p53-based cancer therapies: is defective p53 the Achilles heel of the tumor?

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The tumor suppressor protein p53 plays a pivotal role in the DNA damage response and is defective in >50% of human tumors, which has generated substantial interest in developing p53-targeted cancer therapies. Various therapeutic rationales targeting p53 are currently under investigation including attempts to both activate and inhibit p53. Elevation of p53 can be achieved by either reintroducing an exogenous p53 gene or by blocking its association with its negative regulator hDM2. An alternate approach involves reverting mutant p53 to its wild-type conformation. Inhibition of p53 activity can be achieved either by preventing p53-mediated gene expression or by inhibiting the mitochondrial pro-apoptotic interactions of p53. These approaches are based on the concept that activation of p53 in a tumor is cytotoxic while inhibition of p53 in normal cells will protect the patient. However, activation of p53 also induces cell cycle arrest that can protect most normal cells from DNA damage, and this is the reason why many p53-defective tumors are more sensitive to DNA damage. The development of cell cycle checkpoint inhibitors to abrogate DNA damage-induced arrest builds on this observation as p53-defective cells appear particularly sensitive. Thus, normal cells are protected from premature entry into mitosis and the subsequent mitotic catastrophe induced by checkpoint inhibitors, while p53-defective tumor cells are destroyed. These contradictory approaches must be resolved if we are to take full advantage of the frequent p53 defect in tumors.

The tumor suppressor protein p53 is activated in response to stressful stimuli such as DNA damage, hypoxia and oncogene activation. p53 is often referred to as the ‘guardian of the genome’ because it plays a key role in determining a cell’s fate following DNA damage. When DNA is damaged, p53 can trigger cell cycle arrest, senescence (permanent arrest) or apoptosis to eliminate the damaged cell. Cell cycle arrest provides time for repair of the damage thereby allowing the cell to recover and survive. The mechanisms governing whether a cell will arrest or die are not well understood, but these functions are essential to protecting an organism from the effects of aberrant cell divisions, and defects can lead to cancer development and progression. The fact that p53 is defective in >50% of tumors supports the assertion that p53 is an important player in the prevention of tumor development. The pivotal role of p53 in regulating the decision of a cell to live or die makes it an attractive target for cancer therapeutics. However, there are many conflicting ideas and approaches as to which would be the best therapeutic strategy to pursue. Initial attempts were based on the premise that reintroduction or activation of p53 would induce apoptosis in the tumor. Other approaches are based on the observation that cells with defective p53 are more sensitive to certain drugs and combinations. Finally, we are confronted with the confounding situation wherein approaches to both activate or inhibit p53 have been proposed to enhance the therapeutic index of DNA damaging drugs and radiation. This review will discuss these different approaches and suggest which might have the greatest therapeutic potential.

p53-mediated apoptosis

Understanding the factors influencing the decision of a cell to undergo cell cycle arrest or die in response to DNA damage is essential to developing cancer therapies that specifically target tumor cells without damaging normal cells. However, the mechanisms governing this decision are not well understood. There is a huge literature addressing both cell cycle arrest and cell death, but in the context of therapeutic strategies, the widely accepted model is that p53 is required for DNA damage-induced apoptosis. Yet this is a very misleading paradigm. There are numerous cases where p53-defective cells undergo apoptosis, and many cases in which the loss of p53 even sensitizes cells to DNA damage. So pervasive has p53 become that it sometimes appears there are only two pathways of apoptosis, either p53-dependent or p53-independent, and this extends far beyond any role in response to DNA damage. It has frequently been implied that p53 is needed if cells are to succumb to anticancer drugs, yet many years of research have resulted in the discovery of drugs while using p53-defective tumor models, and the drugs have been used successfully to treat many patients with p53-defective tumors. Furthermore, radiation therapy is routinely administered to patients independent of their p53 status as it does not predict outcome. So why has this misleading concept become so well accepted?

