The p53 tumor suppressor gene is a logical target for cancer therapy. Several therapeutic strategies can be envisioned based upon recent advances concerning structure and function of the p53 protein, its interaction with cellular and viral proteins and its roles in repairing DNA, regulating cell division and promoting apoptosis.

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Current Opinion in Biotechnology 1996, 7:592–600
© Current Biology Ltd ISSN 0958-1669

Abbreviations
A-T ataxia-telangiectasia
CDK cyclin-dependent kinase
CTL cytotoxic T lymphocytes
PCNA proliferating cell nuclear antigen
PI phosphatidyl inositol
RER replication error

Introduction
The most intensively studied tumor suppressor gene product is the 53 kDa protein that is affected in the majority of human cancers [1]. Although originally believed to be an oncogene, wild-type p53 is now well established as a tumor suppressor gene. p53 is a sequence-specific DNA binding protein and transcriptional activator of target genes containing two copies of the monomorphic 10-base-pair DNA sequence that conforms to the consensus RRRC(A/T)(T/A)GYYY (where R is a purine and Y is a pyrimidine) [2,3]. Numerous studies have led to the conclusion that p53 elaborates tumor suppressive activity through specific transcriptional activation of genes (see, for example, [4] and references therein) whose products are directly involved in growth arrest or programmed cell death (apoptosis), although some reports suggest that p53 activity is independent of transcriptional activation [5]. Collectively, these observations have led to the concept that p53 is 'the guardian of the genome', monitoring the integrity of the genome for damaged DNA and determining the fate of a cell that has suffered such damage by allowing sufficient time for DNA repair prior to replication, or alternatively triggering apoptosis if defects are not corrected [6]. In the absence of functional p53, there appears to be a lack of response to DNA damage, with the potential for initiation of tumorigenesis or progression of cancer. The pivotal role of p53 in regulating cell proliferation helps to explain why the functional inactivation of this tumor suppressor gene is a common occurrence in cancer and why p53 is an attractive therapeutic target.

The most prevalent mechanism resulting in the loss of p53 function in tumor cells involves a point mutation (Fig. 1) that results in a full-length mutant version of p53. These mutations abrogate specific DNA binding by the p53 protein with the consequent loss of transcriptional activation of target genes [7]. Wild-type p53 functions as a homotetramer and mutant p53 exerts a dominant-negative effect in heterotetrameric complexes containing both wild-type and mutant subunits, thereby compromising p53 function [7–9]. Because at least some genes transactivated by p53 negatively regulate cell division, cells carrying a single mutant p53 allele could be expected to have a reduced ability to regulate cell division resulting in cells that have a proliferative advantage over surrounding cells. To exacerbate the consequences of p53 mutation, loss of p53 function has been correlated with increased genomic instability; therefore even partial loss of p53 function (through a dominant-negative phenotype) could increase the likelihood that a second mutagenic event (usually deletion) of the remaining wild-type p53 allele occurs, resulting in loss of heterozygosity and p53 function. Therefore, therapies directed toward restoring wild-type p53 function in cells that express mutant p53 might correct some damaging defects, but ideally the dominant-negative effect that a mutant monomer has on the wild-type protein must be overcome.

Figure 1

![Distribution of mutations in human p53. The electronic form of the database (90) was used to graphically represent the distribution of point mutations for each codon. The oligomerization, sequence-specific DNA binding and transcriptional activation domains of p53 are identified.](image-url)
A less prevalent mechanism of p53 inactivation that is nevertheless common in many tumor types (including soft tissue sarcomas, neural tumors, bladder, cervical, and breast carcinomas as well as leukemias) is the interaction of wild-type p53 with viral or overproduced cellular proteins. These protein–protein interactions compromise p53 function by preventing specific DNA binding and/or transactivation activity, sequestering wild-type p53 in the cytoplasm, or promoting its degradation.

This review focuses on therapeutic strategies that utilize our current understanding of the crystal structure of p53, mechanisms of p53 function and the molecular pathways under its control. Just as p53 function can be lost in several ways, there are alternative strategies for restoring lost p53 function, depending upon the nature of the loss of function (Table 1). Because the mechanism of functional loss can be determined by appropriate diagnostic testing, it is likely that treatment decisions will consider the p53 status of the patient's cancer.

