to the G1 phase of the cell cycle [6] and showed that accumulation of p53 can also cause apoptosis in certain cell types [7]. Cell-cycle analysis demonstrated that, when subjected to ionizing radiation, cells containing wild-type p53 show an induction of p53 and subsequent cell-cycle arrest in G1 and G2 phases of the cell cycle, whereas cells lacking p53 still undergo a cell-cycle arrest at G2, implying that p53 is required for G1 arrest [45]. Studies showing that DNA-damage induction of p53 leads to cell-cycle arrest suggest that p53 may be an important cell-cycle regulator.

In contrast, overexpression of wild-type p53 leads to apoptosis in human colorectal carcinoma-derived cells [7] or in murine myeloid leukemic cells [46]. These results were confirmed in vivo with the cancer-prone p53 knockout mice. Thymocytes from the p53+/− mice subjected to ionizing radiation were resistant to apoptosis, whereas thymocytes with wild-type p53 were not [47, 48]. Mice that were heterozygous for wild-type p53 showed a slight resistance to thymocyte apoptosis under identical conditions, suggesting that precise expression levels of p53 may be important in p53-mediated apoptosis.

**BIOCHEMICAL PROPERTIES OF P53**

Insights into the complex cellular effects of p53 came from experiments that defined its biochemical properties. First, p53 was found to contain an N-terminal acidic domain that appeared similar to the transcriptional activation domains of other transcription factors [49, 50]. Fusion of the N-terminal acidic domain of p53 to a portion of the GAL4 DNA-binding protein led to transcriptional activation at GAL4 promoters [49], suggesting that p53 might be a transcription activator of specific genes.

Subsequently, p53 was noted to bind DNA in a sequence-specific manner [51, 52]. Analysis of a large number of human genomic clones that bound p53 in vitro defined the consensus p53 DNA-binding sequence as two copies of a 10-bp motif [53]: 5′-PuPuPuC(A/T)(A/T)GPyPyPyPy-3′. Similar results were obtained in experiments using pools of random oligonucleotides [54]. Recently, the p53 core domain (the central portion of p53, which is responsible for DNA binding) has been co-crystallized, bound to its DNA-binding site [37]. The crystal structure at 0.22-nm resolution shows two molecules of the p53 core domain bound to the 10-bp DNA motif through novel structural elements that include a loop sheet helix and two large loops. p53 mutations commonly observed in human tumors were found to occur at p53 residues that serve as contact points with the consensus DNA-binding sequence [37].

If p53 can bind DNA and transcriptionally activate genes, one might expect that placement of a p53 DNA-binding site in the context of a basal promoter upstream of a reporter gene might lead to induction of the reporter in the presence of wild-type p53. Indeed, p53 transactivates reporter genes in such experiments, both in vivo and in vitro [54–56]. As might be expected, the extent of transactivation is correlated with the number of copies of the p53 DNA-binding site [53]. Certain promoters have been discovered to be repressed by p53 [57–61], but this phenomenon appears to occur by virtue of the association of p53 with various transcription factors and not through binding to specific DNA sequences.

The C-terminus of p53 has been known to be necessary for oligomerization [62], given that p53 binds DNA as a tetramer [37, 63]. Recent work has also demonstrated the role of the p53 C-terminus in forming stable complexes with potential DNA lesions (single-stranded DNA, insertion/deletion mismatches), thereby increasing the lifespan of p53 and stimulating its transcriptional properties [64, 65]. The C-terminus of p53 may thus act as a biological sensor that connects DNA damage with p53-mediated growth arrest or apoptosis.

p53 interacts with several viral and cellular proteins (Table 1; see 66 and references therein). Interaction of p53 with SV40 large T-antigen, human papilloma virus (HPV) E6 gene product, or with adenovirus E1B gene product leads to inhibition of p53-mediated transcriptional activation [67, 68]. Apparently, these viruses have evolved mechanisms to inhibit p53 function so as to replicate and (or) transform cells. The cellular oncoprotein MDM2 binds and conceals the p53 transactivation domain, also leading to inhibition of p53 [69]. p53 binding to TATA-box binding protein (TBP) of the TFIID complex as well as to other transcription factors may account for at least part of the ability of p53 to transcriptionally repress some promoters [60]. Interaction between replication Protein A (RPA) and p53 may provide a mechanism for direct inhibition of DNA replication [70]. The physiological relevance of many of the interactions between p53 and various cellular proteins remains unclear. Many experiments have not yet been replicated in vivo, and the precise affinities of the many molecular partners of p53 have not been determined. One measure for assessing the importance of any particular interaction is to determine whether mutant forms of p53 also interact with partners of wild-type p53 [71].

