BASIC SCIENCE REVIEW

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RESTORATION OF WILD-TYPE p53 FUNCTION IN HUMAN CANCER: RELEVANCE FOR TUMOR THERAPY

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Abstract: *Background.* In the majority of human cancers, the tumor suppressor activity of p53 is impaired because of mutational events or interactions with other proteins (ie, MDM2). The loss of p53 function is responsible for increased aggressiveness of cancers, while tumor chemoresistance and radioresistance are dependent upon the expression of mutant p53 proteins.

Methods. Review of the literature indicates that p53 acts primarily as a transcription factor whose function is subject to a complex and diverse array of covalent post-translational modifications that markedly influence the expression of p53 target genes responsible for cellular responses such as growth arrest, senescence, or apoptosis. The ability of p53 to induce apoptosis in cancer cells is believed essential for cancer therapy.

Results. Numerous data indicate that p53 dependent apoptosis is a relevant factor in determining the efficacy of anticancer treatments. Thus, the development of new strategies for restoration of p53 function in human tumors is considered an important issue. Two main approaches for restoration of p53 function have been pursued that impact anticancer treatments: (a) *de novo* expression of wild-type p53 (wt-p53) through gene therapy and (b) identification of small molecules reactivating wt-p53 function.

Conclusions. The extensive body of knowledge acquired has identified manipulations of p53 signaling as a relevant issue for successful therapies. In this context, the recognition of p53 status in cancer cells is significant and would help considerably in the selection of an appropriate therapeutic approach. p53 manipulations for cancer therapy have revealed the need for specificity of p53 activation and ability to spare body tissues. Furthermore, the promising results obtained by using molecules competent to reactivate wt-p53 functions in cancer cells provide the basis for the design of new molecules with lower side effects and higher anti-tumor efficiency. The reexpression and reactivation of p53 protein in human cancer cells would increase tumor susceptibility to radiation or chemotherapy enhancing the efficacy of standard therapeutic protocols. ©2007 Wiley Periodicals, Inc. *Head Neck* **29:** 272–284, 2007

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p53 protein was identified in SV40 transformed cells where it is associated with Large T Antigen,[1] and *TP53* was initially believed to be an oncogene. Subsequently, *TP53* was found mutated in different human tumors,[2] and its protein product was reported to act as a tumor suppressor.[3] The observations that Li-Fraumeni, a syndrome predisposing to cancer, was associated with *TP53* germline mutations,[4] and that *TP53* knockout mice spontaneously developed cancers at a young age[5] added support to the oncosuppressive action of p53.

p53 (Figure 1) is a powerful transcription factor whose function is essential in preventing inappropriate cell proliferation and in maintaining genome integrity following genotoxic stress.[6] The protein has a short half-life, and its expression level in normal conditions is low. In response to cellular stress, such as DNA damage, hypoxia,

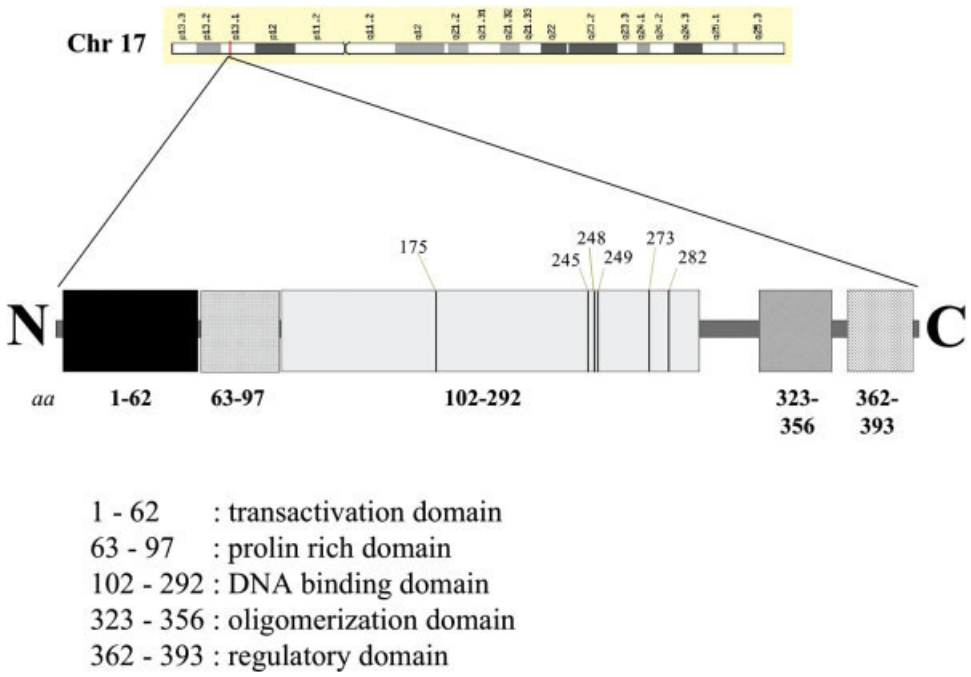

FIGURE 1. Features of human *p53*. Chromosomal localization of the *p53* gene and schematic representation of the domains carried in the p53 protein: -transcriptional activation (aa 1–62); -prolin rich (aa 63–97); -sequence specific DNA binding (aa 102–292); tetramerization (aa 323–356); -and regulation (aa 362–393). Hot-spot missense mutations are reported: DNA binding defective (R273H; R248W), and structural mutations (R175H, G245S, R249S, and R282W). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

oncogene overexpression, or viral infection, the half-life and expression level of p53 rise, and extensive post-translational modifications occur that also increase its sequence-specific DNA binding activity.[7] p53 through its N-terminus domain activates more than 300 different promoter elements,[8] thus modulating the expression of several target genes involved in a number of cellular processes, such as cell cycle arrest, senescence, and apoptosis. Apoptosis is an evolutionary conserved process through which the organisms remove abnormal cells, and the conventional antineoplastic treatments exploit this process to overcome tumor proliferation thus providing successful therapies.

