Pathologies Associated with the p53 Response

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Although p53 is a major cancer preventive factor, under certain extreme stress conditions it may induce severe pathologies. Analyses of animal models indicate that p53 is largely responsible for the toxicity of ionizing radiation or DNA damaging drugs contributing to hematopoietic component of acute radiation syndrome and largely determining severe adverse effects of cancer treatment. p53-mediated damage is strictly tissue specific and occurs in tissues prone to p53-dependent apoptosis (e.g., hematopoietic system and hair follicles); on the contrary, p53 can serve as a survival factor in tissues that respond to p53 activation by cell cycle arrest (e.g., endothelium of small intestine). There are multiple experimental indications that p53 contributes to pathogenicity of acute ischemic diseases. Temporary reversible suppression of p53 by small molecules can be an effective and safe approach to reduce severity of p53-associated pathologies.

INTRODUCTION: EMERGENCY RESPONSES CAN BE DANGEROUS

p53 is generally considered a protein that is beneficial to the organism. Indeed, its absence has disastrous effects: genomic instability, deregulated metabolism of reactive oxygen species, unleashed acute inflammation, cancer, developmental malformations, etc. Commonly used nicknames for p53 such as "Guardian of the Genome," "Guardian of Babies," etc., reflect its importance in protecting organisms and their offspring. p53 plays a critical role in allowing organisms to deal with emergency situations such as genotoxic stress, oncogenic stress, and viral infection, and its multiple specific activities (e.g., induction of DNA repair, growth arrest, and apoptosis) are ideally suited for this role. The activity of p53 in such situations is essential for reducing the risk of accumulation of cells with genetic and epigenetic lesions from which cells with unconstrained growth properties could be selected and form tumors. However, on the other hand, p53 activity can be dangerous to the organism under certain extreme stress conditions. These extreme conditions do not mimic normal environmental or physiological scenarios of stress, and therefore, the potential for unfavorable p53 activity was apparently not eliminated through evolution.

Although most of the information presented and discussed in the other sections of this collection deals with the "useful" functions of p53 and the mechanisms by which p53 exerts

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these functions, here we will focus on *"p53 pathologies"—situations in which p53 activity leads to severe organismal damage*. These pathologies have, in many cases, been revealed through analysis of differences between p53-wild-type and isogenic p53-deficient mice in their responses to life-threatening situations. In large part, the specific activity of p53 that produces pathological results is its ability to induce apoptosis. As would be expected, inappropriate or hyperactivation of this activity can lead to death of cells that the organism would be better off retaining.

The fact that p53-dependent pathologies exist raises the *possibility of using p53 inhibition as a therapeutic strategy* to treat such pathologies. This, of course, is contrary to the prevailing idea of trying to turn p53 "on" as a means to treat cancer, which is solidly based on the fact that p53 deficiency is a bad prognostic factor in cancer. Without questioning this paradigm as a whole, we will present an opposing viewpoint by reviewing cases in which *retaining wild-type p53 is useful for the tumor and p53 suppression may have an antitumor effect*.

To show the feasibility and potential power of pharmacological p53 suppression, we will review data accumulated using the small molecule p53 inhibitor pifithrin-α (PFTα) and related compounds. *PFTs have been used in a variety of experimental models of p53-dependent pathologies and have been successful both in illuminating mechanisms involved in the pathologies and in producing therapeutic effects*.

The critical importance of p53 activity for cancer prevention raises the concern that clinical use of p53 inhibitors could be carcinogenic. We will discuss this and other *potential safety issues associated with the use of p53 inhibitors* and present data aimed at defining which issues, if any, are likely to be real problems.

Finally, we will discuss the *evolution of therapeutic approaches* which stemmed from the idea of pharmacological suppression of p53 and their *progress toward translation into clinical use*.