Conventional anticancer therapies
The exposure of cells to DNA-damaging agents, such as ionizing radiation or chemotherapeutic drugs, results in the activation and stabilization of latent wild-type p53 [10–12]. Recently, hypoxia (a physiologically relevant state for tumors requiring vascularization for growth) has also been shown to induce wild-type p53 in cells, resulting in either an arrest in the G1 phase of the cell cycle or apoptosis [13]. In contrast, cells that contain mutant p53 have been shown to be refractory to the adverse effects of cytotoxic agents [12]. Together, these observations provide a model for the inconsistent response of tumors to anticancer agents whereby effective agents would demonstrate efficacy in cells containing wild-type p53 by inducing p53-dependent apoptosis, whereas cells that have lost p53 function (through mutation or functional inactivation) will exhibit resistance and not undergo apoptosis [12,13]. This model has been extended to suggest that microsatellite instability (acquisition of replication error [RER] phenotype) together with loss of p53 function correlates with chemoresistance. In this study [14], drug-sensitive RER+ tumor cells acquire the RER phenotype by selection for resistance to anticancer drugs. The RER cells can then accumulate additional mutations, including those that result in loss of p53 function, that lead to reduced susceptibility to apoptosis and resistance to multiple chemotherapeutic drugs.

In a study of 32 matched pairs of breast cancer biopsies, those tumor cells containing wild-type p53 demonstrated a p53-mediated checkpoint response to four of the five drugs used, and it appeared that treatment-induced clonal changes were more prevalent in tumor cells that originally contained mutant p53, which is consistent with the view that the p53 status is an important factor in determining whether a positive response will be achieved to anticancer therapy [15]. Indeed, cancers that respond to radiation or chemotherapy include testicular cancer [16], acute lymphoblastic leukemia [17], and primary prostate cancer, all of which appear to retain wild-type p53. Recent studies, however, have challenged the hypothesis that wild-type p53 mediates the cytotoxicity of anticancer agents. In studies with human tumor cell lines, wild-type p53 correlated with chemosensitivity only in ovarian cancer and Burkitt's lymphoma, with no such correlation in leukemic and lung cancer lines [18,19]. This led the authors to suggest that in some tumor lines, chemosensitivity might correlate more closely with p53-independent apoptosis than with p53 status. Additionally, normal human fibroblasts containing nonfunctional p53, and primary fibroblasts from p53-null mice, showed an increased cytotoxic response to the anticancer agent Taxol and an increased percentage of cells demonstrating G2/M arrest and induction of apoptosis when compared with wild-type p53-containing cells [20].

### p53-regulated pathways
A number of genes that are transcriptionally regulated by wild-type p53 are involved in either cell-cycle control or apoptosis (Table 2). p21\(^{WAF1,CDKN1A}\) is a member of the family of cyclin-dependent kinase (CDK) inhibitors that appear to function as a mechanism to arrest the cell cycle in the G1 phase in response to DNA damage or a lack of nutrients. The CDK inhibitors are regulated by p53 through the induction of the p21 gene. The p21 protein subsequently binds to and inhibits CDKs, leading to the G1 arrest of the cell cycle. This arrest allows the cell to repair DNA damage before progressing through the cell cycle. If the DNA cannot be repaired, the cell may undergo apoptosis, which is regulated by p53. Thus, p53 plays a critical role in the regulation of the cell cycle and the induction of apoptosis in response to cellular stress.

<table>
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<th>Table 1</th>
<th>Strategies for restoring lost p53 function.</th>
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In summary, the inactivation of p53 is a common event in human cancers, and its loss is associated with poor prognosis. However, there are alternative strategies for restoring p53 function, and these strategies may be more effective in tumors that have lost p53 function. Furthermore, the identification of p53-regulated pathways, such as the regulation of the cell cycle and apoptosis, provides a basis for the development of new therapeutic strategies to target tumors that have lost p53 function.
has been demonstrated to be induced by, and mediate, wild-type p53 function by the inhibition of G1 CDKs, producing a potent G1 arrest. In adriamycin-treated wild-type p53-containing HCT116 cells, p21wafl,cip1 has been reported to be necessary for G1 arrest [21**, whereas disruption of p21wafl,cip1 function (by deletion of both alleles) led to G1 escape and G2/M block [22**] with subsequent apoptosis [23**]. Thus it appears that in these colon cancer cells, p21wafl,cip1 may play an important role in mediating the p53-induced G1 arrest; however, these and other studies [5,24] suggest that p21wafl,cip1 may not have a role in mediating the apoptotic response to p53.

