Importance of p53 for cancer onset and therapy

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Cancer predisposition, onset and therapeutic response can be critically determined by the integrity of the tumor suppressor p53. The majority of human cancers appear to exhibit either abnormal p53 or disrupted p53 activation pathways. Intervention to restore wild-type p53 activities is an attractive approach for cancer therapy. The manipulation of p53 and its targets is a challenging field that is still in its infancy, but witnessing some notable developments in the areas of p53 gene therapy, mutant reactivation and suppression of the negative p53 regulator Mdm2 using small molecules. In addition, wild-type p53 manipulation in healthy tissues of cancer patients in the context of chemotherapy and radiation therapies is offering the potential of enhanced patient recovery. *Anti-Cancer Drugs* 17:725–732 © 2006 Lippincott Williams & Wilkins.

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Introduction

In the normal cell, p53 is a vital, hypersensitive perceiver of various cellular stresses that reacts by coordinating a plethora of response targets to minimize damage. p53 may promote a pause in a cell's normal cell cycle progression to allow damage repair (a short-term arrest); when damage is more severe, it may induce a permanent arrest of cell division, (senescence), or the termination of cellular activity (apoptosis) when the damage is insurmountable. p53 exerts its effect through both transcriptionally-dependent and -independent mechanisms ([1] and references within).

In the context of this central role for p53 in dictating appropriate cell stress responses, it is not surprising therefore that aberrant p53 is unable to elicit appropriate stress responses. Cancer onset is a critical consequence of p53 malfunction [2]. p53 has been demonstrated to function as a tumor suppressor in multiple animal models [3].

The vital importance of wild-type (wt) p53 as an active tumor suppressor spawned the concept that reinstating wt p53 functions in cancer cells may be of therapeutic potential. In the context of the multiple steps involved in cancer progression, targeting this single entity would seem to be an over-simplistic notion. Some very promising advances, however, have been achieved towards cancer therapy by targeting p53 and its pathways. In the light of recent advances in this field, the material that we will overview is largely focused on innovations that have been described in 2004–2005, with reference to foundation studies for clarity and citation of reviews for background [4,5]. As a result of space limitations, many original important studies have not been cited directly, but rather through recent reviews (Fig. 1).

Influence of p53 structure on its tumor suppression function

p53 activities are acutely related to its structure and even subtle polymorphisms at the level of a single nucleotide (single nucleotide polymorphisms) may exert a profound effect on its performance. The p53 polymorphism at residue 72 has been identified to influence its antitumor function (and consequently cancer predisposition) and sensitivity to cancer therapy. It is now appreciated that the presence of an arginine at codon 72 (72R) as opposed to a proline (72P) confers to wt p53 the capacity to more efficiently induce genes associated with apoptosis (*puma* and *noxa*) and, consequently, exert a superior apoptosis-inducing activity in response to anticancer agents. In the presence of wt p53 72P, G_1 arrest predominates over an apoptotic response. Specifically, in a clinical study of cisplatin-based chemoradiotherapy (that also contained 5-fluorouracil and taxol) in advanced head and neck cancer patients, the highest levels of overall survival and progression-free survival were monitored in the context of a wt p53 72R allele [6].

In addition to these minor sequence variations, p53 protein isoforms that are either transcribed from an alternative promoter (located in intron 4) or generated by alternative splicing have recently been identified. Their expression appears to be tissue-type dependent in

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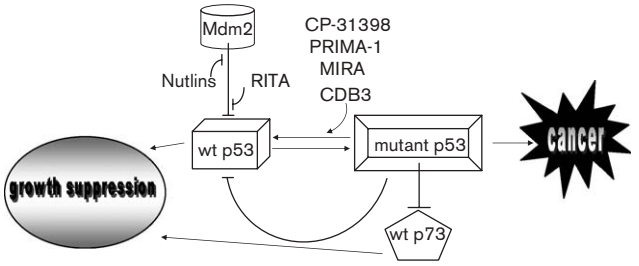
Fig. 1