The seminal work supporting the importance of p53 in mediating DNA damage-induced apoptosis was performed with cells derived from p53 knockout mice (1). Thymocytes isolated from these mice were found to be completely resistant to γ-radiation-induced lethality. Subsequently, an experimental fibrosarcoma derived from the same p53−/− mice was also shown to be resistant to radiation as well as adriamycin, both in vitro and when grown as a transplantable tumor in vivo (2). However, a concurrent paper showed that

Abbreviation: PFTα, pifithrin-α.
γ-radiation was perfectly capable of killing proliferating T lymphocytes and a T cell lymphoma derived from the same p53−/− mice (3). The former observations have clearly dominated scientific thinking in this area since.

How do we rationalize these different observations? It is important to understand the differences in the particular cells and tumors studied. Thymocytes are a quiescent cell type and probably do not activate a cell cycle checkpoint when irradiated as they do not need to protect themselves from proliferation-induced damage; why they should decide to undergo apoptosis rapidly when irradiated is still not fully understood. The fibrosarcoma was derived from p53−/− embryonic fibroblasts that had been transfected with E1A and Ras expression vectors. It is well established that oncogenic changes such as E1A and Ras sensitize cells to apoptosis, and only then does p53 impact response (4,5). Thus, it is unclear whether the increase in apoptosis was truly p53 dependent or if expression of E1A and Ras played a larger role. It has been reported that p53-mediated transcription is required for ‘E1A-induced apoptosis’ (6) but this lexicon has failed to catch on and the literature continues to place the primary role, whether the cells arrest or die, an important distinction (7). However, it is important to note that this study scored drugs for their growth inhibitory potency, not apoptosis, nor any other mode of cell death. Considering that p53 normally arrests cell growth, the apparent sensitivity of p53 wild-type cells is to be expected, but the results should not be extrapolated to conclude that p53 is required for cell death. This study emphasizes that the choice of assay used to determine the fate of cells is very important. Furthermore, many of the assays used today that are purported to score ‘cell survival’ (i.e. mitochondrial stains such as tetrazolium derivatives, protein or DNA stains) are frequently used in a manner that can not discriminate whether the cells arrest or die, an important distinction considering that arrested cells can often recover. These short-term assays have been strongly criticized, and data has been presented that convincingly shows how short-term assays can lead to the conclusion that p53 does sensitize cells to DNA damage, whereas a clonogenic assay shows no such difference (8).

Our own experiments with the topoisomerase II inhibitor etoposide directly measured apoptosis in ML-1 (p53 wild-type) and HL60 (p53 mutant) leukemia cells; the results showed rapid and comparable levels of apoptosis in both cell lines (9,10). In contrast, the consequence of DNA damage in many p53 wild-type epithelial cells is cell cycle arrest rather than apoptosis (11,12).

One commonly used model has been the HCT116 colon cancer cell line because of the availability of isogenic derivatives defective for p53 or its target genes. Deletion of p53 in HCT116 cells led to greater apoptosis induced by adriamycin or cisplatin but resistance to 5-fluorouracil (13). Prior studies had already shown that transfection of HCT116 cells with the human papillomavirus E6 resulted in degradation of p53 and sensitization to cisplatin and nitrogen mustard (14). Parallel studies using HCT116 cells deleted for p21war1 also showed much greater sensitivity to various DNA damaging agents (14). Hence, the mechanism of p53-mediated protection depends on the ability of p21war1 to prevent replication or mitosis on damaged DNA and thereby emphasizes the importance of cell cycle arrest in protecting cells from DNA damage. It is also worth noting that this enhanced sensitivity upon loss of p53 is not unique to HCT116 cells, as similar results have been obtained in a variety of other genetic backgrounds (15–17).

