Dangerous habits of a security guard: the two faces of p53 as a drug target

Andrei V. Gudkov* and Elena A. Komarova

Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

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Being most well-known tumor suppressor that is inactivated in tumors more frequently than any other gene, p53 has been recently recognized as a major player in a variety of pathologies caused by acute stresses of tissues that is responsible for massive cell loss from apoptosis. This created a controversial situation when effective treatment of acute pathology requires inhibition of a major cancer preventive factor that has been traditionally viewed as a target for therapeutic activation. Here we briefly review specific aspects of this problem and discuss the ways of its pharmacological resolution based on detailed knowledge of molecular mechanisms of p53 regulation and activity.

p53 AND ITS TUMOR SUPPRESSOR FUNCTION

p53 was discovered and is mostly known as a tumor suppressor gene. In fact, germline deficiency in p53 leads to early cancer development in mice and men (1–3). p53 is lost or functionally inactivated in the majority of human tumors (4–8) and restoration of its function typically results in tumor cell death (9).

The tumor suppressor function of p53 can be attributed to specific aspects of its regulation and activity in the cell. Although the p53 gene is transcribed in all cell types and tissues, its mRNA expression levels vary among developmental stages and tissues. For example, p53 is only strongly transcribed in middle embryogenesis and in several adult organs such as lymphoid tissues (10). p53 activity is primarily determined at the protein level. Under normal environmental conditions, p53 protein has a short half-life and is barely detectable in cells. p53 degradation is predominantly mediated through its interaction with the ubiquitin ligase Mdm2 (11–13). Transcription of the Mdm2 gene is activated by p53 which creates a classical negative feedback regulatory loop that limits the duration of p53 activity (14). Under conditions of acute stress (e.g. DNA damage, hypoxia, abnormal expression of oncogenes, etc.), interaction of p53 with Mdm2 is decreased. This can be due to either conformational changes in p53 resulting from its phosphorylation by stress-inducible kinases (15,16) or binding of Mdm2 by another tumor suppressor protein, Arf (17), that is positively regulated by many oncogenes. In either case, disruption of p53–Mdm2 interaction leads to accumulation of p53 protein, predominantly in the nucleus where it binds to specific DNA sequences and alters transcription of numerous genes (18,19). Depending upon the structure of particular DNA-binding sites, p53 forms complexes with either positive or negative transcriptional regulators, thereby either activating or inhibiting transcription of different genes, many of which are tissue-specific (20,21). Many p53 responsive genes encode cell cycle regulators, proapoptotic proteins, DNA repair enzymes, etc. Thus, the altered activity of a set of p53-responsive genes determines a cell’s response to a given stress. Depending upon the type and strength of stress and, more significantly, upon the cell type, the end result of p53 activation can be proliferation arrest (either temporary or irreversible, i.e. senescence) at cell cycle checkpoints, induction of apoptosis, induction of DNA repair, secretion of an altered spectrum of factors, etc. With the exception of apoptosis and senescence, which are irreversible by definition, other p53 responses are temporary due to its self-suppression via induction of Mdm2. At a single-cell level, Mdm2-mediated shutdown of p53 activity occurs as an oscillation with gradually decreasing amplitude (22).

Traditionally, these cellular functions of p53 have been interpreted in terms of its tumor suppressor activity (Fig. 1). In fact, it seems reasonable that self-elimination of cells affected by DNA damage through p53-induced apoptosis would contribute to the overall genomic stability of a cell population. In this regard, the extreme sensitivity of immunocytes and spermatocytes to p53-dependent apoptosis (23) makes biological sense since it would be more favorable for the organism to get rid of these cells following DNA
damage than to deal with the consequences of their clonal expansion (immunocytes) or the risk of acquiring a germline mutation (spermatocytes). Consistent with this, T-lymphomas are the predominant type of tumors that develop in p53-deficient mice (1,2). p53-dependent senescence has been also viewed as a tumor suppressor mechanism that forever blocks the ability of connective tissue cells (fibroblasts) to proliferate after severe genotoxic stress (23). Again, the high frequency of sarcomas in p53-deficient mice (1,2) argues in favor of this mechanism being tumor suppressive. The fact that fibroblasts are controlled by p53 in a different way than immunocytes (growth arrest versus apoptosis) also makes a lot of biological sense since elimination of connective tissue cells by apoptosis would have severe negative consequences for tissue integrity.