Activated wild-type (wt) p53 can respond to certain stresses by inducing growth suppression (cell cycle arrest, senescence or programmed cell death). Mutant p53 in contrast can promote the evasion of this response, in part by suppressing wt p53 and p73 activities, and induce cancer onset. The wt p53 and p53 mutants exist in an equilibrium that can be shifted towards a wt state with CDB3. Mutant p53 can be reactivated to perform wt functions using small molecular reactivating molecules such as PRIMA-1 and CP-31398. The wt p53 can be activated by relieving it from the suppression of its negative regulator Mdm2, by preventing their interaction using agents directed towards either Mdm2 (Nutlins) or wt p53 (RITA).

healthy tissues. In breast tissue, the profile of p53 isoforms is distinct in healthy and cancerous tissues. Notably, in breast tumors an N-terminally truncated p53 isoform (δ133p53) was frequently identified to be overexpressed, but was absent from normal breast cells. Strikingly, this p53 isoform curbs p53-mediated apoptosis (through a 'dominant negative inhibition' of wt p53) [7].

Further, in at least 50% of sporadic cancers a profound disruption of p53 integrity is affected by mutation [8]. The inheritance of a mutated p53 allele is common in the inherited Li–Fraumeni cancer syndrome [9]. Significantly, wt p53 is rarely abolished in cancers through gene deletion, but is frequently subjected to missense mutation. p53 is not randomly mutated but rather 80% of its mutations are confined to its DNA binding domain in 'hot spot' zones (residues 110–290: www.iarc.fr/p53/index.html). The two major forms of p53 mutations are DNA binding and structural mutants. These mutations disrupt the contact between p53 and its DNA consensus sequences [two repeats of PuPuPuC(A/T)(A/T)G GyGyGy; in which Pu is purine and Py is a pyrimidine] (reviewed in [10]).

The mutation of a single p53 allele frequently accompanies cancer onset and a loss of heterozygosity accompanies tumor progression. Certain mutations in one allele are sufficient to incapacitate the product of the wt allele through a dominant negative action. The strong selection for p53 mutations has been interpreted to be of benefit to tumor progression. Importantly, some *p53* mutations generate modified proteins with the acquired capacity to promote tumor development through a 'gain of function' and not merely a loss of function (e.g. [11]). Mutant p53 gain-of-function appears to be mediated through its capacity to bind and incapacitate other members of its family, namely p73 and p63 (reviewed in [12]).

p73 and p63 share a significant sequence homology with p53, and are also found in multiple isoforms; however, in contrast to p53, these family members are rarely mutated [13]. Pertinently, p73 influences cellular chemosensitivity and is induced by numerous chemotherapies [14]. An isoform of 73 (TAp73) is able to substitute for p53 in the transcriptional activation of a number of p53 targets [15]. Blocking p73 function in human tumor cells may lead to chemoresistance. Mutant p53 was demonstrated *in vivo* to bind and inactivate p73, and, conversely, down-regulation of mutant p53 enhanced chemosensitivity [16]. Whole animal studies added a new dimension to these findings, with the suggestion that tumorigenesis is critically determined by the ability of mutant p53 to inhibit the transcriptional activities of wt p63 and wt p73. This disruptive capacity of mutant p53 has been suggested to be a possible explanation for the extremely rare mutation of these family members (reviewed by Iwakuma *et al.* [12]).

An interesting detail in this context is the observation that the polymorphism of p53 at codon 72 (Arg or Pro) appears to affect the interaction of mutant p53 with p73. Specifically, mutant p53 with 72R is a more potent inhibitor of chemotherapy-induced apoptosis than the counterpart p53 with 72P (the antithesis of apoptosis sensitivity in wt p53), suggesting that the p53 codon 72 may have a significant impact on the clinical response to chemotherapy [14].

While the p53 structure may be aberrant in a significant proportion of cancers, it has been suggested that p53 pathways are aberrant in all cancers [2]. Specifically, p53 may be maintained in a wt form, but functionally inactive or 'silenced', either because it is inhibited directly or because its pathways of activation are disrupted. The overexpression of Mdm2, which is the major inhibitor of p53, effectively diminishes p53 levels, simulating a p53 null scenario, or inhibiting p53 activities, as associated with a number of cancers [17]. Other p53 targets may also become deregulated and incapacitate the proper signaling from p53, and these have also been associated with cancer (as discussed below in the context of Slug and Puma).

