REVIEW

Structure and Function of the p53 Tumor Suppressor Gene: Clues for Rational Cancer Therapeutic Strategies

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The p53 tumor suppressor protein is involved in multiple central cellular processes, including transcription, DNA repair, genomic stability, senescence, cell cycle control, and apoptosis. p53 is functionally inactivated by structural mutations, interaction with viral products, and endogenous cellular mechanisms in the majority of human cancers. This functional inactivation can, in some circumstances, produce resistance to DNA-damaging agents commonly used in cancer chemotherapy and radiotherapeutic approaches. Current research is defining the biochemical pathways through which p53 induces cell cycle arrest and apoptosis. Knowledge of these fundamental processes is leading to the identification of molecular targets toward which multimodality cancer therapies, using chemotherapeutic, immunotherapeutic, and gene-therapeutic strategies, can be based. [J Natl Cancer Inst 1996;88:1442-55]

The history of investigations of the p53 (also known as TP53) tumor suppressor gene is a paradigm in cancer research. Initially, parallel lines of basic, clinical, and epidemiologic research on p53 are now converging, and research findings will soon be translated into medical practice. The knowledge acquired during this brief history of scientific advancement indicates that the p53 protein is involved in several central cellular processes, including gene transcription, DNA repair, cell cycling, genomic stability, chromosomal segregation, senescence, and apoptosis (programmed cell death) [reviewed in (1-9)]. Since these complex biochemical processes in themselves are performed by multicomponent protein machines, it is not surprising that the p53 protein is included in these molecular machines and that the multiple effects of oncogenic DNA viruses are mediated in part by their targeting the p53 protein for binding and perturbing its functions [reviewed in (2,4,9)] (Fig. 1). Since the number of p53 molecules per cell is limited, i.e., about 10^3 to 10^4 per cell, the physiologic state of the cell and the post-translational modification of p53 must dictate where, when, and how efficiently p53 plays its role as the "guardian of the genome" in response to endogenous and exogenous mutagens (10,11). This review will discuss the current knowledge of the fundamental cellular path-

ways that involve p53, leading to the identification of molecular targets for multimodal cancer therapies.

p53 Structure and Function

DNA Damage and Apoptotic Response Pathways

The p53 protein is clearly a component of one of the pathways activated in response to DNA damage (Fig. 2) (12-17). Cell cycle arrest at the G_1 and G_2 checkpoints prior to DNA replication and mitosis, respectively, aids the DNA repair processes and prevents mutations and aneuploidy, whereas apoptosis can be considered a fail-safe mechanism to rid the organism of cells either with severely damaged DNA or cells with a low apoptotic threshold. Double-stranded DNA breaks are especially efficient in causing p53 protein accumulation, possibly by reducing its degradation through the ubiquitin-dependent proteolytic pathway (12-14,17-21). The molecular pathway between DNA damage and p53 protein accumulation is not understood. p53 protein may be involved as one of the sensors of DNA damage. The carboxyl-terminus of p53 can bind nonspecifically to ends of DNA molecules and catalyze DNA renaturation and strand transfer (22-26). This region of the protein can also bind to extrahelical regions of DNA damage involved in forming insertion/deletion mismatches (27). It will be interesting to determine if p53 recognizes other types of DNA damage, including carcinogen–DNA adducts.

Wild-type p53 protein can transcriptionally transactivate genes involved in cell cycle arrest [e.g., $p21^{waf1}$, a potent inhibitor of most cyclin-dependent kinases (28-30)] and interact either with the DNA repair and synthetic machinery [e.g., proliferating cellular nuclear antigen, GADD45, and $p21^{waf1}$ (31,32)] or proteins modulating apoptosis [e.g., Bax and Fas (33,34)]. Certain other genes generally containing TATA se-


Fig. 1. Schematic representation of p53 molecule. The human p53 protein consists of 393 amino acids with functional domains, evolutionarily conserved domains, and regions designated as mutational hotspots [reviewed in (4)]. A) Missense or nonsense mutation. Functional domains include the transactivation region (diagonally striped block), sequence-specific DNA binding region (amino acids 100-293), nuclear localization sequence (amino acids 316-325, vertically striped block), and oligomerization region (amino acids 319-360, horizontally striped block). Evolutionarily conserved domains (amino acids 17-29, 97-292, and 324-352; black areas) were determined using the MACAW (Multiple Alignment Construction and Analysis Workbench) program. Seven mutational hotspot and evolutionally conserved regions within the large conserved domain are also identified (amino acids 130-142, 151-164, 171-181, 193-200, 213-223, 234-258, and 270-286, checkered blocks). Vertical lines above the schematic, missense mutations; lines below schematic, nonmissense mutations. The majority of missense mutations are in the conserved hydrophobic midregion of the protein that is required for the sequence-specific binding to DNA. The nonmissense (nonsense, frameshift, splicing, and silent mutations) are distributed throughout the protein, determined primarily by sequence context. B) Protein–protein interactions: Cellular (e.g., TBP = TATA binding protein; hsp70 = heat-shock 70 protein; RPA = replicating protein antigen; MDM2 = multiple double minute protein; XPB = xeroderma pigmentosum group B DNA helicase), or viral oncoproteins (e.g., E1B55K = adenovirus protein E1B55K; SV40 large T ag = SV40 viral large T antigen; HBx = hepatitis B viral X protein) bind to specific areas of the p53 protein. Functional domains and protein binding sites (white bars underneath) were compiled from references [reviewed in (4)].
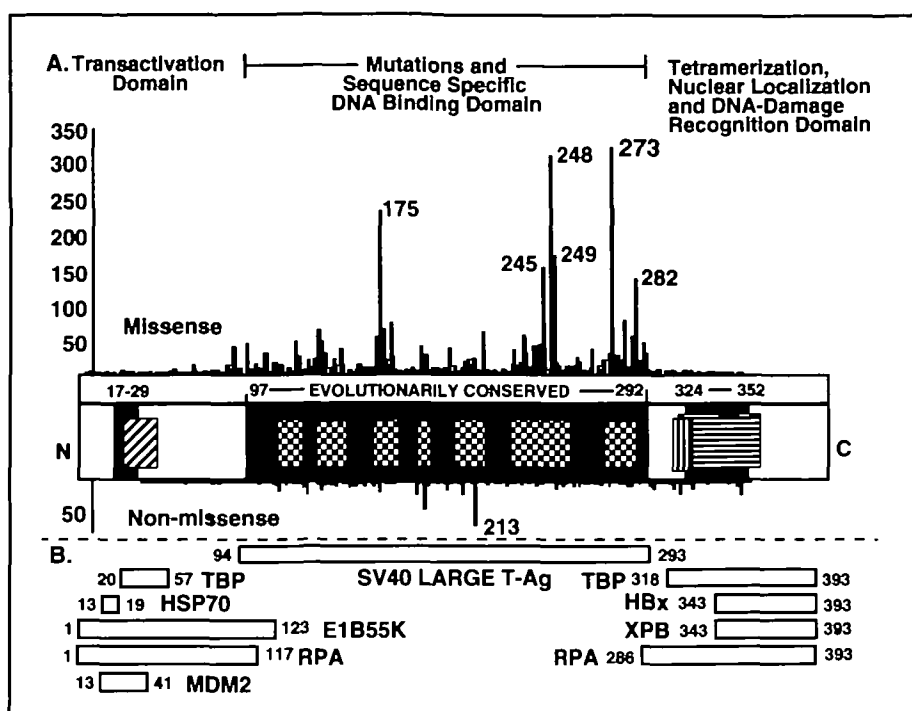
quence in their promoter regions, e.g., bcl-2 (35), can be transrepressed perhaps by p53 binding to the TATA binding protein (TBP) and inhibiting its function as a basal transcription factor (36-39). p53 can also inhibit DNA synthesis by a transcription-independent mechanism binding to putative origins of DNA replication and either prevent initiation or early replication fork unwinding (40,41). p53 forms protein–protein complexes with cellular proteins involved in DNA synthesis [e.g., replicating protein antigen (RPA) (42)], DNA repair [e.g., RPA, xeroderma