Overall, we expect this article to convey the somewhat radical idea that p53 activity is not always a good thing and that development of p53 inhibitors could lead to significant improvement in the treatment of a variety of human pathologies.

p53 AS A DETERMINANT OF ACUTE RADIOSENSITIVITY

p53 activation in response to DNA damage is clearly an advantageous mechanism for the organism that has been selected through evolution. However, p53 activation resulting from extreme conditions of genotoxic stress can lead to p53-dependent pathologies. Not surprisingly, these pathologies usually occur under conditions created by civilization, and therefore, the mechanisms leading to their development have not been subjected to the process of evolutionary adaptation, which eliminates disadvantageous mechanisms. The genotoxic conditions that we refer to here are those associated with the use of ionizing radiation (IR) and chemotherapeutic drugs that target DNA directly (e.g., doxorubicin, 5-fluorouracil, topoisomerase I and topoisomerase II poisons, methotrexate, etc.) or indirectly by affecting the cell cycle (e.g., vinca alkaloids and taxol). In addition to their desired antitumor effect, all of these agents cause significant adverse side effects that limit the level of therapy that can be safely applied. The adverse toxicity observed in these situations predominantly involves the hematopoietic (HP) system and gastro-intestinal (GI) tract. Traditionally, the radio- and chemo-sensitivity of these tissues has been attributed to their high proliferative index. However, experimental data that became available about 12 years ago called for revision of this, as we now understand, oversimplified concept.

The major breakthrough in our understanding of the mechanisms responsible for the tissue specificity of radio- and chemo-sensitivity followed from generation of p53-deficient mice (Donehower et al. 1992; Jacks et al. 1994) and transgenic mice with p53-responsive reporters (Gottlieb et al. 1997; Komarova et al. 1997). Surprisingly, when mice expressing p53-responsive lacZ were subjected to systemic genotoxic stress, the p53 response was found to be highly tissue-specific. There was a striking

coincidence between sites of p53 activity (revealed by in situ detection of the lacZ reporter) and accumulation of apoptotic cells in most radio- and chemo-sensitive tissues (Komarova et al. 2000) (Fig. 1). The observed apoptosis was p53-dependent because it was not seen in p53-null mice. Consistent with these findings, p53--deficient mice were capable of surviving doses of total body γ irradiation (TBI) that were lethal to p53-wild-type mice (Komarov et al. 1999). Interestingly, the rate of cellular proliferation in the tissues of p53-null mice was practically unchanged by TBI (judged by BrdU incorporation). In contrast, DNA replication indicative of blocked proliferation was dramatically reduced in p53-wild-type animals under these conditions (Komarova et al. 2000) (Fig. 2).

Taken together, these results showed that (1) p53 plays an important role in the radiation-induced cell death that produces radiation sickness, and (2) the proliferative index of a tissue does not necessarily determine its radiosensitivity. These conclusions had a strong impact on the interpretation of historically accumulated data from radiation biology regarding different pathological components of acute radiation syndrome (ARS). For example, p53 was defined as a critical determinant of the HP component of ARS, which involves massive loss of cells in all HP compartments (bone marrow, thymus, spleen, lymph nodes, etc.) (see Fig. 2) (Cui et al. 1995; Wang et al. 1996). This was based on the finding that p53-null mice were found to be resistant to the range of TBI doses that cause lethal HP syndrome in wild-type animals. Thus, the HP component of ARS is not primarily caused by irreversible damage to HP cells, but by their massive "voluntary" apoptotic