This review, after a detailed description of the molecular mechanisms through which p53 regulates apoptosis, will focus on the developments of new strategies for restoration of p53 function and their impact on treating cancer.

p53 AND APOPTOSIS

Apoptotic pathways have been thoroughly dissected in different organisms, and several regulatory molecules including p53 have been identified. The molecular mechanisms through which the wild-type p53 (wt-p53) regulates apoptosis in mammalian cells are not totally elucidated. Indeed, wt-p53 is primarily a nuclear protein, but it might function outside the nucleus through protein-protein interaction. Several reports indicate that wt-p53 modulates apoptosis through its transcriptional-dependent and -independent activity.[9]

p53 Transcriptional-Dependent Apoptosis. In mammalian cells 2 distinct apoptotic pathways (extrinsic and intrinsic) have been defined, and wt-p53 is a major player in both (Figure 2). In Table I (see refs. 10–24) are listed the most relevant wt-p53 target genes involved in these pathways. The list includes genes responsible for death receptor signaling such as Fas/CD95, DR4, DR5,[10,11] and genes directly affecting the apoptotic machinery such as various pro-apoptotic Bcl2 family members (Bax, PUMA, Noxa, Bid), Caspase-6, Apaf-1, PIDD, and other pro-apoptotic effectors with less defined roles as PERP and p53AIP (reviewed in ref. 25).

Upon cellular stress (DNA damage and others), p53 is subjected to a complex and diverse array of covalent post-translational modifications: phosphorylation, acetylation, ubiquitylation, sumoylation, methylation, and neddylation. These modifications,