pigmentosum group B DNA helicase (XPB), xeroderma pigmentosum group D DNA helicase (XPD), p62, topoisomerase I, and Cockayne's syndrome group B (CSB) (42-47)], and apoptosis [e.g., XPB and XPD (48)]. Cellular context determines whether p53 can induce apoptosis independent of or dependent on its transcription–transactivation function and in the absence of RNA and protein synthesis (48-53). Of interest, cycloheximide, an inhibitor of protein synthesis, can induce apoptosis (54-56), and a temperature-sensitive mutant of a basal transcription factor, GG1/TAF$_{II}$250,

Fig. 2. Cell cycle arrest, DNA repair, and apoptosis induced by DNA damage. p53 is a component of a DNA-damage (e.g., which may involve the ataxia telangiectasia gene product [ATM]) response pathway. This simplified model does not consider qualitative or quantitative differences due to either cell type or microenvironment. p53 accumulation leads the regulation of cellular genes involved in apoptosis (e.g., BAX, IGF-1R, IGF-BP3, Fas, and Bcl2), cell cycle arrest (e.g., p21^{Waf1}, an inhibitor of cyclin-dependent kinases, cdk), and DNA synthesis and repair (e.g., p21^{Waf1} and GADD45 [growth arrest and DNA damage factor] binding to PCNA [proliferating cell nuclear antigen]). MDM2 protein can bind to p53 protein and inhibit its functions in a negative feedback loop. p53 can also bind directly to proteins involved in DNA synthesis (e.g., RPA = replicating protein antigen) and transcription, nucleotide excision, and apoptosis (e.g., XPD = xeroderma pigmentosum group D DNA helicase, XPB = xeroderma pigmentosum group B DNA helicase, and p62 of the TFIIH = transcription factor complex IIH). Therefore, p53 may mediate apoptosis by two inactive pathways. One dependent on p53 function as a transcription transactivator and transrepressor and a second pathway independent of its transcriptional activities and dependent on p53 protein–protein interactions. MDMX = X homologue of murine double minute gene; MDM2 = multiple double minute protein; HPV-E6 = human papillomavirus protein E-6; SV-40T = simian virus-40 large T antigen; HBx = hepatitis B viral X protein; Adeno E1A = adenovirus protein E1A.
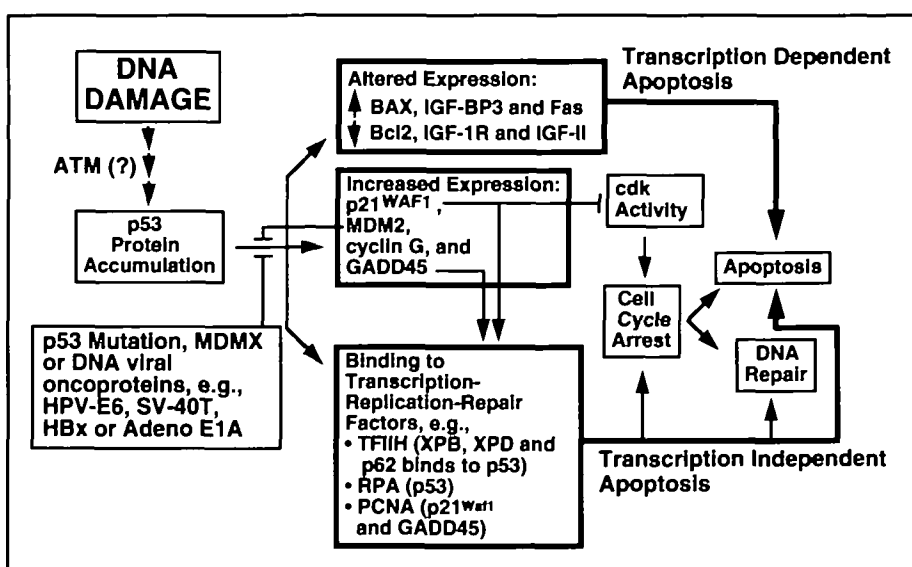
when inactivated at a nonpermissive temperature, induces apoptosis (57). Cells from patients with Cockayne's B syndrome, which are deficient in transcribed strand-specific repair, have increased sensitivity to UV light-induced apoptosis (58). Since the induction of apoptosis was positively correlated with p53 accumulation and inhibition of transcription, Ljungman and Zhang (58) have speculated that blockage of RNA polymerase by UV damage in the transcribing DNA strand initiates the apoptosis response to UV. All of these results are consistent with the hypothesis that the apoptotic protein machinery is constitutively present in a latent state and does not require the synthesis of additional proteins. Nevertheless, p53 regulation of genes, whose products (e.g., Bax, Bcl2, and p21^{Waf1}) may be involved in apoptosis, could modulate a cell's sensitivity to inducers of apoptosis. p53-initiated G_1/S cell cycle arrest is primarily mediated by up-regulation (i.e., increased expression) of p21^{Waf1} (28-30), but p21^{Waf1} is not an inducer of apoptosis in that ionizing radiation induces a p53-dependent apoptosis in p21$^{-/-}$ cells from p21^{Waf1} gene knockout mice (59,60). Therefore, p53 may function by transcription transactivator-dependent and -independent mechanisms in interactive, yet distinct, pathways of cell cycle arrest and apoptosis.