Wild-type p53-based therapies

Gene therapy

The harnessing of p53 into gene therapy has led to 58 clinical trials using recombinant adenovirus (the common cold virus) encoding human p53 (rAD-p53) to treat more than 20 different kinds of cancer (reviewed by Peng

[18]). Although the results of these trials have not all been encouraging (reviewed by Vecil and Lang [19] and Lo *et al.* [20]), the treatment of squamous cell carcinoma of the head and neck (the second most common skin cancer after basal cell carcinoma) has advanced into clinical phase III trials in the US (INGN 201/ADVEXIN; Introgen Therapeutics, Austin, Texas, USA) and, in 2003, it was approved by the State Food and Drug Administration of China. Consequently, in 2004, the world's first gene therapy product, the injectable, recombinant, human adenovirus p53 therapy (trademarked as Gendicine; developed by Shenzen SiBiono GeneTech, SiBiono, Shenzhen, China) was launched in China for the treatment of head and neck squamous cell carcinoma. Gendicine introduces wt p53 into cells in a replication incompetent adenoviral vector that is infectious for only a single cycle (because of a deletion in its E1 region) and does not integrate into the host genome. The virus has been well tolerated in more than 2500 patients treated with the therapy. The antitumor effect of rAD-p53 appears to be contributed by direct apoptosis induction, activation of an immune response, down-regulation of a number of tumor promoting genes (including the genes encoding multi-drug resistance, vascular endothelial growth factor and matrix metalloproteinase), transcriptional inhibition of survival signals and the limitation of glucose uptake.

In combination with the conventional chemotherapy and radiotherapies, gendicine was observed to induce significant synergism with reduced side-effects. Specifically, in a phase II clinical trial for head and neck squamous cell carcinoma, 3 months of treatment with gendicine (a single injection per week) plus radiotherapy resulted in the complete regression of 64% of patients' tumors and partial regression of 32% (93% total response rate); while those receiving radiotherapy alone showed 19% complete regression and 60% partial regression (79% total response rate).

Gendicine is currently being evaluated in clinical trials in China for efficacy in a number of other cancers (including ovarian, non-small cell lung, advanced liver, advanced lung and a number of other solid tumors) [18]. Similarly, the gene therapy product of Introgen is being subjected to extensive clinical trials (reviewed in [20]).

A novel approach to p53 gene therapy using chemotherapy priming has been devised for the treatment of human non-small cell lung cancer (and although it has not been studied beyond animal models, its broader potential merited its inclusion in this discussion). The wt p53 has been introduced into an adenoviral vector in the context of upstream CArG elements from the early growth response-1 (*Egr-1*) promoter that are sensitive to DNA damage and reactive oxygen intermediates. Importantly, cisplatin causes DNA damage and reactive oxygen intermediates. The combined application of cisplatin and this virally infected inducible p53 has demonstrated efficacy in xenografts in nude mice, in which tumor volume was significantly reduced. The suggested applications of such a targeted gene expression include common human neoplasms that are exposed and visible, but poorly treated by conventional therapies. Such tumors could be infused or injected, with improved accuracy of administration being offered with positron emission tomography [21].

Protein transduction therapy using stabilized p53

Protein transduction therapy is under investigation as an alternative approach to gene therapy. The major obstacles to this method are the instability of the potentially therapeutic proteins or peptides and their limited cell penetrance. Preliminary studies with wt p53 were restricted by their rapid degradation in cancer cells [22]. A novel approach for therapy has been prompted by the appreciation of the importance of the p53 C-terminal lysines in its Mdm2-mediated ubiquitination and proteasomal degradation. Substitution of C-terminal lysines (370, 372, 373, 381, 382 and 386) with arginines generated a p53 mutant that is resistant to ubiquitin–proteasome-mediated degradation, with a higher transcriptional activity than wt p53 [23] (where the latter is likely to be favored by the relief of non-specific binding between the non-modified C-terminus and the core domain [24,25]). In a recent study, multiple C-terminal lysines were substituted to convey the combined properties of stability and cell penetrance [26] (a phenomenon conferred by polyarginines as demonstrated for p53 previously [22]). When a p53 mutant bearing six arginines in place of lysines (379, 373, 373, 381, 382 and 386) was transduced *in vitro* into glioma cells lacking wt p53, the mutant protein resisted Mdm2-mediated ubiquitination and inhibited cell proliferation. The p53 target p21 was elevated significantly in response to exposure. In contrast, the growth of primary astrocytes was not inhibited *in vitro* [26].