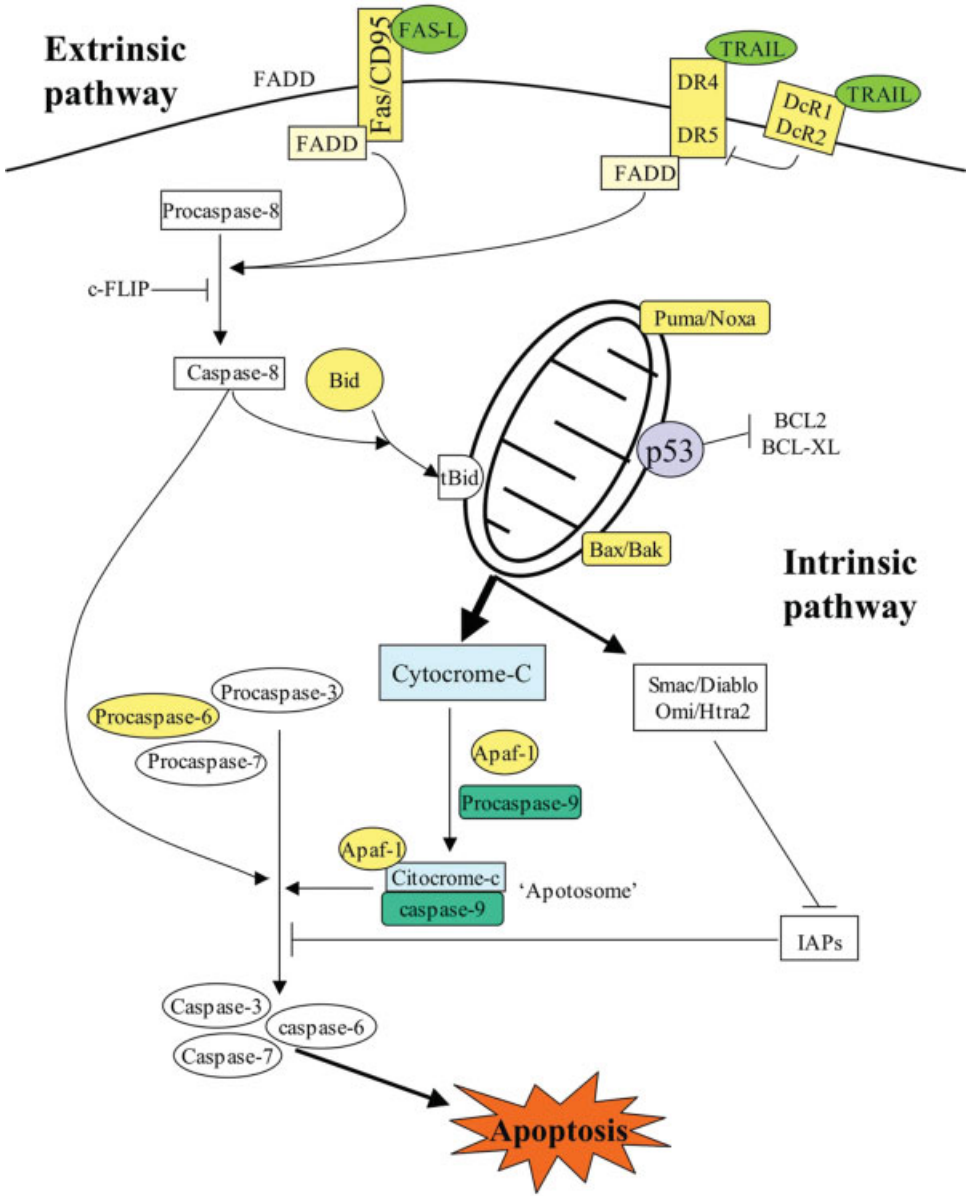

FIGURE 2. The apoptotic pathways. The extrinsic pathway is triggered by the activation of death receptors (Fas/CD95, DR4, and DR5) upon interaction with their respective ligands (FasL, and TRAIL). Once activation occurs, death receptors form the "Death Inducing Signaling Complex" (DISC) that recruits, via the adaptor molecule Fas Associated Death Domain Protein (FADD), multiple procaspase-8 molecules resulting in caspase-8 activation. This signal in some cell types is sufficient to trigger apoptosis. In other cells types, caspase-8 interacts with the mitochondrial pathway activating the BH3-only protein Bid that leads to cytochrome-c release. Negative regulators of this pathway are cellular FLICE-like inhibitory protein (c-Flip), a degenerate caspase homologue that can be recruited by FADD blocking the caspase-8 activation; DcR1 and DcR2 antiapoptotic decoy-receptors that missing the cytoplasmic death domain compete with DR4 and DR5 blocking the TRAIL-induced apoptosis. The intrinsic pathway is used extensively in response to extracellular cues and internal insults as DNA damage. These diverse response pathways converge on the mitochondria altering the balance between pro-apoptotic (Bax/Bak) and anti-apoptotic (Bcl2/BclXL) proteins. The dominance of pro-apoptotic Bax/Bak proteins, induced by BH3-only proteins (Puma; Noxa) and p53, increased the mitochondrial permeability and releasing apoptogenic factors: cytochrome-c and Apaf-1 once in the cytoplasm form the apoptosome complessing and activating procaspase-9 molecules; Diablo/Second mitochondria-derived activator of caspase (Smac) and Omi/high temperature requirement protein A2 (HTRA2) inhibit the cytosolic inhibitor of apoptosis proteins (IAP). The extrinsic and intrinsic pathways converge at the level of caspase-3 activation, which trigger a multitude of subprograms resulting in apoptosis. p53 target genes are shown in yellow. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 1. p53 apoptotic target genes.*

	References
Death receptor pathways	
DR4 (death receptor 4)	10
DR5 (death receptor 5)	11
DcR1 (decoy receptor 1)	12
DcR2 (decoy receptor 2)	13
FAS/CD95	14
Apoptotic effectors machinery	
NOXA	15
PUMA (p53 upregulated modulator of apoptosis)	16
BID (BH3 interacting domain death agonist)	17
BAX (BCL2-associated x protein)	18
Procaspase-6	19
APAF-1 (apoptotic protease activating factor-1)	20, 21
PIDD (p53 induced protein with a death domain)	22
PERP (p53 apoptosis effector related to PMP-22)	23
P53AIP1 (p53-regulated apoptosis-inducing protein 1)	24

The list is not exhaustive

by adding functional groups to the protein, markedly influence the expression of p53 target genes, which regulate cellular responses such as cell cycle arrest, senescence, and apoptosis. Phosphorylation and acetylation usually drive p53 transcriptional activation as a result of stabilization, accumulation, and activation of the protein in the nucleus. Although several different approaches have been used to define the p53 apoptotic programs, we still lack a comprehensive molecular understanding of *p53* target genes in the apoptotic process, and different mechanisms have been postulated for their selective activation.[26,27] Initially, a quantitative model has been proposed,[28] based on the presence of promoters whose activation requires high levels of p53. Indeed, low and high affinity p53 binding sites were revealed in promoters involved in apoptosis and cell cycle arrest, respectively. However, recent analysis of chromatin-immunoprecipitation in cells challenged with DNA damage only partially supported this model. The data reported indicate that some pro-apoptotic target genes (eg, *PUMA*) are regulated by high affinity binding sites, suggesting that the decision to undergo apoptosis also depends on other events. Accordingly, a second model has suggested that p53 selectively activates the transcription of pro-apoptotic target genes upon interaction with transcriptional co-activators, such as p300/CREB-binding protein (p300/CBP), junction-mediating and regulatory protein (JMY), and apoptosis-stimulating protein of p53 (ASPP). The p53/p300/CBP protein complex, through histone acetylation and chromatin remodeling, allows selective transcription of p53-dependent apoptotic genes,[29] whereas the JMY protein,[30] cooperating with p300, enhances the ability of p53 to induce the expression of genes as *BAX*. ASPP family proteins,[31] through direct interaction with p53, enhance the affinity of p53 for promoters of apoptotic target genes such as *BAX* and p53-induced gene 3 (*PIG3*), but not of other target genes (*MDM2*, *cyclin G*, or *p21*). Interestingly, accumulating evidence indicates that p53 interaction with histone deacetylases (HDAC1) and the transcriptional co-repressor mSin3a[32] or with hDaxx,[33] provides a molecular mechanism for p53-dependent transcriptional repression (Figure 3).

p53 Transcriptional-Independent Apoptosis. It has been reported that in some tumor cell lines, inhibitors of mRNA and protein synthesis (eg, actinomycin D, cycloheximide) block the transcription of p53-target genes[34] but do not affect p53-dependent apoptosis. These observations suggested an alternative pathway, transcriptional-independent,