Normal tissue homeostasis is maintained by balancing positive and negative cell growth regulation. Both external and internal signals can initiate or inhibit cell proliferation. Negative regulation also includes entry of cells into a terminally differentiated, senescent, or apoptotic state. During carcinogenesis, genetic and epigenetic lesions that lead to an imbalance between these growth-regulator pathways accumulate in dysplastic and neoplastic cells, leading to clonal selection and expansion, thus giving rise to clinical tumors (61). In this scenario of tumor progression, p53 mutations would occur after the initiating events of carcinogenesis. For example, hypoxia may select mutant p53 cells that are resistant to hypoxia-induced apoptosis (62). Dysregulation and overexpression of certain cellular and viral oncogenes, e.g., myc, E2F, adenovirus E1a, or human papillomavirus E7, stimulate both proliferation and sensitize cells containing normal p53 and Rb tumor suppressor genes to apoptosis and, again, select for p53 mutant cells (Fig. 3) (63-66). Evidence from studies (67,68) of mice with either a homozygous deletion of Rb or a human papillomavirus E7 transgene

indicate that the absence of Rb promotes apoptosis. When Rb is inactivated, the resultant apoptotic response may be dependent on a normally functioning p53 [reviewed in (66)]. Therefore, it is not surprising that (a) oncogenic DNA viruses target both Rb and p53 for inactivation; (b) retinoblastoma, in which Rb is deleted and p53 is normal, is generally sensitive to radiotherapy (69); and (c) p53 is frequently mutated in some human cancer types, e.g., small-cell lung carcinoma and Burkitt's lymphoma, which exhibit deregulated myc expression, a p53-dependent apoptosis inducer [reviewed in (70-72)] (Table 1). In other cancer types, the Rb pathway is often dysregulated either by cyclin D₁ overexpression, cyclin-dependent kinase-4 overexpression or activating mutation, or functional inactivation of p16^{INK4} by various mechanisms (Fig. 4) [reviewed in (73,74)]. Cancer cells harboring cellular or viral oncogenes also may be intrinsically sensitive to the apoptotic response mediated by restored wild-type p53 function. Whereas loss of Rb and many other inducers of apoptosis are dependent on p53, physiologic activators of apoptosis, such as glucocorticoids and the Fas ligand, are independent of p53 (Table 1) and can activate apoptosis in p53 mutant cells.

Phosphorylation of p53

The biochemical functions of p53 may be regulated by reversible serine phosphorylation [reviewed in (75,76)]. p53 protein can be phosphorylated in vitro by at least seven different kinases, including cdc2 (77-79), casein kinase II (80), DNA-dependent protein kinase I (81), a casein kinase I-like kinase (82), protein kinase C (83), mitogen-activated protein kinase (84), and JNK1 (85). Although the precise role(s) of these kinases in regulating p53 function is not understood, recent studies are providing clues. For example, mutation in the casein kinase II phosphorylation site at serine 392 can reduce the antiproliferative activity of p53 (82). The S and G₂/M cyclin-dependent kinase complexes, cdk2-cyclin A and cdk2-cyclin B, phosphorylate serine 315 of p53 and stimulate its sequence-specific DNA binding to p21^{Waf1} and GADD45 sites preferentially (86). The G₁ cyclin-dependent kinase complexes, cyclin E/cdk2 and cyclin D₁/cdk6, do not phosphorylate p53, which is consistent with results indicating that p53 is underphosphorylated at serine
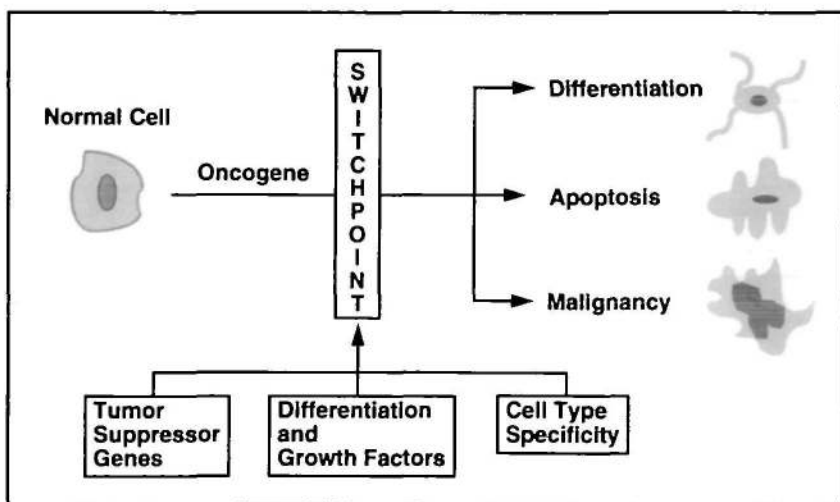
Fig. 3. Cellular switchpoint in response to cellular oncogenes. An inappropriately activated proto-oncogene can lead to differentiation, e.g., Ha-ras in rat PC12 cells, apoptosis, e.g., myc in rodent cells, or neoplastic transformation, e.g., Ha-ras in murine 3T3 cells.