<table>
<thead>
<tr>
<th>Table 1. p53-associated proteins.</th>
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<td><strong>Cellular proteins</strong></td>
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<tr>
<td>CBF</td>
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<td>E6-AP</td>
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<td>ERCC3</td>
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<td>HSP 70</td>
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<td>MDM2</td>
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<td>RPA</td>
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<td>SP1</td>
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<td>TAFII40</td>
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<td>TAFII60</td>
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<tr>
<td>TBP</td>
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<td>WT1</td>
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<tr>
<td><strong>Viral proteins</strong></td>
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<tr>
<td>Adenovirus E1B</td>
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<tr>
<td>EBNA-5</td>
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<td>HPV-E6</td>
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<td>HBX Ag</td>
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<td>SV40 T Ag</td>
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**p53 Target Genes**

DNA binding and transcriptional activation are some of the best-understood functions of p53. Since the elucidation of the consensus DNA-binding site of p53, the human genome has been estimated to contain several hundred p53-binding sites [72]. The observation that nearly every tumor-derived p53 mutant has lost its ability to bind DNA and transcriptionally activate nearby genes [36] suggests that these properties are central to p53's control of cell proliferation. Although the precise number of sites located in gene regulatory regions that may be subject to regulation by p53 is still unknown, this finding suggests that a potentially large number of genes may be under the control of p53 transcriptional modulation. A growing list of p53 target genes has been identified (Table 2; see 66 and references therein). The diverse nature of these targets suggests that p53 may be involved in pathways of cell-cycle control, angiogenesis, DNA repair, differentiation, growth factor signaling, and apoptosis. Of the genes activated by p53, consensus DNA-binding sites have been reported for p21WAF1/CIP1, MDM2, cyclin G, bax, GADD45, MCK, HIC1, and IGF-BP3. The better-understood p53-regulated genes and pathways will be further discussed below.

Table 2. Cellular genes transcriptionally regulated by p53.

<table>
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<th>Genes</th>
<th>Regulation</th>
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<tr>
<td><strong>Transcriptionally activated by p53</strong></td>
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<tr>
<td>Bax</td>
<td>Inducer of apoptosis; member of bcl2 family</td>
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<tr>
<td>FAS/Apo1</td>
<td>Inducer of apoptosis; cell surface receptor</td>
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<tr>
<td>Cyclin G</td>
<td>Cell-cycle component</td>
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<tr>
<td>GADD45</td>
<td>Growth arrest and DNA damage inducible gene; possible repair function</td>
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<tr>
<td>GD-AIF</td>
<td>Angiogenesis inhibitory factor; secreted molecule</td>
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<tr>
<td>HIC1</td>
<td>Hypermethylated in cancer gene; chromosome 17p LOH region</td>
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<tr>
<td>IGF-BP3</td>
<td>IGF-binding protein 3; insulin growth factor pathway inhibitor</td>
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<tr>
<td>MCK</td>
<td>Muscle creatine kinase</td>
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<tr>
<td>MDM2</td>
<td>Mouse double minute no. 2 oncogene; feedback inhibition regulator of p53</td>
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<tr>
<td>p21WAF1/CIP1</td>
<td>Cyclin—CDK and DNA replication inhibitor; cell-cycle regulator</td>
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<tr>
<td>Thrombospondin</td>
<td>Inhibitor of angiogenesis</td>
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<th><strong>Repressed by p53</strong></th>
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<tr>
<td>Bcl-2</td>
<td>Inhibitor of apoptosis</td>
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<tr>
<td>c-myc</td>
<td>Cellular oncogene</td>
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<tr>
<td>FGFI/Fos</td>
<td>Fibroblast growth factor; Cellular oncogene and early response transcription factor</td>
</tr>
<tr>
<td>HSP70</td>
<td>70-kDa heat shock protein</td>
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<tr>
<td>IL-6</td>
<td>Interleukin 6; promoter of B cell differentiation</td>
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<tr>
<td>MDR1</td>
<td>Multi-drug resistance glycoprotein 1; membrane transporter</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen; DNA polymerase processivity factor</td>
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<tr>
<td>TK (HSV)</td>
<td>Thymidine kinase of Herpes Simplex virus</td>
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GADD45. GADD45, a growth arrest and DNA damage inducible gene [73], was one of the first genes to be linked to p53 transactivation [45]. A p53 consensus sequence was found in the intronic sequences of GADD45, and the GADD45 gene was observed to be induced after γ-irradiation in cells containing wild-type but not mutant p53. Cells from p53 knockout mice failed to induce GADD45 or undergo cell-cycle arrest after irradiation [45]. Recent work suggests that GADD45 may participate in the response of cells to DNA damage by binding to the proliferating cell nuclear antigen (PCNA) and promoting DNA excision repair [74]. When evaluated in tumor cell lines, GADD45 overexpression suppressed cell growth [75].