There is another important caveat regarding the requirement for apoptosis following DNA damage and that is the concentration of drug used. Our original observations on apoptosis established that the time to apoptosis at a concentration that killed 90% of the cells was often 2–3 days (18–20). These experiments also showed that apoptosis frequently occurred following mitosis, and that a mitotic catastrophe was an initiating event in the induction of apoptosis (21). Many subsequent papers have failed to consider the importance of cell cycle progression and have assessed apoptosis at much earlier time points. For example, in a comparison of p53 wild-type carcinoma, a 1 h incubation with 20 μM cisplatin induced apoptosis by 72 h (22), while continuous incubation with 100 μM cisplatin induced apoptosis in 8–12 h (23). Apoptosis at this high concentration circumvents the need for cell cycle progression, but the relevance of this rapid apoptosis to the concentration that killed 90% of the cells was often 2–3 days (24). A recent review has also questioned much of the current literature, emphasizing the need to relate observations on apoptosis to the concentrations of drug that reduce clonogenic survival of cells (25).

One potential reason for much of the contradiction regarding p53-mediated apoptosis is that there are many other ways for a cell to die. As discussed above, low levels of DNA damage are frequently slow to kill cells which eventually results from induction of a mitotic catastrophe. It is possible that the death of cells at clonogenic concentrations is mediated by nonapoptotic mechanisms, and that apoptosis requires higher concentrations. If true, then apoptosis may have little relevance to the survival of cells following many insults.

Determinants of response to p53: cell cycle arrest versus apoptosis

As a consequence of the overriding belief that p53 activation will kill cells, one therapeutic approach has been to elevate or activate p53 in tumors while an alternate approach has been to inhibit p53 in normal cells. To predict the outcome of p53 activation, it is necessary to consider the cell type, the
DNA damaging agent and the dose. For example, following whole body γ-radiation, high apoptotic index can be observed in thymus, spleen, intestinal epithelium and oral mucosa. Mice exposed to ~12 Gy typically die as a consequence of bone marrow suppression, whereas gastrointestinal toxicity is critical at higher radiation doses (26). However, activation of p53 can be seen in many tissues, with pro-apoptotic genes being induced in radiation-sensitive tissues, but p21<sup>waf1</sup> being induced in other tissues such as liver which is radiation resistant (27). Interestingly, p53 knockout mice are more resistant to lethal bone marrow toxicity supporting the pro-apoptotic role for p53, whereas gastrointestinal toxicity is increased in p53 knock out mice supporting the protective role for p53 (26). Enhanced gastrointestinal toxicity was also observed in p21<sup>waf1</sup> knockout mice further suggesting this is the critical p53 target gene that protects cells.

It is now evident that the reason different genes are induced in damaged cells may relate to expression of additional co-factors, p53 splice variants or other p53 family members (p63 and p73) that preferentially enhance expression of pro-apoptotic genes or cell cycle regulators depending upon cell type (28–31). It is beyond the scope of this review to discuss all the possible contributors to this differential transcriptional response. However, it is worth noting that low levels of damage, perhaps those that a patient is most likely to be exposed to, could preferentially induce arrest genes while higher levels of damage may induce pro-apoptotic genes (32).

There is another important variable to mention which will impact a potential therapeutic strategy discussed below. Specifically, it has been shown that p53 can also induce apoptosis independent of any transcriptional activity by directly interacting with Bcl-2 family members at the mitochondria (33–36). The relative importance of these two mechanisms of p53-induced apoptosis remains to be resolved, but a very recent study demonstrated that selective inhibition at the mitochondrial level can protect cells from γ-radiation in culture, and more importantly, it can protect mice from a lethal dose of γ-radiation (37).

Extensive research has been performed with γ-radiation, but different damaging agents appear to target different tissues. The profile of toxicity resulting from γ-radiation is not consistent with other DNA damaging drugs. Although most drugs induce myelosuppression, the limiting toxicity for many drugs occurs at other tissues such as kidney (cisplatin), lung (bleomycin) and heart (adriamycin). Even p53-mediated apoptosis induced by γ-radiation is limited in patients both by low level fractionated doses and by localized administration targeted at the tumor such that toxicities observed following a single dose of whole body irradiation in experimental models may have little relevance to the therapeutic situation. This discussion raises the important question as to whether p53 contributes significantly to patient toxicity and whether approaches to protect the patient from p53-induced apoptosis will have any therapeutic benefit.