Table 1. Examples of inducers of apoptosis*

Inducer	Cell type	p53 dependent	bcl-2 antagonists	Reference No(s).
DNA damage				
Cisplatin	Ovarian cancer, Burkitt's	Yes	Yes	(241,242)
Etoposide	Burkitt's, leukemia	Yes/no†	Yes	(241,243)
Ionizing radiation	Burkitt's, lymphoid	Yes	Yes	(220,241)
Mechlorethamine	Burkitt's, lymphoid	Yes	Yes	(241,244)
ADA deficiency	T cells	Yes	Yes	(245)
Various agents	p53-null T-lymphoma	No	Yes	(246)
β-Lapachone	Human prostate	No	No	(247,248)
Oncogene				
c-myc	MEF, leukemia, CHO, HCC	Yes/no	Yes	(51,163,249,250)
E1a	BRK, MEF	No/yes†	Yes	(160,251-255)
E1a-289 R	BMK, MEF	No	Unknown	(252)
E1a-243 R	BMK, MEF	Yes	Unknown	(252)
E1b-19K mutant	Saos-2, rat kidney	No	Yes	(256)
E2F	MEF	Yes	Unknown	(254,257,258)
R-Ras	Jurkat	No	Yes	(259,260)
Survival factor deprivation				(261)
Androgen	Mouse prostate	Enhanced		(261)
Interleukin 3	Lymphoid	Yes	Yes	(184,244,262)
Interleukin 6	Murine myeloma	Unknown	Yes	(263)
IGF (antisense)	Vascular muscle	Unknown	Yes	(264)
Neuron growth factor	Neurons	No	Yes	(265-268)
Protein kinase inhibitors				
B43-Gen	Burkitt's	No	Unknown	(269)
PKC inhibitors	HL-60, B-cell	No	Yes	(269-272)
Cellular membrane receptors				
Fas	Fetal liver, breast cancer, HeLa	No	Yes	(273-276)
Tumor necrosis factor	Breast cancer, lymphoma	Yes	Yes	(273,277,278)
Cytotoxic T-cell killing	Mice B cell		Yes	(279)
Retinoids (HPR and AHPN)	Breast cancer	No	Yes	(280,281)
Steroids	Mouse T cells	No	Yes	(246)
TGF-β	Ovarian cancer, other cell types	No	Unknown	(282-284)
Other factors				
Okadaic acid	Lymphoid	No	No/yes†	(285,286)
Hypoxia	Fibroblasts	Yes	Unknown	(62)

*MEF = mouse embryo fibroblast; CHO = Chinese hamster ovary; HCC = hepatocellular carcinoma; BRK = baby rat kidney; BMK = baby mouse kidney; IGF = insulin-like growth factor; TGF-β = transforming growth factor-beta.
†Cell class and type specificity.

315 in the G_1 phase of the cell cycle (77,79). These data also are consistent with a hypothetic negative feedback loop in which increased cdk2 activity would generate a transcriptionally activated p53 that would increase $p21^{Waf1}$ expression, an inhibitor of cdk2, and consequently reduce cdk2 phosphorylation of p53 and, thus, reduce p53 transcription–transactivation function. Protein kinase C also is likely to regulate p53 function as a transcription transactivator. In vitro protein kinase C phosphorylates serine residue at position 378 in the carboxyl-terminal region of p53 that contains the epitope recognized by the
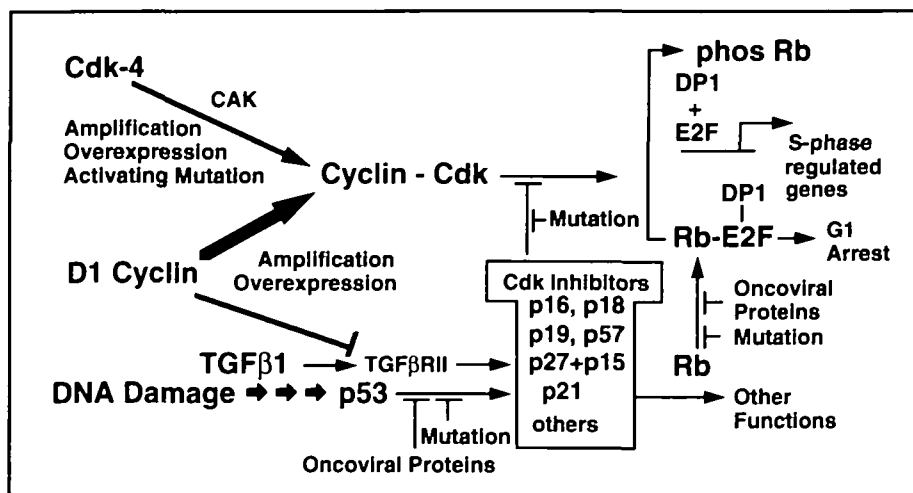

Fig. 4. G_1 cell cycle checkpoint. CAK = cyclin-activating kinase; CDK-4 = cyclin-dependent kinase-4; Rb = retinoblastoma tumor suppressor protein; TGF-β_1 = transforming growth factor-beta1; and TGFβRII = TGFB receptor type II; DP1 and E2F = transcription factors.

monoclonal antibody (Pab421) and the domain for negative regulation of p53 transcription transactivation [reviewed in (87)]. Phosphorylation of this domain blocks Pab421 binding to p53 (88,89), and Pab421 nonreactivity with p53 correlates with growth arrest (75,90). Activation of protein kinase C by phorbol 12-myristate 13-acetate also induces growth arrest at the G_1 checkpoint (89,91). Both cdk2 and protein kinase C can participate in apoptosis (Table 1). The biochemical intersection among these kinases and proteins involved in apoptosis, including p53, remains to be defined.

