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PROSPECTS

Gene-Specific Mechanisms of p53 Transcriptional Control and Prospects for Cancer Therapy

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Abstract The regulation of gene-specific activation is critical to the tumor suppressor function by p53. p53 is a well-characterized transcription factor that responds to DNA damage and other genotoxic stresses by the activation of downstream targets that are involved with repair, differentiation, senescence, growth arrest, and apoptosis. Sequence-specific binding to DNA, conformation, post-translational modifications, cofactor binding, stability, and subcellular localization all influence the performance of p53. The purpose of this review is to define features that play a key role in gene-specific activation and to show that these are often incapacitated in cancer cells. Using such knowledge to design selective strategies for the restoration of p53 wild-type function in cancer cells represents a promising cancer therapy. J. Cell. Biochem. 99: 679–689, 2006. © 2006 Wiley-Liss, Inc.

Key words: p53; DNA binding; tumor suppressor; transcription; gene expression; target site selectivity

p53 AS A TUMOR SUPPRESSOR

Just as the Beatles have forever transformed the musical landscape of our culture, the identification of p53 as a tumor suppressor has equally changed our way of thinking about tumor biology and continues to fascinate tumor and molecular biologists today. It is almost 25 years since two publications quietly burst on the tumor virology scene to identify a T antigen binding protein in mouse cells transformed with the small DNA tumor virus, SV40 [DeLeo et al., 1979; Lane and Crawford, 1979]. What was so provocative about this observation was that this 53 kDa protein was a cellular protein. It was already established that T antigen was a viral protein shown to be essential for the initiation and maintenance of the transformed phenotype. The age-old question: what was their relationship and what was its potential role in tumorigenesis? Although originally considered a tumor antigen because overexpression of the cloned p53 cDNA resulted in oncogenic transformation it was subsequently discovered that the cDNA was actually a mutant version of p53 [Oren and Levine, 1983]. By the late 80s p53 was no longer considered a proto-oncogene as the normal function of wild-type p53 was shown to actually suppress transformation. A continual stream of contributions followed that critical observation. Each added to a body of literature that has helped to define p53 as a transcription factor and a true "guardian of the genome." As a central player in the DNA damage pathway of cells p53 plays a critical role in the regulation of cell cycle, cell death, and DNA repair. Its importance is underscored by the fact that p53 is frequently mutated in human cancers and at the risk of sounding presumptuous, it is likely that tumors containing wild-type p53 probably have some other constituent of the p53 damage pathway altered. In a landmark publication in 1992, it was reported that mice homozygous for the null p53 allele developed normally but by the time they were 6 months of age began to develop a variety of tumors [Donehower et al., 1992]. The genetic basis for cancer predisposition in humans is reflected in Li Fraumeni families that carry germline p53 mutations and

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show an increased frequency of diverse tumor types.

It would be rather impossible to review the extensive labor of all those who have added to the understanding of p53. p53 is the subject of many fine reviews [Ko and Prives, 1996; Bargonetti and Manfredi, 2002; Vousden, 2002]. Therefore, only a salient review of some important features that contribute to the transactivating function of p53 will be discussed. The focus here is to present current ideas and research on the gene-specific target site selectivity of the p53 protein. It will conclude with an overview of the potential therapies that employ different strategies for the treatment of tumors. The ultimate goal of course is to transfer what has been learned at the benchtop to the bedside.

The tumor suppressor protein p53 (Fig. 1) determines the cellular response to a wide array of genotoxic stresses through the induction of growth regulatory and death related genes. In normal cells p53 is a tightly regulated protein that is maintained in low levels due to its short half-life. However, in response to DNA damage, hypoxia, telomeric deterioration, and other inducers of genotoxic stress, increased levels of p53 that accumulate in the nucleus can mediate an outcome of either growth arrest or apoptosis (Fig. 2). In doing so the genome is protected from an accumulation of damaging mutations. The determinants of this outcome are not completely understood although it is hypothesized that cell type, extent of the damage, expression of cofactors and modification of p53 may contribute to the decision [Vousden, 2002].