**Activation of p53 as a therapeutic strategy**

Numerous studies have focused on activation of p53 as a strategy for cancer therapy. These studies have generally not considered the particular oncogenic context of each tumor and whether arrest or apoptosis would be the likely outcome. However, re-introduction of p53 into p53-defective tumor cells has frequently been shown to increase apoptosis. The consequence of overexpression of exogenous p53 may have little bearing on the pathways activated by DNA damage in the presence of a relatively low level of endogenous protein. For example, overexpression of p53 may overwhelm any selective activation of growth arrest genes versus pro-apoptotic genes. Difficulties in this therapeutic strategy lie in controlling the level of p53 expression and in delivering the p53 gene effectively to tumor cells (38,39).

One approach to activate p53 has been to reactivate endogenous mutant p53 (Figure 1). The first reported success in this regard was with the small molecule CP-31398 (40). The mechanism of action appears to depend on the observation that newly synthesized mutant p53 initially assumes an active conformation, before collapsing to the inactive structure. CP-31398 has been shown to stabilize the active conformation of newly synthesized p53, although it cannot reverse the inactive form. Structurally distinct compounds have subsequently been identified such as PRIMA-1 and MIRA-1 both of which appear to differ form CP-31398 in being able to revert the mutant conformation of p53 (41,42). All three compounds have been shown to transactivate p53 response genes and inhibit tumor growth in human xenograft models. At least for MIRA-1 the therapeutic window was very small with toxicity occurring only slightly above doses that had therapeutic activity. Whether these compounds will have any therapeutic application remains to be determined.

An alternate approach that has been generating much recent excitement is the use of small molecules that inhibit the interaction of p53 with its negative regulator HDM2 (Figure 1). The first such inhibitors identified to disrupt this interaction were chalcone derivatives and a fungal metabolite, chlorofusin, although their activity in either cell or animal models does not appear to have been confirmed (43,44). More excitement was generated with the identification of inhibitors such as nutlin (45) and RITA (46) in 2004, while additional inhibitors have subsequently been identified by other groups (47,48). These compounds mimic the binding of p53 to the p53-binding pocket of HDM2. As a consequence, p53 is no longer targeted for degradation but rather accumulates and elicits either arrest or apoptosis. It is unclear, which of these outcomes will predominate; apoptosis would result in tumor regression while arrest might only last as long as the drug was being administered. These compounds have been shown to inhibit tumor growth both in vitro and in vivo, a particularly important observation because it shows that the compounds can be tolerated in vivo while still eliciting an antitumor effect. Hence, the fear that elevation of p53 might kill normal cells appears to be unfounded. One explanation for this observation is that elevation of p53 is not necessarily the same as activation of p53. While much of the drug-induced phosphorylation of p53 is required to dissociate p53 from HDM2, other modifications of p53 that may not occur in the absence of DNA damage may be required for its full activity. An alternate explanation provided for this tumor selectivity is that tumor cells are sensitized to apoptosis because of oncogene addiction (46). This is an important concept for which there is considerable support. For example, tumors almost invariably have elevated levels of Myc which is a known repressor of p21<sup>waf1</sup> (49). Accordingly, p53 wild-type tumors (or those in which p53 has been reintroduced or reactivated) may still be defective in their ability to arrest compared to normal.
cells, and therefore more sensitive to DNA damage. There is further support for the idea that most normal cells will be resistant to elevation of p53. Mice engineered to express reduced levels of MDM2, and as a consequence elevated p53, showed an increase in apoptosis of the thymus and small intestine when irradiated, although there was little toxicity in most tissues (50). From these observations, we can extrapolate that nutlin or RITA plus radiation may only be a toxic combination to a few tissues, and that strategies might be established that could resolve even this concern.