Molecular Archaeology of p53 Mutations

Mutations can arise by either endogenous mutagenic mechanisms or exogenous mutagenic agents and are archived in the spectrum of p53 mutations found in human cancer (2-4,92,93). Errors introduced during DNA replication, RNA splicing, DNA repair, and DNA deamination are examples of endogenous mutagenic mechanisms. The DNA sequence context is an important factor determining these events. Almost all short deletions and insertions occur at monotonic runs of two or more identical bases or at repeats of 2- to 8-base-pair DNA motifs, either in tandem or separated by a short intervening sequence (94). The mechanism that has been most studied is called slipped mispairing, a misalignment of the template DNA strands during replication that leads to either deletion, if the nucleotides excluded from pairing are on the template strand, or insertion, if they are on the primer strand. When direct repeat sequences mispair with a complementary motif nearby, the intervening oligonucleotide sequence may form a loop between the two repeat motifs and be deleted (95,96). More lengthy runs and sequence repeats are more likely to generate frameshift mutations. The deletions and insertions in the p53 gene found in human tumors also may be biologically selected from the broad array of such mutations occurring in human cells. When compared with the distribution of missense mutations, these types of mutations occur more frequently in exons 2-4 (54%) and 9-11 (77%) than in exons 5-8 (20%). The N-terminus of the p53 protein (encoded by exons 2-4) [reviewed in (37,97-99)] has an abundance of acidic amino acids that are involved in transcriptional function of p53 (100,101); it binds to transcription factors such as TBP in the basal transcription multiprotein complex, TFIID (36-38,102,103), and experimental studies have shown that multiple point mutations are required to inactivate its transcription–transactivation function (104). The carboxy-terminus (encoded by exons 9-11) of the p53 protein is enriched in basic amino acids that are important in the oligomerization and nuclear localization of the p53 protein [reviewed in (87,105-107)], recognition of DNA damage (22,108), and induction of apoptosis (48). Multiple point mutations are infrequently found in the p53 gene, which is consistent with the target theory; i.e., exogenous mutagens target the p53 gene within the context of the entire human genome. Therefore, deletions and insertions would be a more efficient mutagenic mechanism than single-point mutations in disrupting these N-terminal and C-terminal functional domains.

The p53 mutational spectrum of hepatocellular carcinoma is an example of a molecular linkage between carcinogen exposure and cancer. In liver tumors from persons living in geographic areas in which aflatoxin B_1 and hepatitis B virus (HBV) are cancer risk factors, most p53 mutations are at the third nucleotide pair of codon 249 (109-112). A dose-dependent relationship between dietary aflatoxin B_1 intake and codon 249^{ser} p53 mutations is observed in hepatocellular carcinoma cases from Asia, Africa, and North America [reviewed in (113)]. The mutation load of 249^{ser} mutant cells in nontumorous liver also is positively correlated with dietary aflatoxin B_1 exposure (114). Exposure of aflatoxin B_1 to human liver cells in vitro produces 249^{ser} (AGG to AGT) p53 mutants (115) (Mace K, Aguilar F, Harris CC, and Pfeifer A: unpublished results). These results indicate that expression of the 249^{ser} mutant p53 protein provides a specific growth and/or survival advantage to liver cells and are consistent with the hypothesis that p53 mutations can occur early in liver carcinogenesis.

Since cellular context may influence the pathobiologic effects of specific mutants of p53, the 249^{ser} mutant may be especially potent in hepatocytes. The enhanced growth rate of p53-null HEP-3B cells by transfected 249^{ser}-mutant p53 indicates a gain of oncogenic function and is consistent with this hypothesis (116). The 249^{ser}-mutant p53 also is more effective than other p53 mutants (143^{ala}, 175^{his}, 248^{trp}, and 282^{his}) in inhibiting wild-type p53 transcriptional transactivation activity in human liver cells (117). One hypothesis concerning generation of liver cancers with 249^{ser} mutation is: (a) aflatoxin B_1 is metabolically activated to form the promutagenic N7dG adduct; and (b) enhanced cell proliferation due to chronic active viral hepatitis allows both fixation of the G:C to T:A transversion in codon 249 of the p53 gene and selective clonal expansion of the cells containing this mutant p53 gene.

In addition to producing chronic active hepatitis, HBV also has other important pathobiologic effects. For example, hepatitis B viral gene products may form complexes with cellular transcription factors, e.g., ATF2 (118), up-regulate transcription of cellular and viral genes (119-123), or activate the ras-raf-MAP kinase signaling cascade (124). Inactivation of p53 tumor suppressor gene functions including DNA repair and apoptosis may be another consequence of cellular protein–HBV oncoprotein complex formation. Since the HBVX gene is frequently integrated and expressed in human hepatocellular carcinomas from high-risk geographic areas (125,126), the X protein has been found to bind p53 (44,127,128) and to inhibit its sequence-specific DNA binding and transcriptional activity (44). HBVX protein also inhibits p53-dependent apoptosis (48). On the basis of the above results, we have speculated that HBVX protein may modulate p53 function in nucleotide excision DNA repair (43), including repair of AFB$_1$–DNA adducts, and we are currently testing this hypothesis. HBV integration also could increase genomic instability, including abnormal chromosomal segregation, and increase rates of DNA recombination (129,130). Therefore, a second hypothesis of liver carcinogenesis emerges in which integration of the HBVX gene is the initial event in these high cancer risk geographic areas and AFB$_1$-mediated 249^{ser}-p53 mutation is the second genetic lesion that leads to further genomic instability.

Structure–Function Relationship of p53

The mutation spectrum can also provide clues to the critical functional regions of the gene, that, when mutated, contribute to

the carcinogenic process. Since about 80% of the missense mutations are in the sequence-specific DNA binding midregion of the protein (2-4), investigators have focused on the transcription transactivator function of p53. However, these missense mutations and the resultant amino acid substitutions can cause aberrant protein conformations (131) that also may alter other functional domains, including those in the carboxyl-terminus of the p53 protein. This positively charged region contains the putative major nuclear localization signal (amino acids 316-325), the oligomerization domain (amino acids 319-360), and a DNA damage-binding domain (amino acids 318-393) (23,86,132,133). p53 sequence-specific DNA binding and transcriptional transactivation can also be modulated by post-translational mechanisms, including serine phosphorylation (86,134) and the redox regulation of the cysteine residues responsible for binding zinc to p53 (135-137). The structure–function relationship revealed by the analysis of the p53 mutation spectrum (3,4), its nuclear magnetic resonance and crystallographic three-dimensional structure (105,106,138) (Fig. 5), and functional studies of wild-type versus mutant p53 activity [reviewed in (97)] have generated both hypotheses for further study and strategies for the development of rational cancer therapy.