It is mainly through its role as a transcription factor that p53 is able to coordinate the appropriate responses to stress signals. Although recent literature suggests that p53 can directly modulate the apoptotic response of tumor cells in a transcription-independent fashion directly at the mitochondria [Mihara et al., 2003; Chipuk et al., 2004]. Location is everything. However, features that define p53 as a transcription factor [Ko and Prives, 1996] include an acidic N terminal region that functions as a bona fide activation domain (residues 1–43) and a central DNA binding domain (residues 100–300). The C-terminal region contains a flexible linker (residues 300–320) that separates the DNA binding domain from the tetramerization domain (residues 320–360) and a highly basic stretch of 30 amino acids that extend from residue 363–393. The carboxy-terminus can bind DNA but its binding is non-specific and does not discriminate between different forms of

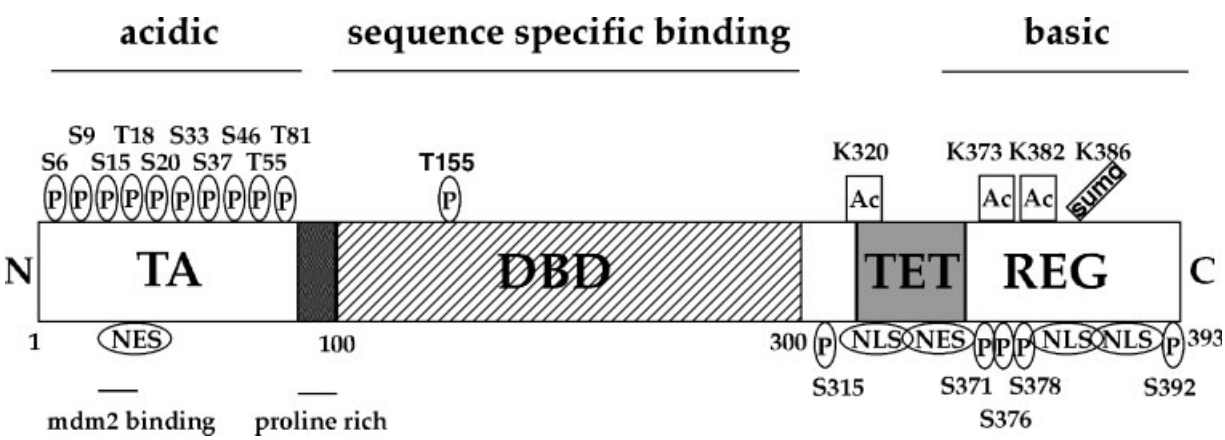

Fig. 1. Bar representation of p53: structure and modifications. The human p53 protein consists of 393 amino acids. The transactivation domain (TA) is located at the N-terminus of the protein. This region interacts with components of the transcriptional machinery that include CBP/p300, a regulator of chromatin remodeling. A basal level of Mdm2, when associated in this region inhibits transcriptional activity. Increased levels of Mdm2 target p53 for degradation. The central domain (DBD) is responsible for sequence specific binding to DNA and is the region in which most point mutations in cancers are found. The carboxy-terminal portion of the protein contains the tetramerization domain (TET). A negative regulatory region has been identified (REG) which extends from amino acid 363–393. Three nuclear localization signals (NLS) are clustered in the C-terminal region. There is a nuclear export signal (NES) located in the amino terminus as well as one in the C-terminus. Both the N and C-terminus contain many sites of post-translational modifications, as shown above and below the cartoon; phosphorylation (P), acetylation (Ac), and sumoylation (sumo). N-terminal phoshorylation may be a prerequisite in order for p300 to bind and mediate acetylation in the C-terminus. Some lysines that are modified by acetylation may also be targets for ubiquitination or sumoylation. The stability and activity of p53 is tightly regulated by these modifications.