<p>| p53 regulated genes with known or suspected growth regulatory properties. |
|-----------------------------|-----------------------------|-------------------------------|</p>
<table>
<thead>
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<th>Target gene</th>
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<td>MDM2</td>
<td>Negatively regulates p53 activity</td>
<td>Induced</td>
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<tr>
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<td>Cyclin/CDK inhibitor</td>
<td>Induced</td>
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<td>GADD45</td>
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<td>Fas</td>
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</tr>
<tr>
<td>Thrombospondin</td>
<td>Angiogenesis inhibitor</td>
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<td>Anti-angiogenic factor</td>
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<td>Induced</td>
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<tr>
<td>HIC-1</td>
<td>Tumor suppressor gene</td>
<td>Induced</td>
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<td>IGF-binding protein 3 by IGF-1</td>
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Whereas the amino terminus of p21wafl,cip1 binds to and inhibits cyclin–CDK complexes [25*,26*], the carboxyl terminus of p21wafl,cip1 has been shown to be necessary to bind proliferating cell nuclear antigen (PCNA) [27*], a processivity factor for DNA polymerase δ that has a role in DNA replication and repair [28,29]. In one study, the interaction of p21wafl,cip1 and PCNA blocked PCNA-dependent DNA replication but not repair [30]. In contrast, adriamycin-treated p21wafl,cip1-/- HCT116 cells were shown to be deficient in the repair of damaged DNA [22**]. This defect was attributed to the lack of p21wafl,cip1 association with PCNA, since the reintroduction of wild-type p21wafl,cip1, but not a carboxyl terminal mutant of p21wafl,cip1, appeared to partially restore the ability to repair damaged DNA in p21-/- HCT116 cells.

Another abnormality observed in adriamycin-treated p21wafl,cip1-deficient cells is endoreduplication of DNA that apparently occurs as a result of successive rounds of S phase without an intervening mitosis [23**]. Because the lack of wild-type p53 activity also results in multiple copies of functionally competent centrosomes generated in a single cell cycle that in turn leads to unequal segregation of chromosomes [31**], it is tempting to speculate that this mitotic infidelity might also be due to the lack of p53-regulated p21wafl,cip1 activity. It remains unclear how a cell containing wild-type p53 and p21wafl,cip1 decides whether to arrest in G1 or undergo apoptosis in response to DNA damage. However, it is conceivable that strategies for independently targeting separate domains of p21wafl,cip1 responsible for interacting with cyclin–CDK complexes and PCNA might result in a cell that has increased genomic instability and enhanced chemosensitivity [25*,27*].

Although several biochemical pathways leading to apoptosis in cells have been described, genes whose products are suspected of playing major roles in regulating apoptosis also appear to be regulated by wild-type p53. The bel-2 family of genes are known to regulate apoptosis (for a recent review, see [32]). Bcl-2 is an inhibitor of apoptosis and appears to be repressed by p53 [33]. Bax, the first Bel-2 associated protein to be identified, appears to function by accelerating apoptosis. Recent studies indicate that Bax is induced by p53 in at least some cell types [34]. Since Bax and Bel-2 appear inversely regulated by p53, the relative concentrations of these two proteins may be critical in determining whether the cell undergoes p53-mediated apoptosis. Fas/APO-1, a member of the tumor necrosis factor receptor superfamily, is known to transduce signals for apoptosis upon specific ligand or antibody engagement. Recently, this cell-surface protein was also demonstrated to be induced by p53 [35] which in turn could initiate apoptosis from extracellular signals.

Because it appears that tumor cells suppress apoptotic signals for survival, dissecting the apoptotic pathways that p53 regulates and identifying compounds that selectively turn on these pathways are primary goals in oncology research.