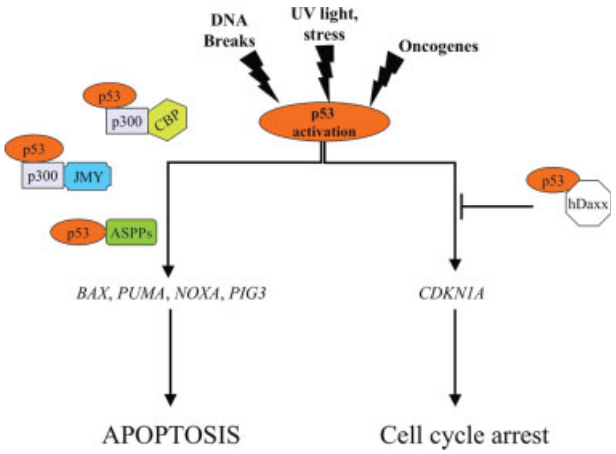

FIGURE 3. Model of cell cycle and apoptosis regulation by p53. Upon different cellular stress, such as DNA damage, p53 became activated through a complex network of post-translational modifications. These modifications allow p53 to interact with different transcription cofactors that help p53 to activate its downstream target inducing apoptosis or cell cycle arrest. Interaction of p53 with cofactors such as p300/CBP or p300/JMY or ASSPs proteins promotes the specific transcription of apoptotic target genes. Interestingly, p53 interacting with others cofactors can repress the transcription of target genes; this is the case of hDaxx, which interacts with p53 to induce the repression of *CDKN1A* transcription, which encodes p21 protein, enhancing the apoptotic response. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

through which p53 can induce apoptosis. Studies corroborating this hypothesis revealed that, upon DNA damage, p53 localizes to the mitochondria and triggers a rapid apoptotic response that occurs before the activation of p53-target genes.[35] Moreover, immune-precipitation experiments in irradiated thymocytes demonstrated that, upon localization in the mitochondria, p53 through its DNA binding domain physically interacts with the BCL-XL and BCL2 anti-apoptotic proteins; this interaction releasing pro-apoptotic BH-3 proteins (BAK, BAX) induces mitochondrial permeabilization and apoptosis.[36]

Studies undertaken to assess the functional role of polymorphism at codon 72 (Pro72/Arg72) also added evidence on p53 transcriptional-independent apoptotic (TIA) activity. Indeed, deletion of the proline-rich domain, where codon 72 is localized, abolishes p53 apoptotic function but maintains p53 transacting activity.[37] Dumont et al[38] by studying the apoptotic response in cell lines overexpressing either variants Pro72 or Arg72, reported that the Arg72 variant induced more efficiently TIA. This effect might be ascribed to a more efficient shuttling of this variant from the nucleus to the mitochondria because of its higher binding affinity for proteins (CRM1; MDM2) involved in p53 nuclear export.

RESTORATION OF WILD-TYPE p53 FUNCTION IN HUMAN CANCER

TP53 is the most frequently inactivated oncosuppressor gene in human malignancies, and its inactivation is beneficial for tumor survival. On this basis, restoration of wt-p53 activity seems one of the most attractive goals for a successful tumor therapy. Data have been reported indicating that senescent program controlled by p53 and $p16^{INK4a}$ is an important determinant of treatment outcome in vivo.[39,40] However, in view of the impact of cell death and apoptosis on therapy outcome, in recent years strategies to restore apoptotic p53 pathways in tumor cells have been thoroughly pursued. Several approaches have been undertaken to restore wt-p53 function. Initially, tumor therapy based on exogenous wt-p53 expression (gene therapy) was exploited. More recently, the use of small molecules for endogenous p53 reactivation, in tumors retaining a wt-p53 gene, was intensively investigated. Similarly, to reverse the mutant p53 action in tumors expressing mutant proteins, a vast number of studies have been devoted to the development of viral particles or of small molecules endowed with the ability to reverse the mutant p53 phenotype.

Reactivation of Endogenous Wild-Type p53

Gene Therapy. Several strategies for cancer gene therapy based on wt-p53 administration have been extensively evaluated both in preclinical and clinical models by using physical or viral vectors. Although preliminary studies in cell culture and in animal models revealed the effectiveness and the low toxicity of these approaches,[41–43] their efficacy in clinical trials is currently debated. Clinical studies carried out in lung, bladder, ovarian, and breast cancers resulted in the absence of additional beneficial effects with respect to conventional treatments (reviewed in ref. 44). Moreover, a recent large international gene therapy study in patients with primary stage III ovarian cancer bearing p53 mutations was closed because of lack of significant benefit.[45] On the other hand, encouraging results were reported for phase II and III clinical trials on 135 patients with late-stage head and neck squamous cell carcinoma (HNSCC). In this study, patients were treated with a combination of recombinant adenovirus-p53 (Gendicine) administration and radiotherapy. The results showed 64% of patients with complete regression and 32% with partial regression. No serious side effects were observed.[46] Moreover, according to a recent review on gene therapy for lung and head and neck cancers,[47] clinical trials of *p53* gene replacement have provided useful information for the design of future gene therapy strategies. Based on several clinical studies, it has been suggested that conventional therapy may provide renewed potential if used in combination with administration of functional *p53* gene in the many clinical settings where local disease control remains suboptimal.