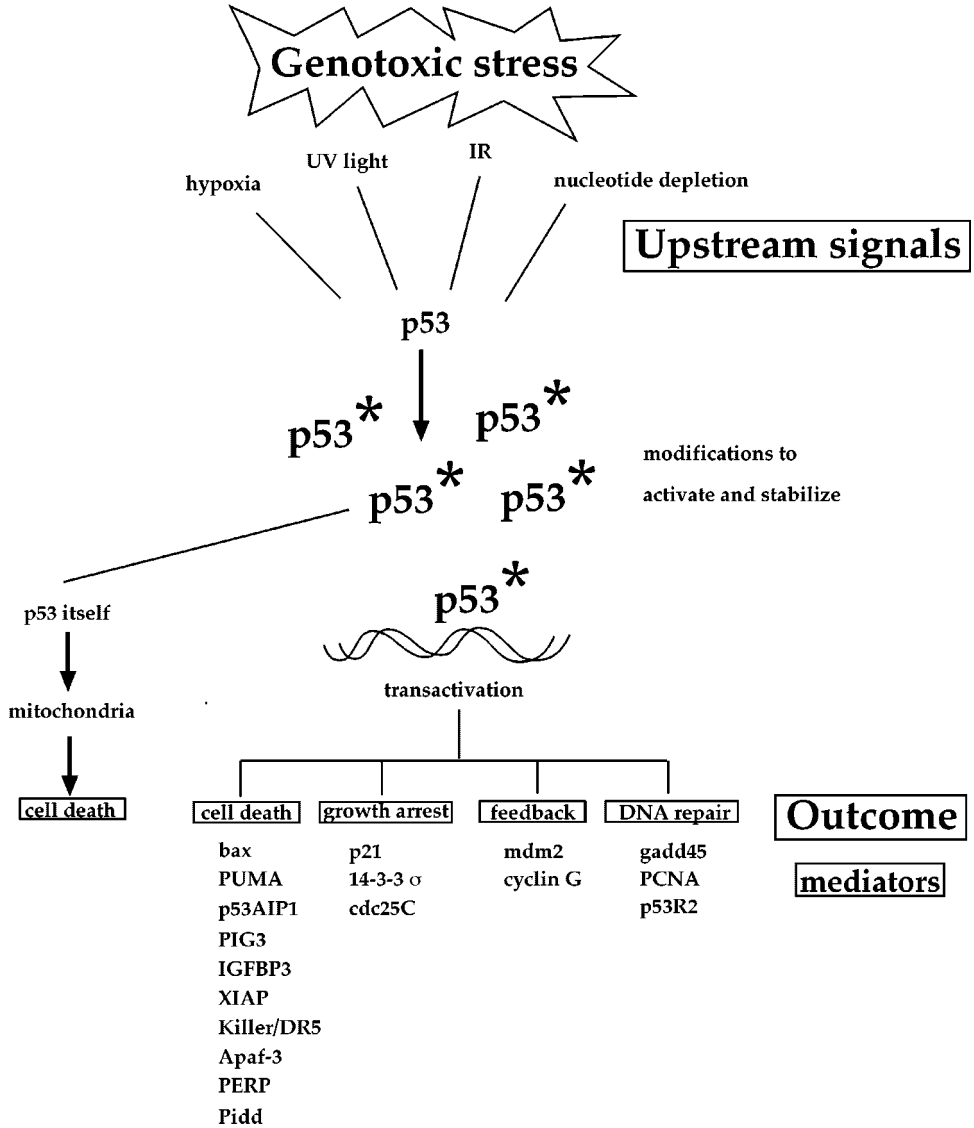

Fig. 2. The p53 pathway: activation of p53-dependent target genes. The initiating stimuli in the p53 pathway are diverse and may include in addition to UV and IR damage, hypoxia, telomeric deterioration, hyperoxia, cytokines, growth factors, activated oncogenes, metabolic changes, anchorage, and cell-cell contact. These upstream signals are recognized by a "sensor" that in turn modifies p53 by phosphorylation and acetylation. Modified p53 becomes stabilized and activated for the transcription of downstream targets. p53 binds in a sequence specific manner to a response element or elements in the regulatory region of the gene. Apoptosis can also be triggered by p53 in a transcription-independent pathway that involves the localization of p53 itself to the mitochondria. The biologic responses that are mediated by p53 targets may result in outcomes that include growth arrest, apoptosis, repair, senescence, and differentiation. As the list of p53-regulated genes continues to grow, so does the possibility of other as yet undefined biologic responses.

DNA. Three nuclear localization signals are clustered in the carboxy region in addition to a nuclear export signal that resides in the N terminal region. Both the N and C terminus contain sites that can be modified by phosphorylation, acetylation, sumoylation, *O*-glycoslyation, and ubiquitination (Fig. 1). The role of modification has been a hot topic of pursuit for many investigators. Understanding how these modifications contribute to the stability, location, binding, activation, and target site selectivity of p53 remains an active area of research.

p53 AS A TRANSCRIPTION FACTOR

The identification of the central domain of p53 as the DNA binding domain has been supported by the solution of the three dimensional crystal

structure that has defined the contact points of this conserved domain with the major and minor grooves of a cognate site [Cho et al., 1994]. The importance of the interaction of the central domain of p53 with sequence-specific DNA is demonstrated by the biologic evidence that tumor cells containing p53 mutations are clustered in the DNA binding region (residues 100–300) of the protein. Hot spot mutants that fail to bind to the consensus sequence fail to transactivate [Kern et al., 1991; Bargonetti et al., 1993]. Recently it has been demonstrated that a variety of agents that include antibodies [Caron de Fromentel et al., 1999], peptides [Friedler et al., 2002], and small molecular weight compounds [Bykov et al., 2002] can restore the DNA binding and transactivation properties of some naturally occurring mutant p53 proteins, thereby providing a potential avenue for cancer therapy. The mechanism of the restoration of activity speaks to the issue of how p53 conformation relates to the binding and transactivation of target genes.