Relief of p53 from its major negative regulator Mdm2

In approximately 50% of tumor cells, p53 is not mutated but is rather tolerated through indirect inactivation. Overexpression of the major negative regulator of p53, Mdm2 (or Hdm2 in humans; Mdm2 will be used throughout the text without distinction) is one approach adopted in some tumors for disabling wt p53 [17]. The most potent small molecules that have been reported to disrupt the p53–Mdm2 binding include the *cis*-imidazoline derivatives (Nutlins) [27] and reactivation of p53 and induction of tumor cell apoptosis (RITA); 2,5-bis(5-hydroxymethyl-2-thienyl)furan (NSC652287) [28]. A different approach to disable Mdm2 is to inhibit its E3

ligase activity towards p53. The potential of these molecules are elaborated below. A number of lead compounds have been identified with this capacity with the potential to serve as leads for drug discovery targeting this aspect of the p53–Mdm2 interaction as well [29,30].

Nutlins

Nutlins are a synthetic class of *cis*-imidazoline derivatives that fit into and fill the p53-binding pocket on Mdm2 and displace p53 [27]. Nutlins were characterized for their efficacy in enhancing the expression of the p53 target genes *Mdm2* and *p21*, inhibiting cell cycle progression and triggering apoptosis in-vitro cultured cells. Nutlin-3 potency *in vivo* was elegantly demonstrated in tumor-transplanted mice orally fed [200 mg/kg twice a day for 20 days, achieving plasma levels in excess of the *in vitro* determined inhibitory concentration 90% (IC_{90}) value of 3.5 μmol/l], in which a 90% inhibition of tumor growth was recorded, without apparent side-effects ([31]; reviewed by Klein [32]).

In acute myeloid leukemia, in which p53 is rarely mutated but rather kept suppressed through the over-expression of Mdm2, Nutlin-3a has been demonstrated to induce apoptosis in primary acute myeloid leukemia samples. Apoptosis induction corresponds to the transcriptional activation of pro-apoptotic Bcl-2 family proteins and mitochondrial permeabilization triggered through transcription-independent mitochondrial p53 translocation. Furthermore, Nutlin-3a contributed synergistically to the cytotoxicity of the chemotherapeutic agents doxorubicin and cytosine arabinoside in acute myeloid leukemia blasts, without detriment to the normal hematopoietic progenitor cells [33].

Further studies have demonstrated that wt p53-bearing cells are prompted to arrest in G_1 and G_2 phases in response to Nutlin treatment. An important application of this phenomenon was suggested in the context of protecting normal cells from paclitaxel-induced mitotic block and apoptosis during therapy. Specifically, pre-treatment with Nutlins, followed by paclitaxel exposure, protected normal fibroblasts from cytotoxicity, while cells bearing mutant p53 underwent mitotic arrest and massive apoptosis [34]. The repertoire of potential Nutlin applications is thus being expanded beyond the wt p53 context alone to include mutant p53.

Despite the very significant impact of these Nutlins, nuclear magnetic resonance modeling of the interaction between p53 and Mdm2 has also offered some important cautionary clauses to the design of inhibitors of the p53–Mdm2 interaction. Sequence alignment data indicate that a drug able to bind Mdm2 and inhibit its interaction with p53 might also have an impact on the interaction of Mdm2 with its other partners p73 and E2F1, owing to sequence similarity at the DNA binding site [35].