Albeit encouraging results, at present further improvements for a safe and adequate wt-p53 in vivo administration are required. Most of the gene therapy strategies have been performed using replication-incompetent adenovirus carrying human wt-p53 cDNA sequences driven by strong viral promoters. Successful tumor gene therapy requires the development of new modified viral vectors proficient in targeting all, or nearly all, cancer cells. This approach is particularly relevant when direct intratumor injection cannot be performed. In the last few years, progress in vector targeting has been obtained, because of the numerous efforts devoted to change the viral tro-

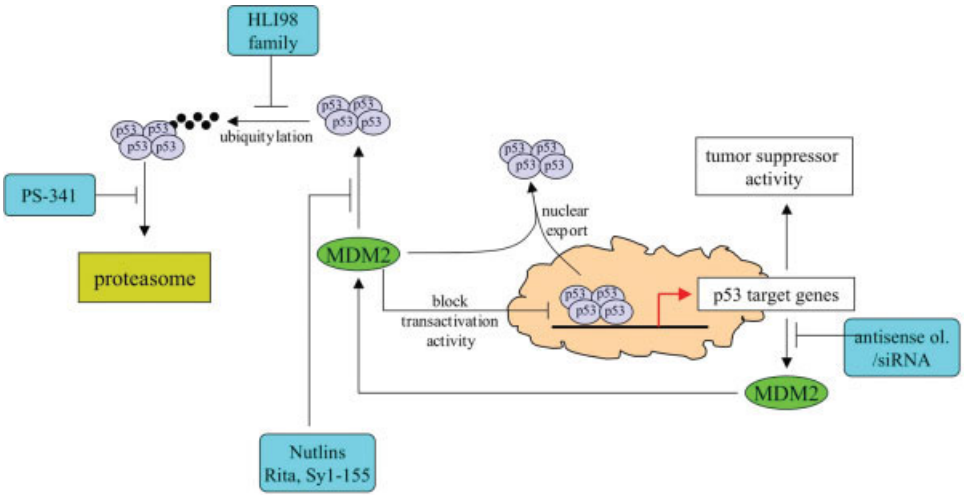

FIGURE 4. The p53 regulatory negative feedback loop MDM2 mediated. Some of the strategies adopted to neutralize the MDM2 functions in tumor cells are shown in blue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pism of adenoviruses through genetic or nongenetic modifications of the viral envelope.[48] At present, however, despite the reported advantages of these modifications, the relevance of such modified viruses for gene therapy approaches are not conclusive.

Neutralization of MDM2 Functions. Cell survival in tumors retaining wt-p53 gene is sustained by other alterations, which impair p53 function and therefore its apoptotic ability. It has been demonstrated that in a significant percentage of human tumors, the mechanism of p53 inactivation is MDM2 overexpression and/or amplification.[49] MDM2 is the major negative regulator[50] of p53 (Figure 4). In physiological conditions wt-p53 has a very short half-life because of the autoregulatory negative feedback loop mediated by MDM2.[51] First, wt-p53 activates the transcription of the *MDM2* gene; subsequently the MDM2 protein, upon interaction with the p53 transactivation domain, impairs p53 transcriptional activity. Moreover, MDM2 through its E3 ubiquitin ligase activity promotes the proteasome-dependent p53 degradation[52,53] and modulates nuclear export of p53.[54] In stressing conditions (DNA damage), p53 reacquires its activity and impairs MDM2 negative regulation. In these conditions, p53 is phosphorylated within its N-terminal domain (Ser15, Thr18, and Ser20) by a number of kinases, and this phosphorylation transiently stabilizes p53, preventing its binding to MDM2 and its subsequent degradation. In the past years, several strategies for tumor therapy have been developed to increase p53 activity by neutralizing MDM2 functions at different levels.

Transcriptional level. Inhibition of *MDM2* gene expression was achieved through the use of antisense-oligonucleotides.[55] These reagents specific for MDM2 have shown antitumor efficacy both in vitro and in vivo.[56] The use of antisense anti-MDM2 oligonucleotides in combination with conventional antineoplastic agents has demonstrated chemosensitizing and radiosensitizing effects in several human cancer cell models.[57,58]

Protein interaction. It has been reported that small peptides derived from p53 were able to inhibit MDM2-p53 protein complexes.[59] Based on these seminal observations, efforts were undertaken to identify new peptide and nonpeptide inhibitors of MDM2-p53 protein complexes as relevant targets for cancer therapy.[60] Several small peptides have been identified whose structure allows them to have high stability in solution and high affinity for MDM2-binding.[61] Experiments have indicated that these peptides were able to induce growth arrest and apoptosis in cancer cell lines and were ineffective in nontumor cell lines.[62] Nonpeptidic or natural inhibitors have also been identified. Natural inhibitors such as chlorofusin,[63] polycyclic compounds,[64] and chalcone derivatives[65] were described to inhibit efficiently the MDM2-p53 complexes, but their biological activity is still unreported. More recently, through extensive screening of diverse libraries of synthetic chemicals, nonpeptidic compounds that inhibit p53-

MDM2 binding have been identified. One of these compounds, a derivative of cis-imidazoline analogs (Nutlin), has been demonstrated as a potent antagonist of MDM2, which activates the p53 pathway in cancer cells leading to apoptosis and growth inhibition in vivo.[66] In the same way, a small molecule named RITA (reactivation of p53 and induction of tumor cell apoptosis) was detected.[67] RITA, via its binding to p53, prevents p53-MDM2 interaction in vitro and in vivo and induces massive apoptosis in various tumor cell lines expressing wt-p53.

A series of nonpeptidic small HDM2 (the human analogue of MDM2) inhibitors were also designed by computer-aided modeling and synthesized by chemical method. Syl-155 is 1 of these inhibitors that induces cell cycle arrest and apoptosis[68] in cancer cell lines expressing wt-p53. In conclusion, nonpeptidic small molecular inhibitors of the p53-HDM2 interaction show promise in the treatment of tumors expressing wt-p53.