Of critical importance to the function of p53 is the ability of tetrameric p53 to bind in a sequence specific manner to canonical sequences in the promoter or introns of response genes [el-Deiry et al., 1992; Funk et al., 1992]. The consensus site, 5′PuPuPuC(A/T)(A/T)GPyPyPy3′, consists of two palindromic 10 bp sequences separated by 0–13 bp. An ever increasing list of transactivated genes, includes $p21^{WAF1/CIP1}$, bax, GADD45, mdm2, IGFBP3, DR5/KILLER, cyclin G, Noxa, PUMA, AIP1, Apaf-1, PCNA, 14-3-3σ, p53DINP1, and others. The absolute sequence for each response element is unique and adherence to the consensus sequence may vary in terms of the number of mismatches. Some studies suggest that it is the size of the spacer region or the number of mismatches in the response element that matters [Qian et al., 2002]. This raises some intriguing possibilities in regard to activation. First, p53 may have a different affinity for response elements due to site topology. Second, when bound to each site p53 may take on different conformations that may or may not support activation, that is, the site is occupied but nothing is happening. Third, specifically modified p53 may bind to a subset of response elements. Fourth, specific cofactors may be required in order for p53 to efficiently transactivate a subset of response elements.

The common thread linking these scenarios is that p53 is a conformationally flexible protein. The carboxy terminus of p53 has been considered a negative regulatory region that inhibits sequence specific binding by the central domain. The notion that conformation may play an important role in the sequence specific binding of p53 to its cognate site originated from a number of observations made after activating modifications were made in the C-terminus of p53 [Prives and Hall, 1999]. Relief of negative inhibition was obtained by partial proteolysis of the C-terminus, binding to the monoclonal antibody PAb 421, association with *E. coli* DnaK protein, association with single-stranded DNA or phosphorylation by casein kinase II or protein kinase C. In addition peptides that span this 30 a.a. region have been shown to stimulate the specific DNA binding activity of full length p53. The concept that p53 exists in a latent conformation and requires modification in order for it to be fully active continues to be debatable. Arguments and experiments have been made to support models proposing steric hindrance or allosteric conversion. More recently this model was challenged by the results of [Kaeser and Iggo, 2002]. Performing quantitative ChIP assay on tumor and normal cell lines that contain a wild-type p53 they found no evidence that p53 exists in a latent state. In unstressed cells they are able to recover substantially more p21 and mdm2 promoter DNA than in p53 null cells. After genotoxic stress the increased binding that was observed reflected the increases in p53 levels. Using NMR spectroscopy Ayed et al. [2001] have shown that dimeric p53 with and without the C-terminus are identical in conformation, yet in a gel shift assay behave as latent and active forms. This finding supports a steric hindrance model for negative regulation. It should be noted that p53 expressed in *E. coli* is not post-translationally modified and requires activation in order to bind the cognate DNA sequence whereas p53 expressed in insect cells or eukaryotic cells is constitutively modified and does not require activation for binding in electrophoretic mobility gel shift assays. This might explain why in tissue culture cells transfection with either the full-length p53 or the terminally truncated form results in similar induction of p21 [Pellegata et al., 1995]. Despite the in vitro biochemical data to support that the C-terminus negatively regulates binding, these experiments tell us that at least in this system it doesn't matter. However, the C-terminus is subject to many different post-translational

modifications. Does overexpression reduce the need for these modifications? What is the underlying importance of phosphorylation and acetylation in cells that are stressed or unstressed? Do either of these modifications play a role in DNA binding and gene specific activation?

Indeed the role of post-translational modification of p53 in activation, stability, and target site selection is still being defined [Appella and Anderson, 2001]. p53 is acetylated in vitro and in vivo in response to stress signals by the coactivators p300/CBP and PCAF histone acetyltransferases (HAT) [Avantaggiati et al., 1997; Gu and Roeder, 1997; Lill et al., 1997]. These HATs bind to p53 in the amino terminus and subsequently acetylate several lysines in the carboxy terminus. Acetylation of 53 is not required for sequence specific binding although it has been shown to stimulate this activity. It remains controversial whether acetylation of p53 or the surrounding histones in the DNA is critical for the activation of transcription [Barlev et al., 2001; Espinosa and Emerson, 2001]. Nevertheless the tethering of p300 facilitates the recruitment of general transcription machinery needed to promote transcription. When this interaction is antagonized by deacetylases such as Sir2, p53-mediated transcription is repressed [Luo et al., 2001; Vaziri et al., 2001]. Some studies suggest that phosphorylation may also be a prerequisite for acetylation [Sakaguchi et al., 1998].