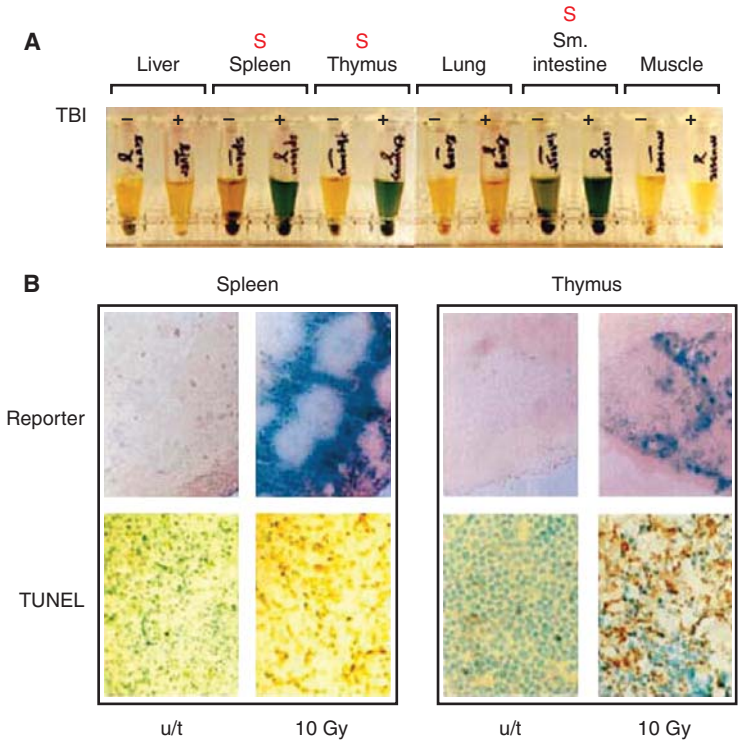

Figure 1. Tissue specificity of p53 activity. (*A*) Detection of β-galactosidase reporter activity in indicated tissue extracts from transgenic mice carrying p53-responsive lacZ and treated with 10 Gy of total body irradiation. Reporter activity is seen only in radiosensitive organs (marked with red "S"). (*B*) Massive apoptosis in tissues demonstrating strong response of p53-dependent reporter 8 h after irradiation detected using TUNEL staining for DNA fragmentation in situ. For details, see (Komarova et al. 1997).

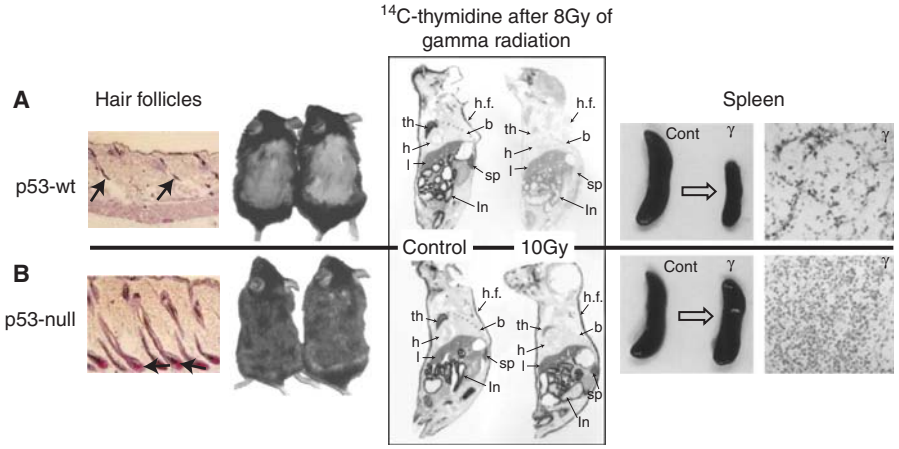

Figure 2. Biological effects driven by p53 in vivo. *Left* panel: mouse model of chemotherapy-induced alopecia shows resistance of hair follicles of p53-null mice to cyclophosphamide-induced apoptosis accompanied with lack of hair loss (Botchkarev et al. 2001). *Middle* panel: DNA replication block observed shortly after TBI (figure shows results obtained 24 h postirradiation) is p53-specific and is not seen in p53-null mice (Komarova et al. 2000). *Right* panel: massive cell loss occurring in the spleen 24 h post TBI (example shown for 10 Gy) because of massive apoptosis is p53-specific and is undetectable in p53-null mice (Komarova et al. 1997).

death triggered by p53. Interestingly, the involvement of p53 in the other major component of ARS, the GI component, does not follow the same paradigm (discussed in more detail later). However, the radiosensitivity of a number of other tissues was found to be p53-dependent.

As for the HP system, p53 was found to be responsible for the radiosensitivity of hair follicles that leads to radiation- and chemotherapy-associated hair loss. p53-null mice did not develop alopecia after treatments with either radiation or cyclophosphamide (a genotoxic chemotherapeutic drug) that caused hair loss in wild-type animals (Fig. 2) (Song and Lambert 1999; Botchkarev et al. 2001).