Most anticancer drugs elicit their toxicities in different tissues than γ-lox-radiation, with the exception of bone marrow. If administration of nutlin or RITA can lead to elevation of p21 in these tissues in vivo, then it may be a useful strategy for protecting normal tissues from the effects of many anticancer drugs. This has been shown for mitotic inhibitors such as paclitaxel. P53 wild-type HCT116, RKO colon cancer cell lines and primary human fibroblasts were protected from the cytotoxic effects of paclitaxel by pretreatment with nutlin (51). In contrast, the p53-defective cancer cell line, MDA-MB-435, responded to paclitaxel treatment with mitotic arrest and apoptosis. These results suggest that nutlin or RITA may be useful in protecting proliferating normal tissues from the effects of mitotic inhibitors by inducing p53-mediated cell cycle arrest. However, it remains to be seen whether they can be used more generally to protect normal tissues from chemotherapeutic agents. As discussed further below, this is an approach that we believe has considerable therapeutic potential.

**Inhibition of p53 as a therapeutic strategy**

An alternate therapeutic strategy is based on the belief that activation of p53 in normal cells will indeed be toxic to the patient. Hence, p53 inhibitors were developed with the intent to protect normal tissues from the adverse effects of chemotherapeutic agents (Figure 1). The p53 inhibitor pithrin-α (PFTα) rescued p53 wild-type cells from apoptosis induced by the DNA damaging agent etoposide and reduced lethality in mice following γ-radiation (52). PFTα displays a number of chemo- and radiation-protective qualities, including neuroprotection, cardioprotection and renal protection (53). These observations have led to the proposal that p53 inhibitors, such as PFTα, could be useful protective agents when used in combination with chemotherapy and radiation.

The mechanism by which PFTα inhibits p53 is not well understood. PFTα blocks p53-dependent transcription and protects many cell types from apoptosis induced by radiation and chemotherapy, although this protection may not depend on p53-mediated transcription. PFTα suppresses caspase activation and decreases levels of nuclear, but not cytoplasmic p53, and may inhibit p53 localization to mitochondria (53). Many p53-independent effects of PFTα have also been observed, including suppression of apoptosis in p53-defective cell lines. Furthermore, PFTα has also been shown to induce apoptosis (54,55). This multitude of effects complicates the development of a therapeutic rationale for PFTα. The precise molecular targets of PFTα must be elucidated in order to determine appropriate treatment strategies with this compound.

Even if PFTα were selective for inhibition of p53-mediated transcription, it is not clear whether it would be useful in treating tumors in patients without significant side effects. If PFTα is used in combination with DNA damaging agents to treat p53-defective tumors, it might serve to protect some normal tissues from p53-dependent apoptosis, but it would also prevent p53-dependent cell cycle arrest that is
essential to preventing most normal cells from progressing through the cell cycle with damaged DNA. A more effective drug strategy may be to develop p53 inhibitors that target either the p53-dependent apoptotic pathway or the p53-dependent cell cycle arrest separately. In this regard, it has very recently been shown that a new small molecule, PFTu, can inhibit the mitochondrial-targeted pro-apoptotic activity of p53 without inhibiting p53-mediated transactivation (37). PFTu also reduced the lethality of whole body irradiation in mice. This is an exciting approach that may have a greater impact in the development of novel therapeutic strategies.