Although p53 is extensively phosphorylated on several serines and threonines in both the N and C termini after DNA damage the nature of its contribution to the regulation of p53 still remains unclear [Ashcroft et al., 1999]. Defining which modifications are important for increasing stability and transcription in addition to those modifications that alter promoter specificity remains a great challenge. Maintained at low levels because of the association with its partner Mdm2, (an E3 ubiquitin ligase which targets p53 for proteosome mediated degradation), p53 becomes stabilized following DNA damage as a result of phosphorylation and acetylation. Phosphorylation in the N-terminus of ser15, 20 by Chk2 and Chk1 hinders Mdm-2 binding and results in stabililization [Shieh et al., 2000]. Phosphorylation of other N-terminal serines may be a prerequisite for binding of p300 and subsequent acetylation of C-terminal lysines [Sakaguchi et al., 1998]. This enhances stability by ensuring the inhibition of the ubiquitination and promotes the transactivation of promoters [Gu and Roeder, 1997]. Mutational analysis is one time-honored approach that many have taken in order to determine the impact that these modifications have on the transcriptional activity of p53. These results have been disappointing at best even though mutations have been made to every known C-terminal site of phosphorylation [Fuchs et al., 1995; Ashcroft et al., 1999]. If redundancy of function accounts for these observations multiple mutations might yield an effect. However, even mutation of all of the C-terminal and N-terminal phosphorylation sites (Fig. 1) has not significantly affected the transcriptional activity of p53 on $p21^{WAF\text{-}1/CIP1}$, mdm2, and bax promoters in transient transfection assays. The authors rightly acknowledge that overexpression in this system may not be the best way to see subtle differences in these mutants and that in the future stable expression of point mutants under the endogenous promoter may be more physiologic and revealing.

DETERMINANTS OF p53 GENE-SPECIFIC REGULATION

The mechanisms that control cell fate remain unclear. Given the number of target genes that fall into the categories of growth arrest or cell suicide you would think that a clear answer would emerge. The simplest explanation to account for differences in expression would be that there are low affinity and high affinity sites for p53 binding and that the levels of p53 determine the order of activation. Many genes from both camps are induced to similar levels whether cells are undergoing growth arrest or apoptosis. In most cases the answer is likely to be more complicated involving an interplay of factors that are cell-type or damage-specific. However, a satisfying example of differential p53 binding is supported in the case of Perp expression in E1A-MEFs undergoing apoptosis [Reczek et al., 2003]. *PERP*, a p53-dependent target gene identified by subtractive hybridization, is expressed in high levels in E1A-MEF cells undergoing apoptosis. When treated with DNA damaging doxorubicin, wild-type mouse embryo fibroblasts (MEF) cells undergo G1 arrest whereas E1A-MEF cells (MEF cells expressing the adenovirus E1A oncogene) cells

are sensitized to apoptose. Using ChIP analysis it was shown that the Perp promoter is occupied by p53 only under the circumstances in which DNA damage induces apoptosis in the E1A-MEF cells, and not in the growth arrested MEFs. The 5′ site in the p21 promoter is occupied during both growth arrest and apoptosis.

As usual, family members emerge to complicate the story. Homologs of p53 that include p63, p73, their isoforms and alternatively spliced forms have been identified that have their own unique and overlapping functions with p53. Indeed it has been shown that they can transactivate p53 target genes and may be required for the binding of p53 to apoptotic target genes [Flores et al., 2002]. Following DNA damage to E1A-MEF cells, p63 was found associated with the Perp promoter, and the loss of p63 and p73 resulted in the loss of p53 binding to the promoter and the inability for these cells to undergo apoptosis. p63 and p73 were not required for the binding of p53 to the p21 and mdm2 promoters or induction of these promoters. Although the mechanism is still unclear these observations suggest that selective transactivation of p53 targets may be influenced by the expression of p53 homologs.

Missense and deletion mutants have been used as tools to analyze differential site selection of p53 target genes. A tumor-derived mutant Ala143 is transcriptionally inactive at 37°C. However when down-shifted to 32°C, p53Ala143 is able to activate several physiologically relevant promoters including GADD45, Cyclin G, p21, and mdm2 but is defective in transactivating a reporter construct containing the bax promoter [Friedlander et al., 1996] and more recently the Perp promoter [Reczek et al., 2003]. That conformation may be important for target site selection is exemplified by the use of 181L and 175P. Although these tumor-derived mutants retain the ability to transactivate the cell cycle arrest gene, p21 WAF/CIP, they are unable to activate apopototic-associated genes like bax and IGF-BP3 [Ludwig et al., 1996]. A different phenotype is observed using 121F. This mutant p53 shows an increased ability to induce apoptosis while failing to activate MDM2 [Saller et al., 1999].