In addition, p53 was found to contribute to radiation damage to the spinal cord through p53-dependent apoptosis of oligodendroblasts (Chow et al. 2000). The lack of apoptosis in these cells in p53-deficient mice was accompanied by reduced frequency of paralysis following severe local irradiation of the spinal cord.

Finally, p53-mediated apoptosis was found to be responsible for the radiosensitivity of early embryos. Decades ago it was shown that there was a dramatic difference in the degree of radiosensitivity of mammalian embryos depending on the stage of embryonic development. For example, in mice, embryos are extremely radiosensitive during the first half of gestation (massive apoptosis is observed following exposure to less than 25% of the minimal lethal dose of TBI determined for adult animals), but highly radioresistant in the second half of gestation (the killing dose is equivalent to or higher than the minimal lethal dose for adults). This phenomenon was attributed to developmental regulation of p53 (MacCallum et al. 1996; Komarova et al. 1997) because (1) p53-null embryos did not show the same degree of stage-dependent differential radiosensitivity seen in wild-type embryos, and (2) strong down-regulation of p53 gene expression at the mRNA level was observed at the time of the switch from high to low radiosensitivity in embryonic development (Komarova et al. 1997) (Fig. 3).

Although the majority of studies of p53 pathologies associated with genotoxic stress have been performed in radiation models, it is likely that their conclusions can be applied to the genotoxicity caused by chemotherapeutic agents with some modifications because of specific pharmacological properties of certain drugs. One study that directly assessed the involvement of p53 in the toxicity of a

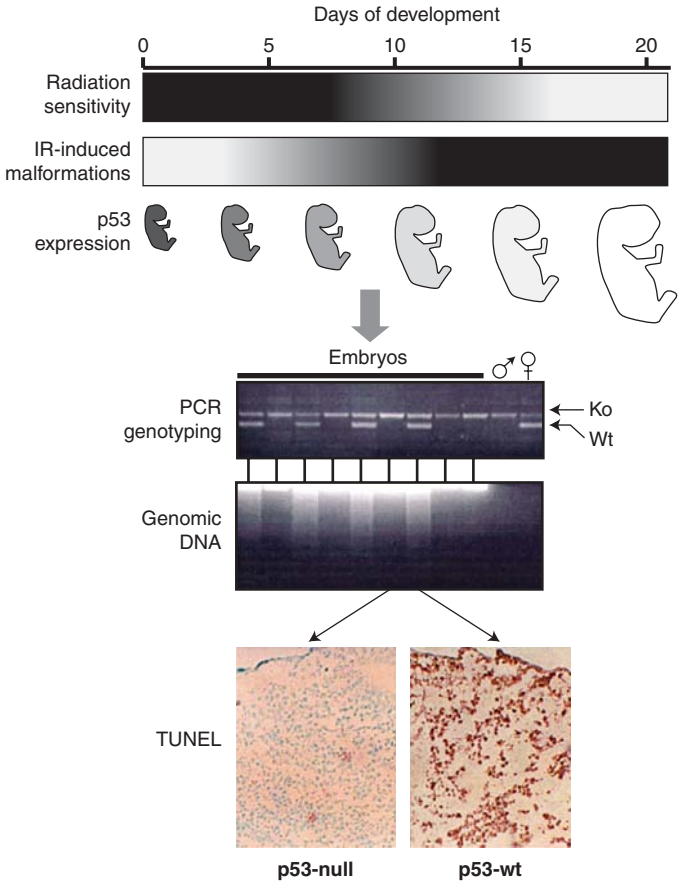

Figure 3. Radiosensitivity of early embryos is p53-driven (Komarova et al. 1997). *Upper* panel: schematic description of the correlation among the time stage-dependence of resistance to TBI (black-higher sensitivity), frequency of radiation-induced malformations and reduction of p53 occurring in mid gestation. *Lower* panel: differential response of embryos to TBI of pregnant female depending on their p53 status. Embryos were either p53-null or p53+/− because they originated from p53-null female and p53+/− male as shown by PCR genotyping. Massive apoptosis detected by TUNEL assay and by electrophoretic assessment of DNA degradation was restricted to those embryos that possess wild-type p53 allele.

chemotherapeutic agent focused on cisplatin and its induction of acute kidney injury, which limits its use and efficacy as an anticancer treatment. The finding that cisplatin-induced nephrotoxicity was abrogated in p53-deficient mice (Jiang et al. 2006; Wei et al. 2007) showed that p53 plays a critical role in the sensitivity of the kidney tissue to genotoxic stress produced by drug exposure.