Proteasome degradation. The ubiquitin-proteasome pathway is the principal pathway for intracellular protein degradation and plays a significant role in cancer growth and metastasis. The development of proteasome inhibitors was seen as a new therapeutic strategy to suppress tumor growth by inducing apoptosis. On this basis, a unique series of potent, selective, and reversible proteasome inhibitors has been developed. These compounds are dipeptide boronic acid analogues that inhibit the chymotryptic activity of the proteasome by blocking the activity of the enzyme.[69] Relevant results have been obtained with a representative of such compounds, the dipeptidyl-boronic acid PS-341 (bortezomib), a potent and specific inhibitor of the 26S proteasome. Studies in vitro and in vivo have shown antitumor activity of bortezomib in a broad range of tumor types including myeloma, prostate cancer, pancreatic cancer, and colon cancer.[70] Although proteasome inhibitors might affect the intracellular level of various cellular proteins, recent data demonstrated that bortezomib stabilizes p53 protein and induces apoptosis in a p53-dependent manner.[71] Phase I and phase II clinical trials with relapsed multiple myeloma refractory to conventional chemotherapy demonstrated the efficacy of bortezomib treatment.[72] Moreover, promising early clinical results in phase I trial of several solid tumors have been reported.[73] At present, bortezomib is under intensive clinical testing in a variety of solid and hematological malignancies.

Protein ubiquitylation. Blockade of HDM2 Ub ligase activity by its natural inhibitor $p19^{ARF}$ leads to increased p53 levels and p53 transcriptional activity. Indeed, it is known that MDM2 promotes the cytoplasmic translocation of p53, not by physically carrying p53 into the cytoplasm but rather by ubiquitylating it.[74] Hence, p53 ubiquitylation may serve not only as a degradation tag but also as a subcellular localization tag. These data suggested that by inhibiting HDM2 Ub ligase activity it might be possible to restore apoptosis in tumors expressing wt-p53. In view of this attractive pharmacological mechanism for inducing cell death in human tumors, studies have been undertaken to uncover small molecules endowed with ability to inhibit HDM2 Ub ligase activity. Initially, 3 chemically distinct types of compounds were identified[75] to function as selective inhibitors of HDM2-mediated ubiquitylation of p53. Very recently, using high-throughput screening of small-molecule libraries, a family of small molecules (HLI98s, 7-nitro-5-deazaflavin compounds) that strongly inhibited HMD2-mediated autoubiquitylation was described. These compounds stabilize p53 protein and activate p53-dependent apoptosis.[76] Pharmacological optimization of these compounds may provide new basis for therapeutic agents in human cancers.

Nuclear export. An attractive approach to enhance p53 function is to induce its accumulation in the nucleus, where p53 can act as transcriptional activator. Leptomycin B (LMB) is a specific and potent inhibitor of CRM1 nuclear exportin, which is involved in the nuclear export of proteins carrying a nuclear export signal (NES).[77] Although the LMB activity impacts the whole cellular proteins trafficking, consistent data indicate LMB strongly activates p53 transcriptional activity even at submolar concentrations.[78] LMB was tested in normal cells (human primary dermal fibroblasts) and on a range of tumor cells. Although LMB induced a mild reversible growth inhibition effect on normal cells, LMB induced a very strong apoptotic response in cultured cells derived from neuroblastomas[79] and from cervical[80] and prostate[81]carcinomas. Besides the intriguing anticancer activity LMB was found highly toxic in phase I clinical trials.[82] At present, studies are focusing on the crystal structure of the CRM1-LMB binding complex for the design of new nontoxic molecules and the identification of strategies to deliver LMB directly into tumor tissues.

Reversing of p53 Mutant Phenotype. Approximately 50% of all human tumors express mutant forms of

p53. These proteins not only have lost the normal p53 functions but also have acquired new functions ("gain of function"), which contribute actively to aggressiveness and chemoresistance or radioresistance of tumors.[83] Thus, mutant p53 represents one of the most important clinical targets for drug intervention. Database[84] of somatic tumorigenic *TP53* mutations indicate that about 95% of these mutations lie in the core DNA-binding domain (Figure 1). Furthermore, most of these mutations occur as single missense mutations, so that mutant p53 in tumors generally is a full-length protein with single amino acid substitution in its core domain. Six mutational hot spots cluster to the DNA binding surface: 2 contact directly DNA (R248Y and R273H) and 4 stabilize the surrounding structure (R175H, G245S, R249S, R282W) giving rise to 2 classes of mutations, "DNA contact" and "structural." DNA-contact mutations result in loss of DNA-binding residues with little effect on folding or stability, while "structural" mutations result in structural changes due to local distortion, mainly in proximity of the DNA-binding site.[85,86] Biophysical studies revealed that a significant number of mutations mainly affect p53 function by reducing the melting temperature of the DNA binding domain below the body temperature.[87]

Two experimental approaches have been primarily employed to target mutant p53 for drug discovery. First, based on the absence of wt-p53 activity in cancer cells, generation of mutated viral vector for tumor cell lysis (Onyx-015) was exploited. Second, based on the attempts to restore some of the wt-p53 activities, development of activating small molecules to target different mutant proteins was pursued.