A small but growing body of evidence suggests that certain modifications of p53 can alter the choice of response elements (Fig. 3). Therefore, subtle differences in the p53 target sites may in fact make a difference in the physiologic outcome following genotoxic stress. An example of how phosphorylation may affect target site selection is seen in the case of Ser46. DNA damage by UV radiation results in the selective phosphorylation of Ser46 by the serine/threonine kinase homeodomain-interacting protein kinase-2 (HIPK2) [D'Orazi et al., 2002; Hofmann et al., 2002]. The authors demonstrate that after damage p53 co-localizes with HIPK2 and CBP to promyelocytic leukemia (PML) nuclear bodies. Binding to HIPK2 facilitates CBP acetylation of Lys382 and results in increased expression of target genes and growth arrest. A sublethal dose of UV will alter the outcome to apoptosis suggesting that additional modification may be required. The UV-induced apoptosis is prevented by the use of anti-sense oligonucleotides. Overexpression of *p53DINP1*, a p53 inducible gene, in combination with DNA

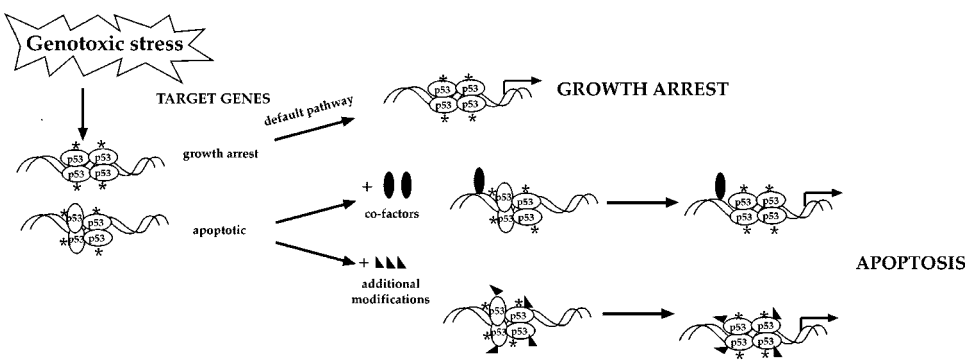

Fig. 3. A model for the differential activation of growth arrest and apoptotic target genes. A scenario for differential expression of target genes involves at least two different groups of genes. One set of genes will be turned on regardless of the particular stimuli. These genes may have a very high affinity for p53 and may not require additional modifications or cofactors. A second set of genes may require very high levels of p53 and may be dependent on cell type-specific modifications or cofactors.

damage by double strand breaks enhanced phosphorylation at Ser46 [Okamura et al., 2001]. Concomitant transactivation of p53AIP1 was followed by cell death. An anti-sense oligonucleotide to p53DINP1 is able to block both the phosphorylation of Ser46 and the induction of p53AIP1. It is likely that p53DINP1 is a cofactor for the kinase that phosphorylates Ser46, which is regulatory for the transcriptional activation of p53AIP1. p53AIP1 [Oda et al., 2000] a protein located in the mitochodrial membrane can act as a mediator of p53-dependent apoptosis by dissipating the membrane potential. Ectopic expression of AIP1 results in apoptotic death even though the transactivation of p21 remains unchanged.

p53 interacting proteins like ASPP1 and ASPP2 may contribute to site selectivity by enhancing the activation of apoptotic rather than growth arrest target genes [Iwabuchi et al., 1998; Samuels-Lev et al., 2001]. In vitro studies have shown that ASPP2 can enhance the DNA binding and transactivation activity on proapoptotic promoters such as bax and pig-3 without significantly affecting binding to mdm2, cyclin G, and p21 promoters. Introduction of ASPP1 or ASPP2 into wild-type p53 containing MCF7 breast carcinoma cells or U2OS osteosarcoma cells induced apoptosis. Targeting p53 for degradation by expressing the viral oncoprotein HPV E6 protein or the inhibition of endogenous ASPP using anti-sense RNA can suppress the apoptotic function of endogenous p53 in response to cisplatin. These observations suggest that growth arrest and apoptotic functions are separable. This hypothesis is supported by a p53 mutant, P98A that is non-responsive to ASPP 1 or 2. It has been shown that this mutant was unable to induce PIG-3, was deficient in apoptosis, yet showed no defect in growth arrest. The importance of the ASPP1 and ASPP2 protein is supported by the observation that in breast carcinoma cells that are wild-type for p53, expression of ASPP proteins is often downregulated.

APPROACHES TO p53 THERAPY

Novel approaches to restore the wild-type activity of p53 differ greatly from the classic chemotherapy approach. The promise of the future lies in the amazing specificity that is offered by the use of small molecules, peptides or siRNA. By knowing the defect in either p53 or other players in the DNA damage pathway one can customize the treatment of tumors (Fig. 4). Is the mutation monoallelic or is there a loss of heterozygosity? If the p53 is wild-type is it being degraded by Mdm2 or HPV E6 associated protein? It may be that p53 is sequestered in the cytoplasm and cannot get to the nucleus. Lastly, can we control the outcome of growth arrest or apoptosis or is that a decision predetermined by the cellular environment or the cell type?