Taken together, the data described earlier (primarily obtained through comparison of p53-null and wild-type mice) show that genotoxic stress-induced p53 activation can lead to apoptotic death of normal cells and produce tissue damage and pathologies that can limit the usefulness of genotoxic treatments as anticancer therapies.

THE ROLE OF p53 AS RADIOSENSITIZER IS STRICTLY TISSUE-SPECIFIC: p53 ACTS AS A SURVIVAL FACTOR IN THE GI TRACT

As mentioned earlier, the role of p53 in the radiosensitivity of the GI tract was found to be fundamentally different from the role it plays in the HP system. Although p53 is responsible for the HP component of ARS, it actually functions as a survival factor in the GI tract under

conditions of severe irradiation. This conclusion is based on studies performed in p53--knockout mice showing that lack of p53 dramatically increases the sensitivity of the small intestine to γ-radiation (Komarova et al. 2004). Although these mice do not develop HP ARS, they do acquire GI ARS and the symptoms of this syndrome are more severe than those that develop in p53 wild-type mice (Fig. 4A). This observation came as a surprise because p53 had been shown to induce apoptosis in several layers of proliferating cells in the crypts of the small intestine (above the presumed stem cell level) and death of these cells is the most obvious radiation-induced event in this organ (Clarke et al. 1994; Merritt et al. 1994) (Fig. 4B). It is now clear that this rapid p53-mediated apoptosis of intestinal crypt cells does not cause serious organ damage or significantly contribute to the GI component of ARS. Rather, it appears that GI radiosensitivity may be determined by vascular endothelial cells within the tissue. The heightened radiosensitivity of the small intestine in p53-deficient mice was found to be due, at least in part, to increased sensitivity of their vascular endothelial cells to genotoxic stress, which results in their p53-independent apoptosis (Burdelya et al. 2006). Vascular endothelial cells were previously defined through the work of Zvi Fukes and Richard Kolesnick as the primary targets of IR responsible for GI injury within the range of doses that damage the GI tract (Paris et al. 2001; Maj et al. 2003). The mechanisms underlying the protective role of p53 in endothelial cells remain unclear, but may involve the ability of p53 to keep wild-type cells arrested at cell-cycle checkpoints whereas p53-deficient cells undergo lethal mitotic catastrophe (Gudkov and Komarova 2003; Komarova et al. 2004). Another function of p53 that may contribute to its GI rescuing effect is its ability to activate the DNA excision repair machinery (Gudkov and Komarova 2003) (Fig. 4C).

These observations indicate that the pro-apoptotic function of p53, if exerted in cells critical for tissue function, can determine general radiosensitivity, whereas other p53 functions (cell cycle checkpoint control, DNA repair activation) contribute to radioprotection.

MECHANISMS UNDERLYING TISSUE-SPECIFIC EFFECTS OF p53

Tissue-specific differences in relative expression of distinct p53 functions (apoptosis, cell cycle checkpoint control, and DNA repair activation) determine the fate of specific cell types after exposure to genotoxic stress. Although some cell types are prone to a predominantly apoptotic response almost regardless of the severity of DNA damage (e.g., HP cells), others never undergo p53-dependent apoptosis, but rather respond to p53 activation exclusively with growth arrest (e.g., fibroblasts). The outcome of apoptosis versus growth arrest might be determined by differentiation-specific or stage of development-specific levels of p53 gene expression (see description of the radiosensitivity of embryos in legend to Fig. 3) or by differential activation of p53-regulated genes (e.g., because of differential availability of cofactors that regulate the ability of p53 to bind to specific subsets of target genes (Gudkov and Komarova 2003)). Recently, two newly identified p53 modulators were added to the list of known p53-interacting proteins that direct p53 to different subsets of target genes (Aylon and Oren 2007). Binding of the Hzf zinc finger protein to p53 favors its association with promoters of growth-inhibitory genes and decreases its association with promoters of proapoptotic genes. In contrast, CAS associates with p53 on the promoters of several proapoptotic genes within chromatin and activates methylation within the transcribed regions of the genes. This increases their transcription and promotes apoptosis (Das et al. 2007; Tanaka et al. 2007). Despite these new findings, tissue-specific regulation of p53 remains poorly understood and will likely be a major focus of future studies.