Recently, Peter Chumakov’s group discovered another powerful activity of p53 that is likely to contribute to its tumor suppressor function. They showed that p53 regulates a family of evolutionarily conserved proteins, named sestrins, that are involved in the metabolism of reactive oxygen species (ROS) (24). Through sestrins, p53 contributes to keeping intracellular ROS levels low, which would be expected to have a cancer-protective effect. The importance of this mechanism for the tumor suppressor function of p53 is supported by work from the same group showing a dramatic reduction in tumor incidence in p53-deficient mice that are kept on a diet rich in antioxidants (25).

PHARMACOLOGICAL RESTORATION OF p53 TUMOR SUPPRESSOR FUNCTION

Provided all of the above-mentioned functions of p53, it is not surprising that historically the p53 field has been predominately focused on the suppression of p53 that is observed in most tumors and the possibility of restoration of p53 activity as a way to induce cancer cell death (26–33). A number of different mechanisms acquired by tumors to inhibit p53 function have been identified. The most frequently observed of them include Mdm2 overexpression (6), loss of Arf expression (5), inhibition of p53 by viral proteins (7,34), etc. Whenever a dominant inhibitor was found to be involved in p53 suppression, the inhibitor was quickly seen as a potential drug target with hopes that suppression of the inhibitor would restore p53 function and thus have an anti-tumor effect (Fig. 2). The validity of such an approach is supported by numerous demonstrations of the anti-tumor effect of p53 activated either by genetic manipulation (35,36) or by small molecules that target its interaction with inhibitory proteins or restore the functionality of mutant p53 (37).

Although this direction of therapy remains a valid approach for certain cancers, its ultimate value is tempered by the genomic plasticity of tumors. Restoration of p53 activity creates conditions favoring selection of cells expressing novel p53-deficient variants that escape the effect of the p53-restoring treatment. In this regard, an interesting window of opportunity was opened by the discovery of a novel NF-κB-dependent mechanism frequently involved in p53 suppression in tumors. NF-κB is a major regulator of innate and adaptive immune responses and inflammation (38), and as opposed to normal cells where it becomes induced only under inflammatory conditions, NF-κB is constitutively active in the vast majority of cancers (39). Although the exact molecular mechanism remains to be determined, it was found that p53, which is functionally attenuated in such cancers, can be ‘woken up’ by either genetic or pharmacological inhibition of NF-κB signaling to induce tumor cell death (Fig. 2) (40). Interestingly, among pharmacological agents capable of simultaneous inhibition of NF-κB and activation of p53, we identified quinacrine (40), a drug historically used for treatment of malaria. The long and successful
effects, pharmacological suppression of p53 has been recognized as a useful approach to treatment of other pathological conditions that are associated with excessive cell loss through apoptosis (48,49). Thus, neuronal death due to ischemic conditions (50,51), Parkinson’s disease (52,53), acute renal and liver failure (54–58), etc., might all be candidates for treatment with p53 inhibitors.

We recently identified another unexpected effect of p53 inhibitors by studying the role of p53 in the responses of tumor stroma to anti-cancer treatments. By comparing tumor models differing in the p53 status of their stroma, we showed that tumors with p53-deficient stroma were significantly more sensitive to experimental chemo- and radiotherapy than tumors with p53-wild-type stroma (59). A similar effect was observed when the anti-cancer treatment was combined with pharmacological suppression of p53 by PFTα. Potentiation of the anti-cancer effect of chemo- and radiotherapy by p53 suppression in the tumor stroma is likely due to the increased sensitivity of p53-deficient vascular endothelium to genotoxic stress, as shown both in cell culture and in experimental tumors. Thus, reversible pharmacological suppression of p53 might be a viable approach to improving anti-cancer treatment via enhancement of the anti-angiogenic effects of chemo- and radiotherapy.

CONCERNS ABOUT PHARMACOLOGICAL SUPPRESSION OF p53

The main concern that has slowed down clinical development of p53 inhibitors is the presumption that their use would be accompanied by an increased risk of cancer. This concern is supported by the high frequency of cancer in p53-deficient mice and men (1–3). However, accumulating experimental evidence argues against the presumption that clinical inhibition of p53 will have similar effects. We did not observe an increase in cancer development in mice rescued from radiation by PFTα injection (41). Moreover, in an elegant genetic model of pharmacologically regulated p53 deficiency in mice, Gerard Evan recently showed that sporadic short-term activation of p53 in otherwise p53-deficient mice is largely sufficient for effective tumor suppression (60). In addition, Evan’s group has come to the conclusion that massive apoptosis occurring in hematopoietic and lymphoid tissues following total body irradiation is not essential for p53-dependent prevention of lymphomas. Thus, both genetic and pharmacological evidence indicates that temporary and reversible suppression of p53 function does not significantly jeopardize its tumor suppression function. Having allayed the fears of carcinogenic effects, these studies significantly strengthen the argument for pharmacological suppression of p53 as a way to prevent and treat several acute pathologies.