More than one half of all human tumors contain mutations in the DNA binding region of p53. Unlike many other tumor suppressor genes in which the protein is deleted or truncated mutant p53 is often shown to be complexed with chaperones in the nucleus supporting the idea that it is a conformationally flexible protein. A number of strategies aim to restore a wild-type function by reactivation of the DNA binding ability of the protein (for a review see [Lane and Hupp, 2003]). Early attempts were based on microinjection experiments using monoclonal antibody PAb 421. This well-known monoclonal antibody binds to an epitope in the C-terminus of p53 and is thought to induce a conformational change which can result in the conversion of a latent to an active form of p53. Microinjection successfully restored the sequence-specific DNA binding of some p53 mutants in vitro. However, antibody treatment remains impractical because it is not easy to get the antibodies into the cell. Using a vector that expresses single chain antibody fragments (scFvs) of PAb421, Caron de Fromentel et al. [1999] present a viable alternative. They are able to show that expression of the single chain PAb421 in three different tumor cell lines containing the His 273 mutation, is able to reactivate the endogenous p53 transactivation function.

A class of small molecules (CP31398) have been identified by high throughput screening that restore a conformation epitope associated with the wild-type protein [Wang et al., 2003]. PRIMA, another small molecule was discovered in an in vivo assay that screened for transcriptional activation [Bykov et al., 2002]. One drawback to the chaperone approach is that the mechanism remains unclear. Another approach has been to develop small peptides that can stimulate the DNA binding activity of wild-type p53. The result of those efforts was the synthesis of a nine-residue peptide, CDB3, which was modeled on BP2, a protein that binds