Molecular Diagnosis of Cancer

In the near future, oncologists will require both knowledge of the traditional TNM criteria used for cancer staging and the genetic and epigenetic lesions in the cancer before initiating rational cancer therapy. Advances in molecular diagnosis of cancer and micrometastasis currently are being translated into clinical practice [reviewed in (139-143)], and issues of bioethics, quality assurance, economics, and timeliness of the molecular diagnosis are important considerations (144-147). Since the strategies to target p53 are all unproven in the clinical setting, the following discussion reviews the rationale for and

current developmental state of such strategies and does not endorse any one in particular.

Strategies for Rational Cancer Therapy

Biochemical Pathways Involving p53

When considering strategies that target a specific gene or its protein for cancer therapy, one should also consider the target as a component of a critical biochemical pathway(s) in cancer cells carrying this defective gene product, so that downstream elements of the pathway also become possible targets. Although the intricate web of interactive pathways controlling cell growth and death complicates this simplistic concept, one can predict that one defective element alone is sufficient to inactivate each pathway. Gene products at the intersection of two or more pathways such as p53 are most likely to be inactivated as a result of selective pressure for clonal growth during the molecular pathogenesis of cancer.

The development of drugs to mimic the tumor suppressor function of p53 and to target other components of the pathway(s) is a challenging task that is being aided by advances in studies of p53 molecular mechanisms (discussed above). Strategies to screen potential drugs are suggested by the development of assays reflecting biologic functions of the p53 protein: its binding to specific DNA sequences, its function as a transcription factor, its function as an inducer of apoptosis, and its ability to form complexes with cellular or viral oncoproteins (Fig. 1). Since certain p53 missense mutants demonstrate an increase in potential oncogenic function (148-153), the identification of drugs inhibiting this acquired activity is a second and complementary strategy to those focusing on the restoration of its tumor suppressor function.

Tumors With Wild-Type p53

Apoptosis is a cell death pathway that can be enhanced in tumors by anticancer therapies [reviewed in (154-159)]. Ionizing radiation or drugs such as doxorubicin, etoposide, or cisplatin produce DNA damage and a p53-dependent apoptotic tumor cell response in laboratory studies (160-164). However, these therapeutic agents can also induce apoptosis by a p53-independent pathway in certain cells, notably p53-null HL60 cells (165). Anecdotal evidence from the clinic has also emerged, indicating that the status of p53 in the tumor is an important prognostic and therapeutic response indicator. p53 mutation is generally associated with a poorer prognosis in the most common types of human cancer [reviewed in (1,139)]. In addition, Wilms' tumor, retinoblastoma, testicular cancer, neuroblastoma, and acute lymphoblastic leukemia, which are some of the most curable cancers, rarely contain p53 mutations, and Burkitt's lymphoma, containing p53 mutations at the time of diagnosis or occurring during a relapse following therapy, generally responds poorly to therapy (166-174). Clinical studies (175-180) testing the hypothesis that these anticancer therapies mediate their apoptotic response by a p53-dependent mechanism have so far provided equivocal results, which may indicate cell type and agent differences and that anticancer therapies can also activate a p53-independent apoptotic pathway (181). Both retrospective
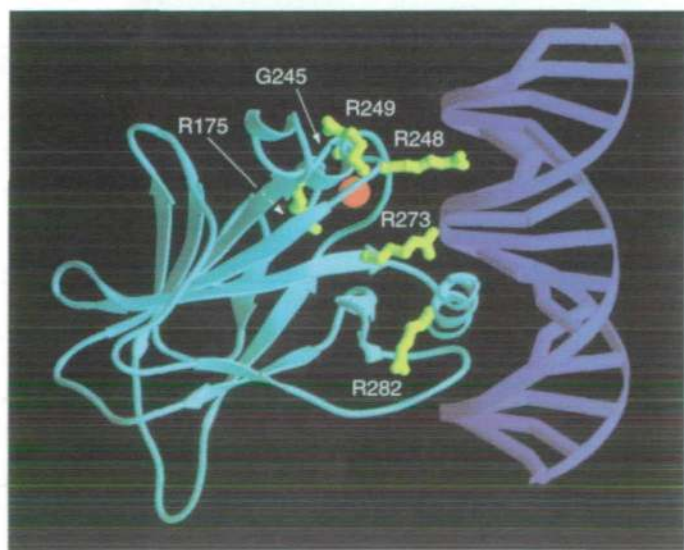

Fig. 5. Ribbon model from the crystal structure DNA-binding domain and its interface with DNA [reprinted with permission (138)].

and prospective clinical studies are needed to test further these hypotheses.

Other inducers of apoptosis are dependent on p53 (Table 1) and could be used in novel anticancer strategies. For example, certain growth factors may act as survival factors of cancer cells so that their depletion or reduced activity would produce apoptosis (182-185). The use of anti-EGF-receptor monoclonal antibodies, which block the EGF-mediated growth signal cascade, have been shown to act synergistically with anticancer drugs in killing cancer cells in laboratory studies [reviewed in (186)]. Apoptosis activated by tumor necrosis factor (TNF) can be dependent on wild-type p53 (187,188), a fact that suggests that cancers with wild-type p53 would be more sensitive to TNF therapy. p53 may down-regulate the expression of survival factors including interleukin 6 (IL-6) (189) or it may inhibit the cellular response to survival factors, such as insulin-like growth factor (IGF), by up-regulating IGF-binding protein-3 (190). Overexpression of certain survival factors, e.g., Steel factor, a ligand of the kit receptor tyrosine kinase, can inhibit p53-mediated apoptosis without affecting the G_1 checkpoint function of p53 (191). Overexpression of the transcription factors E2F-1 and DP-1 that are sequestered by hypophosphorylated Rb (192-195) can override the inhibitory effect of the interleukin 3 survival factor in p53-mediated apoptosis and provide a functional link between p53 and Rb tumor suppressors (196). As discussed above, phosphorylation of the carboxyl terminus of p53 by serine kinases, e.g., cdk2 and protein kinase C, may regulate the transcription transactivator function of p53, including up-regulation of p21^{Waf1} that encodes a G_1 checkpoint protein. Enhanced phosphorylation of the carboxyl terminus of p53, either by activation of these kinases or by inhibition of the protein phosphatases responsible for the dephosphorylation of p53, may have an anticancer effect. These interactive apoptotic pathways suggest novel strategies for anticancer therapy on the basis of modulating survival factors, the survival factor pathway including their cellular receptors and inhibitory proteins, and the phosphorylation of p53.