Onyx-015. McCormick at Onyx Pharmaceuticals introduced the hypothesis that an adenovirus deleted of the E1B region could only replicate and generate cellular lysis in cells lacking functional p53 because of the putative need for p53 inactivation for adenoviral replication. Accordingly, the Onyx-015 reagent, a p53-targeting oncolytic mutant adenovirus, has been developed for clinical application.[88] The use of this mutant adenovirus is not perceived as delivering a therapeutic gene (gene therapy), but it does take advantage of the genetic abnormalities of tumor cells. A potential advantage of this viral therapy is that infected cells will provide neighboring cancer cells with high titers of new virus particles when their lysis occurs. In vitro and in vivo experiments indicated that the virus has antitumor efficacy and that this efficacy is significantly enhanced by combination with chemotherapy.[89] Contradictory observations have been reported for Onyx-015 replication independent of the p53 mutational status in tumor cells.[90] However, these observations were partially explained[91] by the discovery that replication of Onyx-15 is facilitated in cells where loss of $p14^{ARF}$ and high MDM2 levels inactivate wt-p53. Moreover, a more accurate definition of the repertoire of susceptible p53 mutants[92] may now offer criteria for a more appropriate selection of tumors to be treated. Evaluation of numerous clinical trials thus far performed indicates that the administration of Onyx-015 as single agent produces marginal benefit, whereas its administration in combination with conventional therapy is more effective.

Activating Molecules. Restoration of wt-p53 has been demonstrated using antibodies, peptide constructs, and small synthetic molecules. Activating molecules have been identified by either library screening or rational drug design. In recent years, experimental data emphasized the possibility of restoring the wt conformation of mutant-p53 through the employment of small molecules.[93] These findings raised the possibility of developing drugs that restore the tumor suppressor function of mutant p53, thus selectively eliminating tumor cells. In cancer cells, where mutant-p53 is abundantly expressed, restoration of wt conformation would trigger a massive apoptotic response not predicted in normal cells where wt-p53 is expressed at very low levels. Currently, a number of reactivating molecules have been identified and the results obtained are intriguing. In this review, some of these molecules are described and their mechanism of action discussed.

CP-31398. A library of 100,000 chemicals was screened for compounds that could stabilize the native conformation of wt-p53 core domain upon thermal denaturation. The CP-31398 molecule was shown to enhance the stability of Ala-173 and His-273 core domains in vitro and to restore the native conformation and the transcriptional activity of mutant proteins in living cells promoting p53 target gene transcription. In nude mice, CP-31398 has been reported to inhibit the growth of tumor carrying the p53 mutants Arg-249 or Ser-241 in the absence of toxicity at therapeutic doses employed.[94] Further studies have confirmed that CP-31398 treatment causes: (1) stabilization of wt-p53 levels, (2) apoptosis-related changes, (3)

induction of p53 target genes. Moreover, CP-31398 was demonstrated to increase the levels of wt-p53 protein by inhibiting the MDM2-mediated ubiquitylation and degradation.[95] The observation that CP-31398 stabilizes wt-p53 suggested that CP-31398 interacts with newly synthesized p53 in vivo changing its folding.[96] Moreover, data indicating that CP31398 acts as a DNA intercalator stabilizing wt-p53, suggested that CP31398 may work as a DNA-damaging agent.[97] However, by using chromatin immunoprecipitation assays, it was demonstrated that CP-31398 promotes mutant p53 to bind to p53 response elements in vivo. Indeed, CP-31398 functionally restores the ability of p53 mutant proteins to interact with both high (p21) and low affinity (BAX) promoters in cells carrying either DNA contact defective (His-273) or structural defective (Ser-249) mutants.[98] How CP-31398 restores the wt-p53 functions is currently not well understood. A model suggests that CP-31398 acts as a chaperone that binds mutants during biosynthesis, enables them to fold in the active wt-p53 conformation and, by inhibiting ubiquitylation and degradation, allows p53 activities.

PRIMA-1 (p53 reactivation and induction of massive apoptosis). The screening of chemical libraries for compounds that selectively inhibit the growth of Saos-2 cells expressing TET-regulated mutant p53 protein (His273) allowed the identification of PRIMA-1.[99] PRIMA-1 has been shown to restore: (1) the wild-type conformation of mutant p53 proteins both in vitro and in living cells, (2) the DNA binding and the transactivation activities of p53 on target gene promoters such as p21 and MDM2 and PUMA.[100] Interestingly, PRIMA-1 does not induce any effects in cell lines p53-null or expressing wt-p53, as in nontransformed diploid human fibroblasts, suggesting its effects are dependent on the presence of mutant p53 proteins.[101] Experiments in vivo reported PRIMA-1 to reduce tumorigenicity of human tumor xenografts (Saos-2-His-273 cell line) without toxic effect in mice after IV injection.[102] At present, it is not well understood how PRIMA-1 reactivates mutant p53 proteins; in particular a direct interaction of PRIMA-1 and p53 has not been demonstrated as yet. More detailed studies would elucidate this process and provide important details for the design of new p53-targeting agents for therapy of malignant cells.

CDB3. CDB3 is a peptide that was identified through a rational approach for searching molecules that stabilize the native form of p53. Indeed molecules that bind the native, but not denatured protein, will shift the equilibrium away from the denatured form increasing the native one.[103] Following this approach and studying the binding between wt-p53 core domain and the p53-binding protein 2 (53BP2 or ASPP) a nine-residue peptide[104] CDB3 was designed and found useful. CDB3, derived from residues 490–498 of 53BP2, has been reported to restore in vitro and in living cells in the wild-type DNA binding activity of different mutant p53 proteins (Ile-195; His-175; His-273; or Ser-249) with significant transactivation of wt-p53 target genes (*MDM2*, *gadd45* and *p21*), and partial restoration of p53-dependent apoptosis.[105] The mechanism through which CDB3 reactivates mutant p53 proteins is not well understood. At present, it has been suggested that CDB3 has chaperone functions[104] like CP-31398. Indeed, these molecules (drugs), which rescue the conformation of unstable mutants of p53, have to act during or immediately after biosynthesis. They should maintain the mutant protein in a folded conformation and prevent its aggregation, allowing p53 enough time to reach the nucleus and bind specific DNA sequences or proteins that will stabilize it.[106] Another way to understand the action of CDB3 is offered by recent data reporting that ASPP1 and ASSP2 are able to activate p53 family members,[107] thus suggesting that CDB3, which is a fragment of the ASSP protein, has a broader range of targets in addition to p53. In this view, it might be speculated that suppression of mutant p53 through CDB3 may relieve p73 anticancer activity.