Selective killing of p53-defective tumors as a therapeutic strategy

As discussed above, p53 can act as a survival factor through its ability to induce p21waf1 and thereby prevent cells from progressing through the cell cycle with damaged DNA. While this might appear to provide a selective means to kill p53-defective tumor cells, there is a limitation to this approach. p53 is not the only checkpoint pathway that is activated in tumors, and a redundant system exists that concurrently protects tumor cells from the cytotoxic action of drugs. These alternative checkpoint pathways provide a novel and potentially selective therapeutic strategy that has been termed ‘synthetic lethal’, a term derived initially from yeast genetics. In its simplest form, if a cell uses two redundant pathways for survival, loss of either pathway has no consequence whereas loss of both pathways is lethal (56). In chemotherapy, inhibitors could be developed to target both pathways, but the strategy is particularly effective when a tumor is already defective in one pathway such as p53; inhibition of the other pathway can then selectively kill the tumor.

In response to DNA damage, cells halt progression through the cell cycle to allow adequate time for the damage to be repaired. The phase of the cell cycle in which the cell arrests depends, in part, on their p53 status; cells with wild-type p53 arrest predominantly in G1, while cells with defective p53 fail to arrest in G1 but rather arrest in S or G2 phase. Once repair is complete, cells may recover and proliferate. Inhibition of cell cycle checkpoints limits the time available for repair, thereby forcing cells to prematurely progress through S and G2, and undergo an aberrant and frequently lethal mitosis.

The central regulators of cell cycle checkpoints are the protein kinases ATM and ATR (Figure 2). While one target of these kinases is p53, alternate regulation of arrest is mediated via the checkpoint kinases Chk1 and Chk2. ATM is primarily activated by DNA double-strand breaks, while ATR is activated by stalled replication forks. ATR directly activates Chk1, while ATM directly activates Chk2 and, using ATR as an intermediary, also activates Chk1 (57). While both Chk1 and Chk2 were originally thought to act on similar substrates, it now appears that Chk1 is primarily responsible for arrest of cells in S and G2 phase (58,59). In S phase, Chk1 phosphorylates the protein phosphatase CDC25A, thereby targeting it for degradation (60,61). In G2, Chk1 phosphorylates CDC25C which is then sequestered in an inactive complex with 14-3-3 (62,63). As a consequence, CDC25A/C are unable to dephosphorylate and activate cyclin E/Cdk2 in S phase or cyclin B/Cdk1 in G2.

The first checkpoint inhibitor recognized was caffeine which was shown to drive G2-arrested cells through a lethal mitosis (21,64). This activity was subsequently shown to be selective for p53-defective cells (65). The target for caffeine was eventually identified as ATM and ATR (66). Unfortunately, caffeine cannot be administered safely to patients at concentrations high enough to abrogate cell cycle arrest. We identified another checkpoint inhibitor, 7-hydroxystaurosporine (UCN-01), as being 100,000 times more potent than caffeine and it also abrogated arrest selectively in p53-defective cells (12,67,68). The molecular target of UCN-01 for checkpoint abrogation is Chk1 (69,70).

Despite its promise as a therapeutic agent in combination with DNA damaging agents, UCN-01 was found to bind avidly to human plasma proteins which may limit its therapeutic potential (71,72). However, in a Phase I clinical trial, we have recently found that administration of UCN-01 following cisplatin can markedly reduce the number of tumor cells in S and G2 suggesting that it can elicit a biological effect despite its plasma binding (73). An additional concern with UCN-01 is that it inhibits a variety of other kinases including protein kinase C and PDK1, and can arrest cells in G1 alone at higher concentrations. Hence, it remains difficult to prescribe sufficient UCN-01 to abrogate arrest without compromising its desired activity by causing arrest. There is
tremendous interest in identifying other checkpoint inhibitors that can overcome these limitations. We identified G66976 as a potent analog that abrogates arrest in the presence of human plasma, while many companies have preclinical and clinical studies with alternate Chk1 inhibitors.