Tumors With Inactivated Wild-Type p53

Certain DNA viruses have oncoproteins that bind to p53 and inactivate its functions. The E6 protein of the oncogenic strains of human papillomaviruses binds to p53 via E6-AP, a specific ubiquitin protein ligase (197), and enhances the proteolytic digestion of p53. Drugs that inhibit either the formation of this protein complex or the digestion of p53 might have therapeutic benefit in tumors associated with human papillomavirus infections, including cervical, penile, and rectal carcinomas. Since p53 mutations in cervical carcinomas are associated with aggressive cancer and occur late in tumor progression [reviewed in (4)], these chemopreventive agents may be efficacious in early cancers and may inhibit preinvasive lesions. Alternatively, inactivation of p53 by the E6 protein can lead to the enhanced sensitivity to chemotherapeutic agents in a model system using human fibroblasts (198).

p53 can also be inactivated by cellular proteins. The prototypic example is mdm-2, which is overexpressed and amplified in a subset of sarcomas (199). One approach would be to target the mdm-2 gene directly by antisense or triple DNA helix therapy (200-203). A second strategy could involve drugs that specifically inhibit mdm-2 from binding to p53.

Tumors With Mutant p53

Human cancers frequently harbor p53 mutations [reviewed in (3,4)]. Of the approximately 6.5 million new cancer cases worldwide each year, 2.4 million are estimated to involve p53 mutation (1). In the most common types of lethal cancers found in the U.S. population, it is estimated that more than 300 000 cancer cases per year involve p53 mutations (Table 2). These are crude estimates because the mutation frequency differs among populations because of dissimilar exposures to carcinogens and, perhaps, ethnic differences in cancer susceptibility genes [reviewed in (4)]. The high frequency of p53 mutations in human cancers attests to its importance as a target of rational cancer therapy. Furthermore, one can select tumors with p53 mutations for therapeutic agents, e.g., antimicrotubular agents, such as paclitaxel (Taxol) or vincristine, that mediate apoptosis by a p53-independent pathway (O'Connor P, Fan S: personal communication). Novel strategies using a combination of agents can be envisioned: e.g., a low dose of a DNA-damaging agent to arrest normal cells in G_1 of the cell cycle and a delayed dose of an antimitotic agent to target the mutant p53 tumor cells that continue to progress into S phase, G_2, and mitosis.

Healing the Mutant p53 Protein

Tumor-derived p53 mutations target amino acid residues that contact either the DNA or residues that are important for the structural integrity of the core domain of p53. Failure of mutant proteins to bind to DNA has been attributed to the loss of critical DNA contacts, whereas failure by structural mutants to bind to DNA has been attributed to structural defects in the proteins, such as structural rearrangements, local unfolding of the structure, or denaturation of the core domain (138). Therefore, mutant p53 can have altered sequence-specific DNA binding and function as a transcription factor either by inhibiting its transactivator activity or by changing its specificity of DNA binding and the repertoire of genes transcriptionally transactivated [reviewed in (4,138)]. On the basis of biophysical principles, it would seem difficult to reverse mutant conformations to the wild type. However, laboratory studies have provided

Table 2. Incidence of some common cancers in the United States: estimated number of cases with p53 mutations*

Cancer	No. of new cases	No. of estimated cases with p53 mutations
Lung	169 900	95 000
Prostate	244 000	73 000
Colorectal	138 000	68 000
Breast	183 400	44 000
Head and neck	40 000	18 000
Lymphoma	24 000	10 400
Pancreatic	24 000	10 400
Stomach	22 800	9500
Melanoma	34 000	3000

*American Cancer Society, U.S. Estimates, 1995 (287).

results warranting continued effort to develop this strategy. First, certain p53 mutant proteins have temperature-sensitive phenotypes, including increased transcription-transactivator and growth-inhibition activities at the lower permissive temperature, e.g., 32 °C when compared with the nonpermissive higher temperature, e.g., 37.5 °C [reviewed in (6)]. Second, microinjection of certain monoclonal antibodies, e.g., Pab421, recognizing the carboxyl-terminus of p53, can restore the transcriptional transactivator activity of the 273^{his} mutant of p53 (204). Third, certain peptide drugs can alter the conformation of mutant p53 in cells (205,206). Fourth, certain p53 mutants can still form tetramers and cooperate with transfected wild-type p53 in the transcriptional transactivation of reporter gene constructs (117,207). The p53 missense mutants most likely to assume a wild-type protein conformation appear to be those with a substituted amino acid in the sequence-specific DNA binding site (Fig. 3). Examples include the amino acid residues 273 and 248 in the mutant proteins of p53, which are among the most commonly occurring in human cancer (Fig. 1). Mutations resulting in amino acid substitutions in the interior of the p53 protein may be a thermodynamically less stable folded structure and require other strategies. Tumors carrying these interior p53 mutations may be candidates for p53 gene therapy (208) (discussed below). Last, certain p53 mutants also bind to cellular proteins (43,44), which could lead to either dominant negative or gain of oncogenic activities. Therefore, strategies such as targeting the mutant gene by triple DNA helix and antisense approaches [reviewed in (200-203,209)] could result in diminishing these activities and have a therapeutic benefit.