MDM2. The mouse double minute no. 2 gene, MDM2, is amplified in a subset of human sarcomas and can interact directly with p53 [76, 77]. The MDM2 oncogene binds the N-terminal activation domain of p53 and prevents p53-mediated transactivation [69]. Subsequent work has shown that MDM2 contains p53 DNA-binding sites in its first intron and can be transcriptionally activated by p53 [78]. Because p53 induces MDM2, but MDM2 prevents further transcriptional activation by p53, it has been proposed that these two genes form a feedback-inhibition regulatory loop that prevents overactivity of p53 transcriptional induction [79], e.g., during embryonic development [80, 81].

Nonstandard abbreviations: PCNA, proliferating cell nuclear antigen; CDK, cyclin-dependent kinase; IGF-BP3, insulin growth factor-binding protein 3; SSCP, single-strand conformation polymorphism; and IHC, immunohistochemistry.

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1 Nonstandard abbreviations: PCNA, proliferating cell nuclear antigen; CDK, cyclin-dependent kinase; IGF-BP3, insulin growth factor-binding protein 3; SSCP, single-strand conformation polymorphism; and IHC, immunohistochemistry.
**Other genes.** Recent experiments have suggested a possible direct link between expression of the 61-2 homolog bax and p53-mediated apoptosis [87, 88], and some experiments have provided evidence for a possible role for bax in explaining why some cells undergo apoptosis after exposure to DNA-damaging agents whereas other cells merely undergo cell cycle arrest [89]. More recent evidence suggests that bax may not be required for DNA-damage-induced apoptosis in thymocytes, given that "bax-knockout" thymocytes underwent cell death after γ-irradiation in an identical manner as bax+/+ cells [90].

Other experiments have identified insulin growth factor-binding protein 3 (IGF-BP3) as a direct transcriptional target of the p53 tumor suppressor activity [91]. Secreted IGF-BP3 binds to IGF1, thereby inhibiting mitogenic signaling.

The precise number and expression levels of the various p53 target genes in different cell types remains unclear. Still to be determined are which genes or combinations of genes induced by p53 are responsible for the variety of p53 responses in different human cells.

**Diagnostic and Prognostic Value of p53 Mutations**

**DIAGNOSIS**

The high prevalence of p53 mutations in human cancer suggests that p53 could be used as a marker in malignancy (Fig. 2). p53 mutations may occur as early or late events in tumor progression, depending on tissue type. p53 mutations have been found in late or advanced stages of various tumor types, including gastrointestinal cancer [92, 93], prostate cancer [94], ovarian cancer [95–97], bladder cancer [98], cervical cancer [99], endometrial cancer [99], and liver cancer [100, 101]. On the other hand, p53 mutations occur in early stages of lung cancer [102, 103], head and neck cancer [104, 105], and breast cancer [106, 107]. In addition to transformation of follicular lymphoma described below, p53 alterations have been associated with evolution of chronic phase to blast crisis in chronic myelocytic leukemia [108, 109], and with the progression of Barrett epithelium to invasive esophageal cancer [110].