**Cancers containing wild-type p53**

In general, therapeutic strategies for correcting the loss of p53 function depend upon the presence or absence of a wild-type p53 gene in the tumor cells. Lack of function in cells containing wild-type p53 seems to be almost exclusively attributable to sequestration of p53 protein through inappropriate interaction with inactivating proteins. Under these circumstances, agents that prevent p53 from these interactions might restore normal function to p53. Therapeutic intervention of this sort represents utilization of existing resources within the cell to prevent further uncontrolled growth.

p53 can be inactivated through interactions with any of a number of proteins. MDM2 was the first of such cellular proteins discovered. MDM2 is a 491 amino acid protein containing a ring-finger domain [36], a nuclear localization signal and an acidic transcriptional transactivation domain [37,38]. In cells and cancers that contain amplified and/or overexpressed MDM2, p53 is wild-type [37,39]. The introduction of MDM2 into cells containing wild-type p53 prevents irradiation-induced G1 arrest and/or apoptosis similar to that seen in cells containing mutant p53 [40*,41**]. Whereas no function has been defined for MDM2, nullizygous MDM2 transgenic mouse embryos...
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This lethality was not observed in embryos that also lacked wild-type p53. Thus it was suggested that MDM2 normally functions to prevent growth suppression by p53 during development. Other studies have indicated that MDM2 also interacts with E2F-1 and/or retinoblastoma protein (Rb) [44,45]. However it is not clear whether those interactions are important for the normal cellular functions of MDM2.

Studies that mapped the interacting domains of MDM2 and p53 initially demonstrated that the first 118 amino acids of MDM2 bind the acidic activation domain of p53 and prevent transcription factors from binding to p53 [38]. Further work demonstrated that the amino acid sequence TFSDLW (single letter amino acid code) in p53 is crucial for high affinity interaction [46]. It is notable that a similar amino acid sequence (DFSG1L1) in E2F-1 has also been shown to bind MDM2 [45]. These data suggest interacting domains that could be targeted in drug discovery efforts. Furthermore, a single agent capable of binding the region on MDM2 that interacts with both p53 and E2F-1 may demonstrate a synergistic effect by releasing functional wild-type p53 and E2F-1, leading to apoptosis [47-49]. Drug design efforts could benefit from initial crystallization analyses of minimal interacting domains from p53 and MDM2 showing that p53 binds a hydrophobic pocket in MDM2 that is accessible to small molecules (N Pavletich, personal communication). Thus, a drug that blocked the inappropriate interaction between MDM2 and p53 could have utility for treating those tumors (including sarcomas, gliomas, breast and bladder cancers) containing amplified and/or overexpressed MDM2.

Other proteins that have been shown to bind and inactivate wild-type p53 include the viral proteins HPV-E6, HBV-X, and CMV-IE84 (HPV, human papillomavirus; HBV, hepatitis B virus; CMV, cytomegalovirus). HPV-E6 together with E6-associated protein (E6-AP) binds p53 and promotes its degradation [50]. Because neither HPV-E6 nor E6-AP by itself can stably associate with p53, it might be appropriate to target the HPV-E6/E6-AP complex. However, recent studies demonstrate that HPV-E6 also stimulates telomerase activity resulting in an extended lifespan of cells [51,52], perhaps indicating that additional antiviral agents may be necessary to effectively eradicate HPV-transformed cells.

HBV-X has been shown to bind p53, inhibit its sequence-specific DNA binding and transcriptional activity and inhibit p53-dependent apoptosis [53-55]. Because p53 has been shown to bind the Xeroderma pigmentosum B (XPB) and Xeroderma pigmentosum D (XPD) subunits of the TFIIH-based transcription-repair complex that results in inhibition of helicase activity and induction of apoptosis [56], it was suggested that HBV-X interferes with these p53-dependent functions, thereby abrogating p53-dependent transcriptional activation and apoptosis [57]. Hepatocytes expressing HBV-X protein therefore would be expected to have a selective growth advantage.

CMV-IE84 is expressed in a subset of patients that developed restenosis after coronary angioplasty [58]. The presence of overexpressed CMV-IE84 in cells containing wild-type p53 abolishes p53-mediated transcriptional activation. These observations suggest that CMV-IE84 might promote restenosis in these patients by blocking the p53 regulation of cell division. CMV-IE84 therefore provides a potentially relevant target for preventing restenosis in patients undergoing angioplasty.