Reactivation of p53 and induction of tumor cell apoptosis

RITA is a small molecular weight molecule selected for its capacity to promote apoptosis in cancer cells harboring wt p53. RITA was demonstrated to diminish the interaction between p53 and its major negative regulator Mdm2. Initial fluorescence correlation spectroscopy studies indicated that RITA bound to the p53 N-terminus and the identified activation of p53 was thus proposed to involve RITA binding to the p53 N-terminus which leads to relief from Mdm2 [28]. The mode of action of RITA has been questioned in the context of nuclear magnetic resonance studies, in which core binding was insinuated [36]. The precise mode of RITA's action remains to be resolved, however, as controversy surrounds the influence of p53 preparation on the studies undertaken [37].

Therapies directed towards mutant p53

The mutation of p53 predominantly involves missense coding in the central core DNA binding domain, at preferred 'hot spots'. These mutations impair the proper interaction of p53 with its responsive elements either by substituting residues that interact directly with DNA, and are termed DNA contact mutants (e.g. R273H, R248W), or by introducing residues that disrupt the exposure of the DNA binding interface and reduce its thermal stability, and are referred to as structural mutants (e.g. R249S, R282W, R175H, G245S) [10].

Activation of mutant p53 using small molecules

Three prototype molecules have been described that are able to promote DNA binding by mutant p53. The mode of their action has become the subject of many studies and while the net result on the p53 mutants may be similar, the means of invoking this induction appear distinct. Importantly, these molecules appear to stabilize both mutant and wt p53; however, the high levels of reactivated mutant p53 appear to be the critical determinant of apoptosis induction.

CP-31398

CP-31398 is a fascinating example of a molecule with clear p53 activating capacity, whose mode of action is still being defined. This styryl quinazoline is a small synthetic molecule that was selected from the Pfizer drug library for its ability to stabilize p53 against thermal denaturation *in vitro* [38]. CP-31398 was demonstrated to stabilize and activate both wt p53 [39] and mutant p53, without influencing the DNA-binding activity of the p53 family members p63 and p73 [40]. Although CP-31398 can act as a DNA intercalator [41], this DNA-damaging effect does not induce phosphorylation of Ser15 and 20, and consequently DNA damage has been discounted as the primary mode of its influence on p53 stabilization [39]. Importantly, while CP-31398 does not interfere with the interaction between p53 and Mdm2, it does, however,

protect p53 from degradation by inhibiting Mdm2 mediated ubiquitination. Further, despite this continued association between p53 and Mdm2, high levels of transcriptionally active p53 accumulate in response to CP-31398 treatment inducing expression of p21, and members of the mitochondrial extrinsic [41] and intrinsic death pathways [42,43].

CP-31398 does not bind the core domain of recombinant wt or mutant p53 *in vitro* [44]; however, it can restore DNA binding to p53 mutant core domains (p53 R273H and R249S). It has been proposed that CP-31398 binds p53 [40], possibly to its tetrameric form, at a site away from the protein–DNA interface. Through allosteric effects it promotes the formation of a stable p53 conformation capable of binding DNA (reviewed in [40,45]). A model whereby CP-31398 locks p53 in a tetrameric active form is yet to be demonstrated.

Further studies suggest that CP-31398 can induce death via two pathways, one that is affected early and involves newly synthesized p53, and a second that is p53 independent, requires calcium release and is affected later. It is likely that future drug designs based on CP-31398 will attempt to develop a modification that results in the stimulation of p53-dependent pathways exclusively [46].

In nude mice, the growth of human melanoma and colon carcinoma cells bearing p53 mutants, p53Arg249Ser and p53Ser241Phe, was inhibited by CP-31398, with no noted toxicity [38]. Importantly, further studies of melanoma cell lines have defined that while CP-31398 is able to promote cell death in lines bearing either wt p53 or a single point mutation, the drug is ineffective on cells bearing multiple p53 mutations [43]. These results emphasize the importance of the p53 background in a tumor for determining the likelihood of a response to therapy.