HSP90 inhibitors. Heat shock protein 90 (HSP90) is a molecular chaperone highly expressed in cancer cells. HSP90 stabilizes misfolded proteins including mutant p53[108] and contributes to their accumulation into the cells. A naturally occurring compound, geldanamycin, has been identified to specifically inhibit the intrinsic ATPase activity of HSP90 compromising its chaperone functions. Treatment of cells that express mutant p53 proteins with geldanamycin lead to mutant p53 destabilization, ubiquitilation, and subsequent proteasome degradation. Geldanamycin, in preclinical model systems, showed promising antitumour activity, and its derivative 17-allylaminogeldanamycin (17-AAG) has been used in phase I clinical trials reporting encouraging results.[109] Currently, several geldanamycin analogues have been identified, and their antitumor activity is under investigation.[110]

Chimeric adaptor protein. Represent an innovative approach of selective mutant p53 protein re-activation. One-hybrid adaptor protein was originated from the DNA binding and tetramerizing portions of the p53-homologue p73 fused to the oligomerization domain of p53. This chimera binds to the DNA of p53-responsive promoters through the p73-derived portions, and it binds to mutant p53 by the p53-derived oligomerization domain. Through this one-hybrid system, mutant p53 is re-enabled to activate transcription. When the adaptor was expressed in tumor cells that contain mutant p53 (His-273, Trp-248), expression of p53-responsive genes was activated, and growth was inhibited. No such effects were observed in cells that contain wt-p53 or no p53 at all. The adaptor molecule efficiently reactivates the "DNA contact" p53 mutants (His-273, Trp-248), but cannot reactivate the "structural" (His175) p53 mutants.[111]

Ellipticine. Ellipticine has been discovered by an anticancer drug discovery program in which more than 70,000 low-molecular-weight compounds have been screened in vitro against a panel of 60 different human cancer cell lines.[112] Ellipticine belongs to a group of molecules called ellipticiniums which show preferential activity toward mutant p53-carrying tumors cell lines. Indeed, ellipticine induces p53 target gene expression (p21, MDM2, and Bax) and G1 arrest or apoptosis in tumors expressing mutant p53 but not in the corresponding p53 null cells both in "in vitro" and "in vivo" conditions.[113] The mechanism of ellipticine action is still not elucidated; some evidence suggests ellipticine restoring the wild-type conformation of newly synthesized mutant p53 protein.[113]

Ribozyme. The trans-splicing ribozymes have been reported as an alternative strategy that can simultaneously reduce mutant p53 expression and restore wt-p53 activity in various human cancers. The ribozyme accomplished such conversion by repairing defective p53 mRNAs with high fidelity and specificity. The corrected transcripts translated to produce functional p53 can transactivate p53-responsive promoters and down-modulate expression of the multidrug resistance (MDR1) gene promoter.[114] Ribozyme from the self-splicing group I intron of *Tetrahymena thermophila* have been generated for p53 transcripts. Ribozyme p53-specific recognizes accessible uridine residues upstream of the mutation target in the mutant p53 transcript by base pairing through the internal guide sequence (IGS). The ribozyme cleaves the target residue of the RNA, releases the downstream mutant p53 RNA sequence, and replaces the sequence with its 3′ exon which encodes the correct wt-p53 sequence, "*via*" trans-splicing reaction. Studies also reported p53-ribozyme specifically repairs His-273 in 2774 ovarian cancer cells inducing p53 target genes expression (p21, Bax) and apoptosis.[115]

CONCLUSIONS

Since its discovery as a tumor suppressor, p53 has been extensively studied. Biochemical studies, albeit not exhaustive, contributed to identify functions through which p53 exerts its oncosuppressor activity and its functional pathways. The extensive body of knowledge acquired about p53 activities and its relevance in cancer formation and progression has indicated the manipulation of p53 signaling is relevant to successful therapies. Accordingly, identification of the p53 status in the target cells is imperative, and the knowledge of additional oncogenic events contributing to a particular cancer would significantly aid in the selection of an appropriate therapeutic approach. Indeed, p53 manipulations could be helpful when pathways upstream of p53 are defective but not if defects are downstream to p53 signaling. In addition, the relevance of p53 manipulations for cancer therapy resides in the specificity of p53 activation and in the ability to restrict its activity to cancer cells sparing healthy body tissues.[42,43] Lastly, the promising results obtained by investigating the possibility of identifying molecules competent to reactivate wt-p53 functions in cancer cells provide the basis for the design of new molecules with lower side effects and higher anti-tumor efficiency. Currently, high toxic side effects and resistance often accompany chemotherapy and radiation-therapy protocols for cancer treatment. The reexpression and reactivation of p53 in human cancer cells would increase tumor susceptibility to radiation or chemotherapy, enhancing the efficacy of standard therapeutic protocols.

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