Another potential mechanism preventing wild-type p53 function occurs in inflammatory breast carcinomas and undifferentiated neuroblastomas where wild-type p53 is retained in the cytoplasm [59,60,61]. The lack of p53 mutations (such as in the nuclear localization signal) in these instances would suggest that an interacting cytoplasmic protein is responsible for preventing p53 from reaching its nuclear site of action. Identification of such a protein could provide an additional therapeutic target.

A defect in the regulation of wild-type p53 activity occurs in individuals suffering from ataxia-telangiectasia (A-T). A-T is an autosomal recessive disorder characterized by progressive cerebellar ataxia, immunodeficiency, radiosensitivity and cancer predisposition. A-T heterozygotes have an increased risk of cancer, particularly breast carcinoma, whereas A-T homozygotes have up to a 700-fold increase in the incidence of leukemia and non-Hodgkin's lymphoma [62,63]. Cultured cells from A-T patients demonstrate defective G1/S and G2/M checkpoints of the cell cycle, and undergo apoptosis in response to nonlethal levels of radiation-induced DNA damage [64,65]. The defective checkpoint controls are apparently attributable to failure to induce wild-type p53 because the rise in p53 levels in response to ionizing radiation is delayed in A-T cells. The recently cloned A-T gene (ATM) predicts a protein of 3056 amino acids containing a leucine zipper and a PI-3 kinase domain (PI, phosphatidylinositol) [66,67]. Interestingly, the PI-3 kinase inhibitor wortmannin has been demonstrated to radiosensitize cells containing either mutant or wild-type p53, as well as to decrease the induction of p53 DNA-binding activity and activation of p53-dependent transcription following DNA damage [68]. It remains unclear whether wortmannin is an inhibitor of ATM and whether reintroduction of the wild-type ATM gene to A-T cells containing mutant ATM would restore p53-dependent cell cycle controls, thereby reducing radiosensitivity. It has been suggested that the radiosensitivity of A-T cells is a consequence of the inability to inhibit p53-dependent apoptosis. Restoration of normal G1 checkpoints in A-T cells by stable reintroduction of wild-type ATM might be
beneficial to A-T patients, whereas inhibition of function of the A-T gene product in combination with localized radiation might provide an effective anticancer protocol.

Finally, it is conceivable that in some tumors containing wild-type p53, one or more of the genes normally induced by p53 (Table 2) are defective or inactivated. However, to date, very few, if any, cancers containing wild-type p53 have revealed mutations in p21\textsuperscript{waf1,cip1} [69], the most intensively studied of the p53-induced genes. It remains to be determined whether other downstream target genes in the p53 pathway are inactivated.

Cancers containing mutant p53

Gene therapy with wild-type p53 provides perhaps the most intuitively obvious strategy for treating cancers that express mutant p53 or that are p53 null [70]. To be clinically useful, transfer of the wild-type p53 gene into target cells should be efficient enough to transduce a high percentage of malignant cells in a tumor. However, initial data from cell culture studies suggest that overexpression of wild-type p53 in cells lacking functional p53 results in a bystander effect that reduces the growth rate of neighboring cells not receiving the gene [71]. Although preliminary characterization indicates that the factor(s) eliciting the bystander effect might be secreted into the extracellular medium, its biochemical and biological properties have not yet been elucidated. Furthermore, it is not clear whether such a bystander effect will occur and have a beneficial outcome in vivo. However, in addition to promoting apoptosis of tumor cells, p53 has been demonstrated to induce inhibitors of angiogenesis that might suppress tumor growth in vivo [72,73].

The most common delivery vehicles for p53 gene therapy have been viral vectors; although recent advancements in developing liposomes for delivery appear promising [74,75], these delivery vehicles might not permit an adequate transduction efficiency. Fujiwara et al. [76], using an orthotopic human lung cancer model, demonstrated a therapeutic effect that greatly reduced tumor cell growth with intratracheal installation of a retrovirus containing wild-type p53, despite the relatively low titers. A clinical trial for the treatment of non-small cell lung cancer with retroviral p53 gene therapy is in progress [77].