The p53 reactivation and induction of massive apoptosis-1 and its derivatives

PRIMA (p53 reactivation and induction of massive apoptosis)-1 is a small molecular weight compound that is cytotoxic to cell lines expressing both DNA or contact p53 mutants [47]. PRIMA imposes growth suppression preferentially in tumor cell lines harboring mutant p53 as compared with those with wt p53 [48]. PRIMA-1 exerts its growth inhibition through the coercion of mutant p53 to transcriptionally activate the p53 targets *mdm2* and *p21*. Further, PRIMA displayed an antitumor effect in mouse experiments, without detected toxicity [47].

A methylated version of PRIMA-1 (PRIMA-1^{MET}) was selected for its enhanced potency and identified to up-regulate the expression of the BH3-only pro-apoptotic factor *puma*. It was demonstrated to act synergistically with a number of chemotherapeutic agents [the DNA alkylator cisplatin, the topoisomerase (Topo) I inhibitor camptothecin, the Topo II inhibitor adriamycin; and drugs that influence RNA/DNA synthesis, 5-fluorouracil; cytoskeleton polymerization, paclitaxel and cytoskeleton depolymerization], both *in vitro* and in xenografts in nude mice. Interestingly, PRIMA-1 efficacy appears to be greatest when the second drug induces the elevation of mutant p53 levels. [49]. This finding is consistent with PRIMA-1 efficiency correlating with p53 expression levels [48].

Other small molecules have been synthesized that are based on PRIMA-1 with respect to the arrangement of its functional groups, but in the context of a more malleable frame than can be modified to generate analogs. These analogs have allowed the definition of the important functional components of PRIMA-1 (namely its phenyl group and primary amine) [50]. While the precise mode of mutant reactivation remains to be defined, one possible role for PRIMA-1 has been suggested to involve facilitating the interaction of mutant p53 with heat shock protein 90α (HSP-90α), which is then translocated into the nucleus in which transcriptional activation of p53 targets can occur [51].

Maleimide-derived molecule MIRA-1 (1-propoxymethyl)-maleimide

The maleimide-derived molecule MIRA-1 (1-propoxy-methyl)-maleimide (MIRA) drug series are maleimide-based compounds selected for their capacity to engender mutant p53 with the capacity to transcriptionally activate wt p53 targets and induce cell death. Active compounds are distinguished by a common 3–4 double bond in the maleimide group, which prompted the suggestion that interactions between these reactive double bonds and cysteines and lysines of p53 may promote its stabilization through alkylation. They are more potent than PRIMA-1 and their kinetics of death induction are distinct, with (1-propoxy-methyl)-maleimide (MIRA-1) promoting death within 6–12 h and PRIMA-1 after 24–48 h. These compounds are thus proposed to act via distinct mechanisms that remain to be characterized.

MIRA-1 has not universally been able to reactivate DNA binding in the mutants evaluated and compounding mutations in addition to the classic hot spot substitutions may influence its potency in some instances (such as SW480 bearing mutations at residues 273 and 309), which indicates its specificity, but also its limitations *in vivo* for tumors bearing more than the target mutation [52] (reminiscent of the limitations described for CP-31398 [43]).

Core domain binding 3

The discovery that p53 structural mutants exist in equilibrium with their wt (native) form prompted Alan

Fersht and his team to design an approach for shifting the equilibrium away from the denatured form towards the more thermostable, correctly folded form [10]. The candidate molecule named core domain binding 3 (CDB3) that was selected for this purpose is a nine-residue peptide fragment of a partner that naturally interacts with p53, the p53-binding protein 2 (53BP2 or ASPP) [53]. CDB3 was proposed to act as a chaperone that is able to interact with and thermostabilize newly synthesized p53 and its mutants to allow proper folding and promote its interaction with DNA [53,54]. CDB3 (modified with a fluorescent tag) was demonstrated to promote the proper native folding of p53R273H contact and p53R175H structural mutants, induce p53-dependent expression of the target genes *mdm2*, *gadd45* and *p21*, and stimulate apoptosis. Importantly, treatment with CDB3 alone did not induce apoptosis in wt p53- bearing cells, but increased their sensitivity to immunoreactivity-induced apoptosis [55]. In addition, the missense mutant R249S (prominent in liver carcinoma provoked by aflatoxin consumption) was also submissive to structural correction through association with FL-CDB3 [56].