Nevertheless, the multifunctional nature of p53 and its involvement in numerous regulatory processes raised another problem with the use of p53 inhibitors in patients. In contrast to its well-known role as a mediator of cell death, we found that p53 can be a survival factor in vitally important organs. Specifically, lack of p53 dramatically increases the susceptibility of small intestine to gamma radiation as shown in studies with p53-knockout mice (61). Although these mice do not, as mentioned above, develop hematopoietic radiation

PATHOLOGIES ASSOCIATED WITH p53 ACTIVITY AND THE VALUE OF p53-SUPPRESSIVE DRUGS

p53 activation remains a valid anti-cancer approach that will likely result in development of new cancer treatments in the near future. However, accumulating observations of p53 activity in vivo in experimental animals (predominantly mice) indicate that the same p53 functions that are so important for tumor suppression can be harmful under conditions of systemic genotoxic stress such as total body irradiation or injection of genotoxic anti-cancer drugs (Fig. 2). In 1997, in our work on ‘blue mice’ (mice carrying in their germline a p53-responsive beta-galactosidase reporter gene), we made an observation that implicated p53 in the side effects associated with radio- and chemotherapy: we noticed that the p53-responsive reporter was most active and apoptosis was most prominent in those tissues that are known to be radio- and chemo-sensitive and failure of which determines cancer treatment side effects (hematopoietic and lymphopoietic sites, small intestine and hair follicles) (10). Indeed, we later showed that p53-deficient mice do not die or develop acute radiation syndrome when exposed to up to 10 Gy of gamma irradiation, a dose that kills 100% of p53 wild-type mice (41). Similarly, the p53-deficient animals were resistant to chemo- and radiotherapy-associated hair loss (42,43). The power of p53 as a tissue-killing agent is underscored by genetic work demonstrating the consequences of suppressing Mdm2, the major natural inhibitor of p53, in mice. Germine deletion of Mdm2 results in p53-dependent embryonic lethality (44,45) and conditional stimulation of p53 function in mice without functional Mdm2 leads to rapid loss of lymphoid tissues and subsequent death of the animal (46). These data might be interpreted as indications that p53 activity in normal cells is an underlying cause of the adverse side effects that accompany radiation and chemotherapy and support our hypothesis, which we suggested earlier, that pharmacological suppression of p53 might be used as an approach to reduce these side effects (47). This hypothesis was validated by isolation of a small molecule inhibitor of p53 named pifithrin-α (PFTα) that appeared to be a powerful radioprotectant (41) that is ineffective in p53-deficient cells and therefore selective for normal tissues. Thus, p53 was defined as a major determinant of cancer treatment side effects that is responsible for the general chemo- and radiosensitivity of mammals.

In addition to amelioration of anti-cancer treatment side effects, pharmacological suppression of p53 has been recognized as a powerful drug to prevent and treat several acute pathologies. Thus, a p53 drug might be used in skin disorders, such as psoriasis and vitiligo, where it has already shown clinical promise (62). Furthermore, p53 inhibition might be beneficial in ischemic conditions (50,51), Parkinson’s disease (52,53), acute renal and liver failure (54–58), etc., might all be candidates for treatment with p53 inhibitors.

The advantages of NF-kB inhibition as an approach to p53 activation include (i) its specificity for cancer cells (since only cancer cells have deregulated NF-kB) and (ii) its potential usefulness even against cancers with mutant p53 (there are more benefits to tumor cells from constitutive activation of NF-kB than ‘just’ suppression of p53, e.g. NF-kB protects cells from TNFα-induced apoptosis).
syndrome, they do acquire GI radiation syndrome and their symptoms are more severe than those that develop in p53 wild-type mice. This property of p53-deficient mice, at least in part, is associated with the increased sensitivity of their vascular endothelial cells to genotoxic stress (59). Vascular endothelial cells were defined through the work of Fuks and Kolesnick as the primary targets of ionizing radiation responsible for GI injury within the range of doses that damages the GI tract (62,63). The mechanisms behind the protective role of p53 in endothelial cells are not completely understood but may involve the ability of p53 to keep wild-type cells arrested at cell cycle checkpoints while p53-deficient cells undergo lethal mitotic catastrophe (23,61). Another function of p53 that presumably contributes to its rescuing effect is p53-dependent activation of the DNA excision repair machinery (23). Interestingly, high radiosensitivity of the GI tract is observed in p53-null mice, despite the lack of p53-dependent apoptosis in the epithelium of their small intestines. This indicates that apoptosis is not always a marker of organ failure.