Apoptosis

Many of the currently successful cancer therapeutic agents inhibit tumor growth by increasing the rate of tumor cell death by apoptosis [reviewed in (154-158,210-215)]. Cells exposed to agents that produce DNA damage, such as double-strand breaks, frequently use the p53-mediated pathway of apoptosis (Fig. 2). However, other pathways of apoptosis exist, and normal cell types differ in their sensitivity to inducers of apoptosis (216). This cell-type variation in sensitivity may be determined by the balance between enhancers and inhibitors of apoptosis. In addition, cells of the same type may physiologically alter the balance of enhancers and inhibitors. As discussed above, p53 may mediate apoptosis by both transcriptional transactivation of genes that enhance apoptosis and transcription transrepression of genes that inhibit apoptosis. These genes and their encoded proteins can be considered targets for therapeutic strategies. In addition, components of the p53-independent apoptotic pathway(s) are viable targets in combination with targets in the p53-dependent apoptotic pathway. For example, enhanced expression of the bcl-x$_s$ gene, an enhancer of apoptosis in cancer cells, can either increase their sensitivity to the cytotoxicity of etoposide or paclitaxel (217) or directly induce apoptosis in cell lines with either wild-type or mutant p53 (218). Decreasing the activities of inhibitors of apoptosis that may be overexpressed in cancer cells, such as raf (219), IL-3 (220), or IL-6 (221), is a second strategy for combined rational cancer therapy. Because p53 may also mediate apoptosis by a transcription transactivator-independent pathway through protein–protein interac-

tions (48-50,57,222), the identification of these protein partners of wild-type p53 and the respective binding sites could lead to the development of small molecules that mimic wild-type p53 functions.

Immunotherapy

Tumor rejection in mice has been shown to be mediated predominantly by cytotoxic T lymphocytes (CTL), which recognize peptides derived from a variety of proteins expressed by the tumor cells and presented on the tumor cell surface in association with class I MHC molecules (223,224). In recent years, a series of CTL-defined human tumor antigens has been identified as peptides derived from ectopically expressed or lineage-specific wild-type (nonmutated) cellular proteins that are overexpressed by tumors relative to their normal counterparts, and efforts are being made to develop peptide-based vaccines for cancer immunotherapy (225). The ideal cancer vaccines would target a CTL-defined tumor antigen, which commonly occurs in human cancers and can be presented by a class I MHC molecule expressed in large patient populations. In this regard, the missense mutations in the p53 gene represent attractive candidates for therapy (161,226,227) applicable to a wide range of patients, and an immunotherapy trial to test this hypothesis is in progress (Carbone D: personal communication).

The potential for targeting p53 mutations, however, resides in the ability of a peptide containing the missense mutation to be processed and presented by a particular class I MHC molecule. Unfortunately, an immunoselection process against tumors expressing mutations capable of being processed and presented by HLA-A2.1 limits the potential of targeting p53 mutations for immunotherapy (228). Important consequences of p53 mutation, however, are overexpression and the potential for enhanced presentation of peptides derived from nonmutated regions of the mutated p53 molecule. Such antitumor therapy would be independent of the particular p53 mutation in an individual and dependent solely on the identification of naturally processed and presented wild-type sequence p53-derived peptides. The efficacy of p53 wild-type sequence peptide-based immunotherapy has recently been demonstrated in mice. A vaccine consisting of bone marrow-derived dendritic cells pulsed with H-2K^d-binding wild-type sequence p53$_{aa232-240}$ peptide has been shown to induce rejection of a murine sarcoma expressing a p53 mutation outside the region encoding the wild-type sequence p53 epitope (229). The translational potential of this immunotherapy is enhanced by the identification of a naturally processed wild-type sequence human p53-derived peptide that can be presented by HLA-A2.1 molecules (230). A second strategy has used canary pox virus vectors expressing p53 as a cancer vaccine in mice (231). The immunoprotective response was not dependent on any particular p53 mutation, and either wild-type or mutant p53 was equally effective in the live virus vaccine.

Gene Therapy

Laboratory studies have demonstrated the efficacy of p53 gene therapy in human cancer cells in vitro [reviewed in (1,232)] or as a xenograft in athymic nude mice (233-235). The p53 gene, i.e., a p53 complementary DNA expression vector, was successfully transferred by transfection or infection using

Table 3. Approved and pending p53 gene therapy protocols*

Human gene transfer protocol (status)	Cancer type	p53 delivery vehicle	Route of administration	Institution
9403-031 (approved)†	Non-small-cell lung cancer	Retroviral	Intratumor	The University of Texas M. D. Anderson Cancer Center, Houston; University of Alabama at Birmingham
9406-079 (approved)	Non-small-cell lung cancer	Adenovirus serotype 5	Intratumor	The University of Texas M. D. Anderson Cancer Center
9412-096 (approved)	Head/neck squamous cell carcinoma	Adenovirus serotype 5	Intratumor	The University of Texas M. D. Anderson Cancer Center
9412-097 (pending)	Hepatic metastasis of colon and other types of cancer	Adenovirus serotype 5	Hepatic artery infusion	University of California at San Francisco

*Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, MD 20892. Status of approved and pending protocols, June 1, 1996.
†See (288).

either a replication-defective retroviral or an adenoviral vector, and tumor cell growth was inhibited. A phase I, retrovirus-mediated wild-type p53 gene therapy of lung cancer has recently been reported (288). No clinically significant vector-related toxicity was noted. Whereas local tumor regression was reported in three of nine lung cancer patients who had previously failed conventional therapy, the efficacy of p53 gene therapy will be determined in studies designed to address this issue. p53 gene therapy can be coupled with either cancer chemotherapeutic agents or ionizing radiation. The mechanism of cell death mediated by p53 was shown in some studies to occur via the apoptotic pathway [reviewed in (232,234-236)]. Da Costa et al. (237) have devised a novel strategy of gene therapy in which the mutant p53 in tumor cells binds to exogenously introduced gene products, resulting in transcriptional activation of a toxic gene.

The results of these successful laboratory studies using retroviral and adenoviral p53 expression vectors have led to the approval of phase I protocols in humans (Table 3). Whereas gene therapy is conceptually simple and the laboratory results are encouraging, significant obstacles, e.g., incomplete targeting of the tumor cell population, may limit the success of the current human trials [reviewed in (238-240)]. Nevertheless, improvements in the biotechnology of gene therapy can be anticipated, and the strategy of combining p53 gene therapy with other therapeutic modalities may be more efficacious.

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