Selective depletion of mutant p53 by using histone deacetylase inhibitors

The histone deacetylase (HDAC) inhibitors FR901228 and trichostatin A have been characterized for their remarkable cytotoxicity in cells with mutant p53, without significant detriment to those lacking p53 and with far less impact on cells bearing wt p53 than predicted. This tolerance of HDAC inhibitors in wt p53 cells was surprising as the transactivation functions of wt p53 are inhibited by HDACs. The unexpected cytotoxicity in cells with mutant p53 is preceded by the activation of the wt p53 targets Mdm2 and p21, which appear to be instrumental in the subsequent complete diminution of mutant p53 levels. Although not formally proven, it has been speculated that these mutants undergo structural reformation to a wt configuration that enables the transcriptional activation of these wt p53 targets. These studies indicate that, in cancer cells, the functional reactivation of high levels of mutant p53 by HDAC inhibitors may be sufficient to offer selective cytotoxicity in the context of a normal cellular background. These observations offer a new dimension to the current clinical trials of the HDAC inhibitor FR901228 (FK228, depsipeptide) [57].

Manipulating p53 targets

A fascinating perspective on blood cancer has recently been contributed by an elegant delineation of the induction of p53 in the hematopoietic system. In committed blood cell lineages such as thymocytes, p53-mediated apoptosis has been identified to be critically mediated through the induction of *puma* [58,59]. In contrast, when blood progenitor cells are exposed to γ-irradiation they do not die, but rather apoptosis is inhibited by a second p53 target Slug, which suppresses the induction of *puma*. Importantly, while Slug is expressed in progenitor blood cells it is not expressed in normal mature blood cells. Strikingly, however, Slug elevation has been associated with a number of leukemias (reviewed and elaborated in [60]). The elucidation of this pathway has provoked the concept of manipulation of Slug for therapy. Unfortunately, animal studies indicate that Slug-initiated cancers become Slug independent with time and that the elimination of Slug in such cancers is inadequate to halt their development [61]. In this context, it is fascinating that Puma is receiving attention for its potential in gene therapy, as initially demonstrated in gliomas [62]. One may speculate that under the control of a relevant promoter, Puma gene therapy may also have the potential for treating leukemias, where *puma* is suppressed by Slug.

Suppression of p53 and its targets for healthy tissue protection during therapy

Conventional chemotherapy and radiotherapies are still routinely applied for cancer treatment, either as adjuvants to surgery or in their own right. Reliance on p53 to mediate death induction in response to these genotoxic treatments risks the well being of sensitive, normal highly proliferating cells, such as those of the bone marrow, hair and the intestinal epithelia. To allow a localized treatment and promote more rapid patient recovery the approach of healthy tissue 'sparing' has been proposed (reviewed in [63]). Gudkov and Komarova [64] proposed an approach to reversibly inhibit wt p53 activities in healthy tissue during chemotherapy and radiation therapy of tumors lacking functional p53 using the 'p53 inhibitor' or pifithrin-α. An alternative candidate was subsequently reported: 'peptide 14' that is a 22mer peptide derived from the p53 core domain (amino acid residues 105–126) that is also able to reversibly block p53-induced apoptosis and inhibit p53-induced transactivation [65].

In light of the new understanding of the role of the p53 targets Slug and Puma in determining the fate of both blood progenitors and mature cells, the concept of elevating Slug levels to protect blood cells in patients undergoing radiotherapy has been proposed. The success of these proposed protective approaches will be dictated by the capacity to selectively impose these measures on healthy cells while targeting the cancerous tissues for eradication.

Conclusion

The remarkable concentration of attention on the activities of p53 has spawned the birth of multiple novel approaches to cancer therapy. The individual peculiarities of each cancer and the context in which it develops are now recognized to dictate the response to any given therapy. Tailoring therapies to given cancer types is the

direction of the future, and p53 and its pathways are appealing targets for manipulation to achieve this aim. The emerging recognition of the interaction between p53 family members in dictating drug efficacy is likely to provoke new approaches to future drug designs.

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