Recently, adenoviruses have also been utilized to transduce p53 genes. A major advantage of using adenoviral vectors is the ability to produce titers up to \(10^{12}\) infectious units per ml. However, adenoviruses are maintained episomally in target cells and are immunogenic in humans, and both of these properties could limit their efficacy. Despite these limitations, a number of investigators have developed adenoviruses encoding p53 and have been successful in obtaining growth suppression in cell culture or animal models of small cell lung [78], non-small cell lung [79], ovarian [80], head and neck [81] and prostate [82] cancers, apparently without significant effect on normal cells containing wild-type p53 [79]. Furthermore, the combination therapy of p53 adenovirus with cisplatin has been evaluated in animals [83], and is being explored clinically [84].

As outlined above, mutations in p53 constitute the majority of defects among cancers that have lost p53 function. An underlying feature in all cells containing p53 mutations is the accumulation of mutant p53 protein (normally wild-type p53 protein is very low to absent in cells). From the recent elucidation of the co-crystal structure of the core domain of p53 with a consensus DNA-binding site, it appears that the majority of p53 mutations occur in residues that are at or near the protein–DNA interface [85], decreasing the overall affinity of mutant p53 for the cognate DNA-binding site. The second most prevalent class of mutations affects the hydrophobic core of p53 resulting in structural alterations. A strategy that makes use of expressed mutant p53 protein is applicable when certain p53 mutants of this latter class are present in cancer cells. Structural and biochemical studies suggest that alterations of hydrophobic core mutant p53 proteins are subtle, and that the structural integrity might be retained through interaction with a stabilizing molecule. In one such example, the V143A mutant of p53 has a \(T_m\) of 35°C, whereas the wild-type protein has a \(T_m\) of 40.5°C (N Pavletich, personal communication). The decreased \(T_m\) in this p53 mutant might result in a rapid destabilization of the protein and consequent loss of wild-type p53 activity in the cell at 37°C. We (T Stoller, G Beaujard, unpublished data) and others [86] have demonstrated that V143A has a specific activity similar to wild-type p53 when produced at 31°C. Accordingly, a molecule that is able to stabilize the wild-type structure of V143A that is transiently present at 37°C might adequately sustain wild-type p53 activity in cancers containing this particular mutant. Since several hydrophobic core mutants exhibit properties similar to V143A, including a decreased \(T_m\) (N Pavletich, personal communication), such a molecule may stabilize many members of this class of mutants. In contrast, approaches to restore wild-type activity to ‘contact mutants’ of p53 are less obvious and may be confined to molecules that can act as a bridge between the altered amino acid and the consensus DNA binding site to increase the overall affinity of the mutant p53 with DNA.

An interesting new approach to p53 therapy exploits the presence of elevated levels of mutant p53 protein within the cell to supply specificity [87]. In this particular method, a gene therapy strategy was utilized to generate a novel intracellular protein complex in cells expressing mutant p53. The resultant complex then activated expression of a toxic gene that converted an innocuous substrate into a cytotoxic agent [87]. Since the toxic product was able to freely diffuse to surrounding cells, a potent bystander effect was generated. Theoretically,
this strategy could be applied to any gene product that is expressed or overexpressed specifically in a cancer cell.

Table 3

| Potential opportunities for combination therapy. |
|------------------|----------------------------------|
| p53 therapy      | Radiation or cytotoxic drug      |
| Gene therapy     |                                 |
| p53              | Yes                              |
| Downstream targets of p53 | Yes                              |
| Oncogene-specific interacting protein | No                              |
| Viral therapy    |                                  |
| Vaccine          | No                               |
| Small molecule drugs restoring p53 function | Yes                              |
| Small molecule drugs blocking inactivating proteins | Yes                              |

Another strategy that might be successful for certain p53 mutations involves the generation of specific vaccines. This approach makes use of the observation that cells expressing mutant p53 process and present the mutant epitope on the cell surface bound to the MHC [88]. Cells presenting mutant p53-specific peptides on their surface could then be targets for specific CD8+ cytotoxic T lymphocytes (CTL). Genetic immunization with minigenes expressing the single mutant epitope [89], or mutant peptide [88], was demonstrated to induce mutant p53-specific CTL and an antitumor response. However encouraging, it remains to be determined whether human tumors are able to present cell-surface mutant p53 peptides at levels sufficient to trigger killing by mutant-specific CTL.