Detection of mutations can be performed by polymerase chain reaction (PCR) of DNA or RNA, followed by direct sequencing, immunohistochemistry (IHC) to detect increased amounts of mutant p53 in tumor tissue, or single-strand conformation polymorphism (SSCP) analysis. The most precise method for detecting p53 mutations is direct sequencing of the p53 gene, which comprises 11 exons encoding a 393-amino-acid protein. Because the majority of p53 mutations occur in the DNA-binding domain, located in exons 5–8 (codons 126–306), sequencing these exons alone detects >85% of p53 mutations [38]. p53 sequencing may be performed on tumor biopsies or on the tumor cells present in minute quantities in body fluids such as urine or sputum. DNA sequencing has also been useful in determining the presence or absence of a germ line p53 mutant allele in family members of patients with Li–Fraumeni syndrome. Unfortunately, DNA sequencing is both time- and labor-intensive for routine clinical testing. Less expensive and simpler screening methods have therefore been evaluated for
**Fig. 2. Consequences of p53 mutation in neoplasia; current diagnostic methods; and potential therapeutic interventions.**

Mutation of the p53 gene can lead to loss of cell-cycle control and apoptosis, which may result in genetic instability, polyploidy, chemotherapeutic drug resistance, and tumor growth. Current methods for detecting p53 mutations include DNA sequencing, PCR-SSCP, or immunohistochemistry. Although no routine therapeutic intervention currently exists specifically for cancers with p53 mutations, conceivably in the not-too-distant future chemotherapy may be designed to take advantage of endogenous p53 status to achieve therapeutic benefit. Gene transfer technologies may offer another useful way to introduce tumor-growth-suppressing genes such as p53, p16, or p21 into cancer cells and thereby control their growth.

Detection of p53 mutations; positive results by these methods can then be confirmed by DNA sequencing.

The use of IHC to detect mutant p53 relies on the fact that the amounts of mutant p53 protein are usually greatly increased in tumor cells [33]. Wild-type p53 protein has a lifespan of several minutes, leading to nearly undetectable amounts of this protein in normal cells, whereas the longer lifespans of many p53 mutants (several hours or more) make them easier to detect. Uniform nuclear immunostaining has been identified in dysplasias of the breast, esophagus, bronchus, and larynx [35]. A compilation of 84 studies in which IHC and sequencing experiments were performed on the same tumor sets revealed that sensitivity of IHC for mutant p53 was 75% and the positive predictive value was 63% [38]. The large number of false positives and false negatives observed may result from the fact that nonsense mutations, which lead to truncated forms of p53, do not result in increased concentrations of p53; moreover, p53 concentrations are increased in some tumors, including melanomas and testicular carcinomas, without containing any mutations [35].

SSCP has been used to detect mutations through the altered electrophoretic mobility of DNA sequences containing point mutations. SSCP has a sensitivity and specificity of ~90% for detecting p53 mutations [111]. Thus, IHC or SSCP analyses can be useful in screening for p53 mutations, but must be interpreted cautiously. Negative results cannot be used to exclude p53 mutations, and positive results should be confirmed by DNA sequencing.

**P53 MUTATIONS AND PROGNOSIS**

The presence of p53 mutation has been associated with worse prognosis in some tumor types, in part through the loss of wild-type p53 tumor suppressor function, in part because of radio- or chemoresistance, and in part through the acquisition of oncogenic properties that have been observed with certain p53 mutants.

P53 mutations have been associated with aggressive behavior of various tumor types, including brain tumors [112], invasive bladder cancer [113], aggressive breast cancer [114–116], metastasizing gastric cancer [117], invasive head and neck cancer [105], metastatic melanoma [118], invasive ovarian cancer [96], aggressive prostate cancer [94, 119], aggressive thyroid carcinomas [120, 121], and anaplastic Wilms tumor [122].