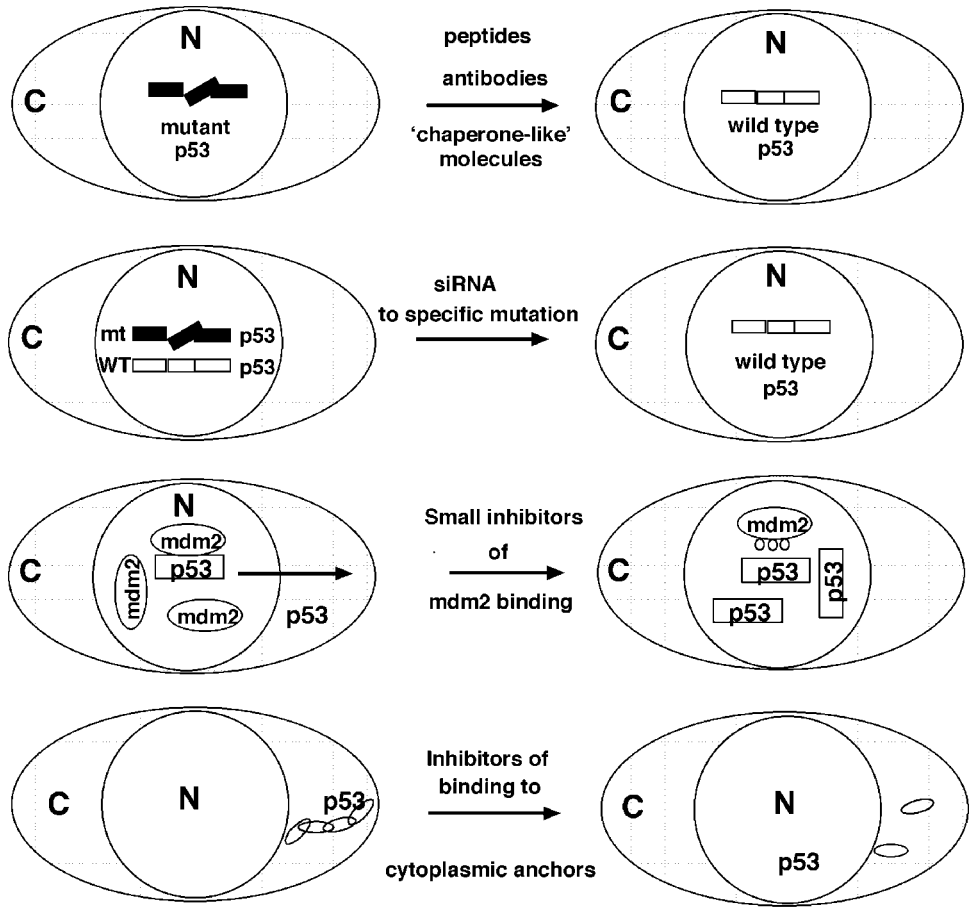

Fig. 4. Strategies for the restoration of wild-type p53 activity. A number of different mechanisms for p53 inactivation in tumors have been identified. Some strategies for restoration are aimed at mutant p53s that are defective for DNA binding and functional wild-type p53 that is being degraded by amplified Mdm2 or is sequestered in the cytoplasm. (1) Conformational. Using 'chaperone'-like small molecules, single chain antibody fragments and small peptides a wild-type conformation can be conferred on some mutant p53 proteins. This correction of conformation restores the binding to p53 response elements. (2) Downregulation of dominant negative p53 in cells that contain one wild-type and one mutant allele. Using siRNA that is specific to a point mutation in p53, the mutant form of p53 and its dominant negative effect on the wild-type p53 can be eliminated. Despite the dosage effect, p53 is functional and results in the activation of growth arrest and apoptotic targets. (3) Inhibition of the p53 and Mdm2 interaction. Small molecules (Nutlins) that interact with the Mdm2 pocket to which p53 binds can be given in cases where Mdm2 is amplified and as a result the p53 is being continually degraded. The prevention of this interaction results in an apoptotic response in tumor cells and a temporary growth arrest in normal cells. (4) Release of p53 that is sequestered in the cytoplasm. In some tumors that are wild-type for p53, the protein is unable to localize to the nucleus. In neuroblastomas, some breast carcinoma cells, and teratocarcinomas, p53 may be anchored in the cytoplasm by the mot-2 protein. The restoration of p53 function is based on the use of inhibitors that release p53 from their anchors permitting translocation to the nucleus.

to the core domain of p53 and stabilizes it in vitro. CDB3 was able to restore the sequence-specific binding activity to I195T. Although the mechanism remains hypothetical, it appears to act by stabilizing the active conformation by binding to the edge of its DNA-binding site.

The power of synthetic small inhibiting RNAs (siRNAs) is being exploited in the lab and promises to be a selective tool for tailoring anti-tumor therapy. It has been reported that a single base difference placed right in the middle of an siRNA molecule can discriminate between mutant and wild-type p53 [Martinez et al., 2002]. If you could target monoallelic mutations in p53 such as those seen in Li-Fraumeni families it would form the basis for eliminating mutant p53 proteins and restoring only wild-type expression. Additionally, it would eliminate any gain of function activity and reduce the interaction that mutant proteins might have with regulatory cofactors. The authors show that siRNA to mutant p53 is highly transfectable (>90%), highly selective, and is very efficient at reducing the levels of endogenous mutant

protein. In vitro experiments also showed that concomitant with repression of the mutant p53 is the induction of both a p53-responsive reporter construct and the endogenous p21 protein.

Other strategies focus on tumors containing wild-type p53 that is sequestered in the cytoplasm. These include neuroblastomas, breast carcinomas, and teratocarcinomas. One report demonstrated that in tumor cells in which sequestration is due to retention by mot-2, an hsp70 family protein, a cationic rhodacyanine dye analog called MKT-077 is able to abrogate the interaction with p53 [Wadhwa et al., 2000]. Translocation to the nucleus resulted in restoration of transcriptional activation and a selective growth arrest in the tumor cells and not the normal cells. The selective toxicity of this agent is enhanced or perhaps complicated by a second p53-independent phenomenon. Cancer cells have a higher mitochondrial membrane potential which accounts for the preferential retention of MKT-077 in the mitochondria and hence its toxicity.

An exciting approach has focused in on the site of interaction between p53 and its negative regulatory partner, MDM2 [Vassilev et al., 2004]. Small synthetic compounds (Nutlins) were identified that were able to displace p53 from its pocket in MDM2 protein. This would be of great consequence to tumor cells that contain wild-type p53 and amplified MDM2. Indeed these compounds were shown to increase the levels of p53, activate p53 regulated genes and cause cell cycle arrest and apoptosis only in tumor cells that were wild-type for p53. What seems remarkable is that in animal xenograft experiments in nude mice, there appeared to be little toxicity to the normal tissue whereas growth of the tumors was suppressed compared to the control animals. It is provocative to consider that p53 signaling in the cancer cells is inherently different from normal cells resulting in the observed different outcomes. This hypothesis is supported by the finding that p14ARF does not negatively regulate Mdm2 in normal cells as in tumor cells. If these results speak to the issue of latency they would suggest that at least in some tumors it is not an issue and simply raising the levels of p53 are sufficient to induce an apoptotic response. The differential outcome in normal cells and cancer cells is compelling. Identification of factors that contribute to the activation of pro-apototic targets would be of critical importance. Such findings will provide a deeper understanding of the cancer cell and afford new possibilities for treatment.

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