Finally, a novel approach to treating cancers that have lost p53 activity utilizes a defective adenovirus that lacks the viral-encoded protein (E1b) that binds to and inactivates p53 [P1]. Specifically, wild-type adenovirus utilizes E1b to inactivate p53 function, permitting viral replication in wild-type p53-containing cells. A nonproductive infection (or productive infection with poor virus yield) may result when the defective (i.e. therapeutic) virus lacking E1b encounters wild-type p53-containing cells because wild-type p53 becomes activated as a result of the infection. In contrast, the infection of cells containing mutant p53 results in a productive infection, killing the cells and releasing progeny virus to infect and kill any other mutant p53-containing cells that are present. Although the immunogenicity of adenovirus as well as the unknown consequences of a mutant adenovirus that may be capable of replicating in an individual remain potential problems, the possibility of selectively eradicating all mutant p53-containing cells within a primary tumor or at distant metastatic sites represents an exciting prospect for treating cancers containing mutant p53 regardless of the site of the mutation.

Conclusions

Novel approaches to therapeutic strategies involving p53 have been suggested by the recent elucidation of the structural and biochemical properties of wild-type p53. Genes that are regulated by p53 provide additional insights into the pathways that p53 regulates, demonstrating the pivotal role that p53 plays in regulating cell division and genetic stability. Some of the more innovative strategies for treating cancers containing nonfunctional p53 involve gene therapy or novel approaches to the discovery and use of small molecules. Additionally, in some instances, the described therapeutic efforts to target p53 might be more effective when used in combination with conventional cytotoxic therapies. Table 3 proposes therapeutic combinations that might reasonably be expected to be more effective than single agent treatment. It is anticipated that therapeutic strategies will become more refined as we further our understanding of the biochemical pathways that p53 regulates, of how p53 responds to DNA damage, and how this tumor suppressor gene elicits cell-cycle arrest or apoptosis.

Acknowledgements

We would like to thank Nikola Pavlctich (Memorial Sloan-Kettering) for providing us with results prior to publication.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Hypoxia, which is found in poorly vascularized parts of tumors, is demonstrated to induce p53-dependent apoptosis. Highly apoptotic regions in tumors were found to correlate with both hypoxia and wild-type p53. The authors propose that hypoxia provides a physiological selective pressure in tumors such that cells containing mutant p53 escape apoptosis and have a proliferative advantage that contributes to tumor expansion.


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See annotation [43*].


See annotation [43*].


See annotation [43*].


This paper, together with [40*]-[42*], directly confirms one function of MDM2 to block p53 induction of G1 arrest and apoptosis. It was known prior to these reports that MDM2 could bind p53 and prevent p53-dependent transcriptional activation of target genes, that tumor cells that had elevated MDM2 also contained wild-type p53 and that tumor cells with elevated MDM2 were defective in G1 checkpoint control. References [42*] and [43*] directly addressed the role of MDM2 in early embryonic development and showed that wild-type p53 activity is lethal to embryos at day 5 unless MDM2 is present to suppress this activity. References [40*] and [41*] demonstrate that MDM2 inhibits wild-type p53-mediated G1 arrest and apoptosis. Together these reports establish a role of MDM2 in tumorigenesis and validate MDM2 as an important therapeutic target for drug discovery efforts.


Despite early reports (see reference [50]) demonstrating cytoplasmic retention of wild-type p53, little information was available relating cytoplasmic sequestration to functional inactivation. The authors in this report demonstrate that the cytoplasmic retention of wild-type p53 seen in neuroblastoma cells is sufficient to impair p53-mediated G1 arrest in response to DNA damage. Interestingly, the authors also show that cytoplasmic retention of p53 is saturable, because nuclear accumulation of p53 occurs at high doses of DNA-damaging agent. These data suggest that the molecule(s) responsible for cytoplasmic retention of p53 might be an appropriate target for drug discovery.


An ingenious gene therapy strategy utilizing endogenous cancer-specific genes. Although significant improvements need to be addressed in this approach for technical feasibility, the authors have successfully addressed two major issues in the gene therapy approach to treatment of cancer: specificity of targeting to cancer cells and generation of a cytotoxic bystander effect.


Patents