Mutations of p53 have also been associated with decreased survival in patients with various types of cancer, as an independent prognostic factor. In breast cancer, accumulation of p53 protein is correlated with both p53 mutation and shortened survival [106], and has predicted decreased overall survival in node-negative patients [123], although a recent study has found that IHC detection of p53 was not particularly reliable in node-negative breast cancer [124]. In colorectal cancer, accumulation of p53 correlated with high risk for disease recurrence and decreased survival [125–127]. p53 accumulation in node-positive colon cancer is a useful independent prognostic factor [128], and accumulation in soft tissue sarcomas is correlated with decreased patient survival [129]. p53 mutation predicted shortened survival in gastric cancer [130], chronic myelocytic leuke-
mia blast crisis [131], non-small-cell lung cancer [132], and renal cell carcinoma [133].

The future holds promise that the status of p53 as a molecular marker might be a useful basis for stratification of patients in clinical trials, and eventually might play a role in the standard care of patients with cancer.

CHEMO- AND RADIORESISTANCE

Experimental evidence indicates that p53 activates a cell suicide pathway after treatment with chemo- or radiotherapy [134, 135, and refs. therein]. Treatment of p53+/+ mouse thymocytes with radiation resulted in apoposis (programmed cell death), whereas p53−/− thymocytes were resistant. Treatment of mouse p53+/+ fibroblasts transformed by adenoaviral E1A protein and the ras oncogene with either γ-irradiation or chemotherapeutic agents resulted in apoptosis, whereas p53−/− fibroblasts were resistant [136]. Treatment of Burkitt lymphoma cell lines with radiation or chemotherapeutic agents was less effective if the tumor cells contained p53 mutations [137].

Follicular lymphomas respond to chemotherapeutic regimens initially but, frequently, chemoresistant lymphomas evolve and lead to therapeutic failure. p53 mutations have been found in the later, drug-resistant stages and have been implicated in the pathogenesis of histological transformation of follicular lymphoma. One study uncovered mutations of the p53 gene in 4 of 5 cases displaying histological transformation from follicular to diffuse-type non-Hodgkin lymphomas and in 0 of 16 cases displaying clinical progression in the absence of histological transformation [138]. Additionally, and not unexpectedly, high expression of p53 (>45% cells positive by immunostaining) was observed in 9 of 15 transformed lymphomas but in 0 of 10 de novo large cell lymphomas and in 1 of 7 follicular lymphomas [139].

Some evidence, however, suggests that p53 status may not necessarily predict therapeutic outcome. Experiments in which p53 in tumor cells was targeted for degradation by HPV16 E6 via ubiquitin-mediated proteolysis did not give rise to radio- or chemoresistance [140, 141]. In fact, some studies have shown that inactivation or mutation of p53 renders cells more sensitive to cytotoxic agents whose primary mechanism of action is DNA damage [142–144]. Mutations in the p53 gene did not correlate with radioresistance in a series of human squamous carcinoma cells [145]. Careful studies are needed to compare in vitro and in vivo chemosensitivity and to prospectively determine the role of p53 in therapeutic outcome.

Prospects for p53 Gene Therapy in Cancer

Given that the loss of p53 function is a common event in human neoplasia, one emerging strategy involves replacement of this gene or its transcriptional targets in preclinical models with viral vectors. Some in vitro studies have suggested that it may be useful to combine p53 replacement with chemotherapy [146].

To replace lost or dysfunctional p53 (or any gene) and to achieve a beneficial anti-tumor effect in vivo, several steps must be successfully accomplished, including: (a) delivery of the gene of interest to the target tumor cells, (b) introduction of the vector into the majority (assuming a bystander effect [147, 148]) or perhaps 100% of the tumor cells (assuming cure is the goal and there is no bystander effect), (c) sustained functional expression of the exogenous protein in the tumor cells, (d) tumor cell death, (e) acceptable toxicity to normal cells, (f) absence of a deleterious host immune response, and (g) increased survival of the host. Several studies have begun to demonstrate the power of adenovirus as a vehicle for gene replacement in human disease, including cancer [149–158]. A developing approach for cancer therapy involves attempts to replace such genes as p53, p16, or p21 [149] by using recombinant, replication-defective adenovirus for delivery in preclinical models of cancer (Fig. 2) [159–169].

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