The selectivity we have seen for p53-defective cells has been questioned, and there are several reports that UCN-01-mediated abrogation of arrest does not correlate with p53 status. We have re-evaluated these papers and have found a critical difference in the strategy used. In each of these papers, UCN-01 was added concurrent with the damaging agent, or within 3 h of adding insult. In this sequence, UCN-01 prevents the activation of the checkpoint before a protective p53 response can be enacted. In contrast, our strategy is dependent on p53 being induced prior to addition of UCN-01; under these conditions, p53 wild-type cells are completely resistant to abrogation. We have investigated these different sequences in our own laboratory and have confirmed that concurrent addition of UCN-01 and a DNA damaging agent to p53 wild-type cells prevents S phase arrest and enhances cytotoxicity.

We have directly addressed the impact of p53 on checkpoint abrogation using isogenic cell lines created by stable expression of shRNA that suppressed p53 expression. The parent lines in this experiment were two immortalized breast cell lines, MCF10A and IMEC. The topoisomerase I inhibitor SN38 arrested these cells in mid-S phase independent of their p53 status; the failure to arrest in G1 occurs because SN38-induced DNA damage depends on DNA replication. Addition of UCN-01 had no impact on the S phase-arrested p53 wild-type cells, but their p53-suppressed counterparts were forced through S and G2. Prior to the general availability of siRNA technology, we had used an alternative approach to inhibit p53. Specifically, we expressed a small fragment of p53 that prevented p53 tetramerization. This cell line also arrested in S phase in response to SN38, while p53 accumulated and was phosphorylated on serines 15 and 20 as seen in the parent line. However, unlike the parent cell line, p21WF1 failed to increase consistent with inhibition of p53 activity. Upon addition of UCN-01, this cell line abrogated S phase arrest but failed to abrogate G2 arrest. This was a very unexpected observation but the failure to abrogate G2 was found to correlate with repression of cyclin B suggesting that this p53 repressor function is retained in the cells. Consequently, we have concluded that both the transactivation function of p53 that elevates p21WF1, and the p53 repressor function that suppresses cyclin B are required for full protection from checkpoint abrogation.

The separation of the transactivation and repressor functions of p53 has been previously observed in cells expressing the adenovirus E1B 19kDa protein, except in this case, the cells retained transactivation but alleviated p53-mediated repression. We have observed a similar phenomenon in several p53 wild-type tumor cell lines. For example, SN38-damaged HCT116 cells induce p21WF1 but fail to repress cyclin B, p21WF1 and p53 repressor function that suppresses cyclin B are required for full protection from checkpoint abrogation.

HCT116 cells exhibit defective repression of p53 response genes remains to be determined. However, it should be kept in mind that these are tumor cells and, from a therapeutic perspective, it would be advantageous if they were to abrogate arrest. In contrast, our experiments with isogenic lines described above used nontumorigenic lines that may better reflect the outcome in a patient’s normal tissues.

The need to induce the p53 protective response before addition of a checkpoint inhibitor raises the possibility of another intriguing therapeutic strategy. We suggest that prior treatment with nutlin or RITA to induce a p53 response can protect the normal cells from DNA damage. Once the normal cells have been protected, it may be possible to combine a DNA damaging agent and checkpoint inhibitor to further enhance the therapeutic index. If there continues to be concern for systemic toxicity associated with activation of p53, it may be further possible to administer PFTalpha to protect normal cells without impeding the protective induction of p21WF1.

Concluding remarks

It is clear that no single p53-targeted therapeutic strategy will be sufficient for the treatment of all tumors. Dissecting the p53 pathways will be instrumental to the development of any p53-targeted therapeutic strategy, since different tumors will display different reactions to p53 activation, inhibition or checkpoint abrogators. Ideally, such therapies will be customized to patients based on the status of p53, checkpoint proteins, and oncogenic changes. Of the treatment strategies described above, the strategy involving the use of a DNA damaging agent followed by a checkpoint inhibitor shows the greatest promise, since it can be used not only for the >50% of tumors with defects in p53, but in some p53 wild-type tumors that contain other defects in the p53 pathway. Whether this strategy can be further improved by combining with compounds such as nutlin or RITA that could further enhance the p53-dependent protection of normal tissues remains to be determined.

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References