Another negative aspect of p53 deficiency for radiosensitivity is the increased susceptibility of p53-null animals to radiation-induced fibrosis (E.A.K. and A.V.G., unpublished data). This is likely the result of deregulated cytokine secretion due to altered NF-kB responses (64). In fact, we found that p53 plays the role of a general suppressor of NF-kB activity, acting as a ‘buffer’ that reduces the severity of inflammation (the major contributor to fibrosis development) which is largely regulated by NF-kB (38).

Despite the evidence that p53 is a survival factor in the GI tract, treatment with PFTα does not sensitize the GI tract of p53 wild-type mice to total body irradiation (and does not protect as well) (61). This is presumably because the p53 inhibitor is suppressing inducible but not steady-state effects of p53, whereas both types of effects are suppressed in p53-null mice. The effect of PFTα treatment on development of radiation-induced fibrosis remains to be determined. However, regardless of the outcome of these studies, it is clear that there are important p53 functions other than induction of apoptosis that are useful and need to be preserved for patient safety. This creates a difficult and perhaps seemingly unresolvable situation requiring pharmacological dissection of the ‘good’ and ‘bad’ properties of the same target molecule.

**PHARMACOLOGICAL DISSECTION of p53 ACTIVITIES: PIFITHRIN-μ**

Moll’s laboratory published the papers that described a new mechanism through which p53 could induce apoptosis (65,66). This mechanism did not employ the transactivation function of p53, but involved p53 binding to mitochondria which resulted in the opening of mitochondrial pores, leakage of cytochrome c and induction of caspases (Fig. 3). Further work demonstrated that this mechanism was the primary cause of radiation-induced death of thymocytes and presumably other cells from most radio-sensitive tissues (67). These findings indicate that protection of such cells from apoptosis would not require inhibition of the transactivation function of p53. However, at the same time, the majority of tissue protective functions of p53 (growth arrest at cell cycle checkpoints, induction of DNA repair enzymes, etc.) are thought to be exerted through its transactivation function. This situation created an interesting opportunity for pharmacological dissection of cell protective and at least some of the apoptotic functions of p53 by isolating compounds that would block p53 binding to mitochondria (or more specifically, to the mitochondria-bound anti-apoptotic Bcl-2 family members Bcl-2 and Bcl-xL). Thus, we have isolated a small molecule named pifithrin-μ, (PFTμ) that inhibits p53 binding to Bcl-xL and Bcl-2 and reduces its association with mitochondria but has no effect on p53-dependent transactivation (68) (Fig. 3). PFTμ has a high degree of p53 specificity and does not protect cells from pro-apoptotic factors downstream or independent of p53. PFTμ rescues primary mouse thymocytes from p53-mediated apoptosis caused by radiation and protects mice from doses of radiation causing lethal hematopoietic syndrome. These results demonstrate that selective inhibition of the mitochondrial branch of the p53 pathway is sufficient for radioprotection in vivo and validate our approach to development of clinically useful p53 inhibitors.

**SUMMARY**

In conclusion, the prospects for pharmacological targeting of p53 for a variety of therapeutic purposes can be summarized as follows (Figs 2 and 3).

1. (1) Activation of p53 can be useful for cancer treatment and prevention but should be carried out carefully by selective targeting of tumor-specific mechanisms of p53 suppression in order to avoid adverse side effects in healthy tissues; and

2. (2) Temporary and reversible suppression of p53 can be beneficial for prevention and treatment of acute conditions associated with severe genotoxic stress, but again should be applied carefully to selectively target only the pro-apoptotic function of p53 in order to preserve its tissue protective functions.

Significant progress in our understanding of the molecular genetic, biochemical and pharmacological aspects of p53 has provided solid proof-of-concept for these two major directions of exploration of p53 as a drug target. Thus, the collective work of dozens of laboratories has resulted in maturation of the p53 field to a level that justifies expectations of drugs targeting p53 in the foreseeable future.
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