The rapid p53-dependent death of p53-wild-type animals exposed to severe systemic genotoxic stress from HP syndrome does not mimic any likely naturally occurring evolutionary scenario and in itself does not

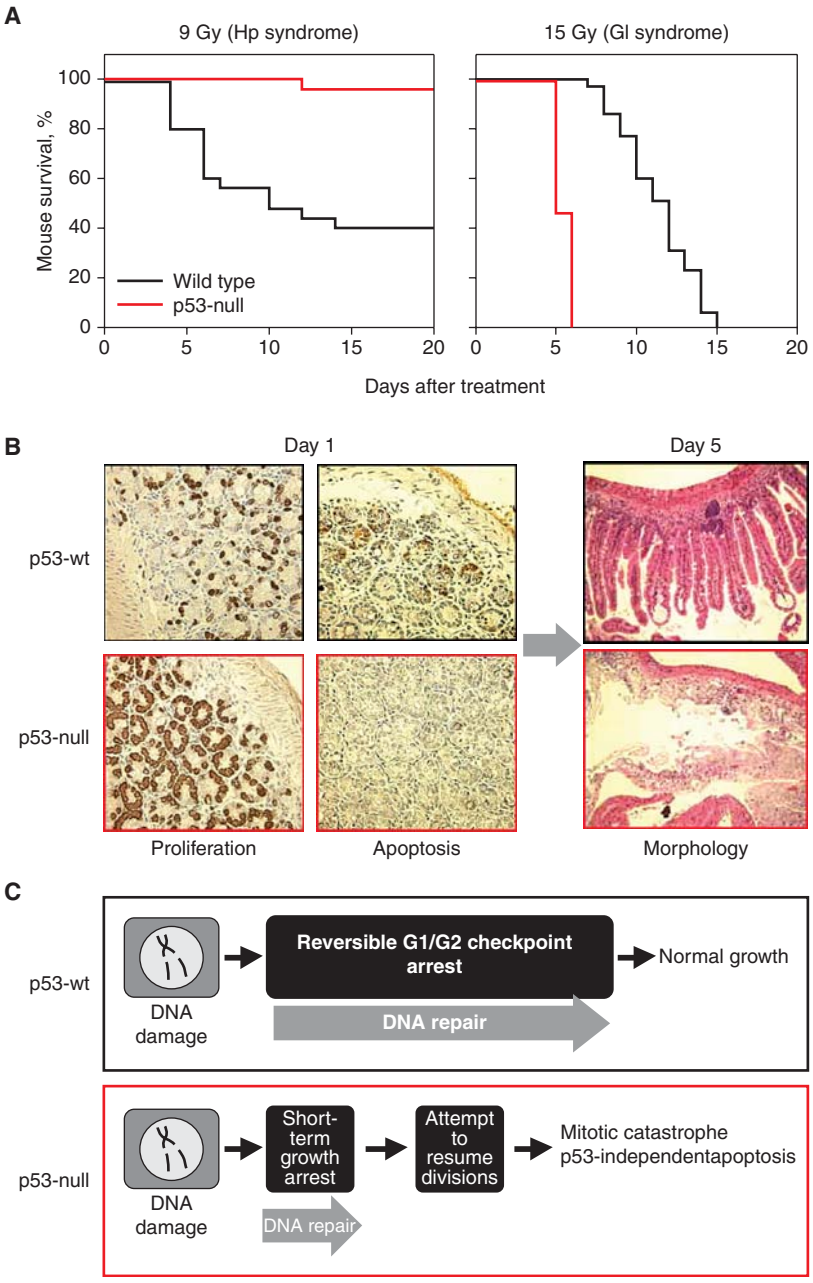

Figure 4. p53 is a survival factor in irradiated small intestine (Komarova et al. 2004). (*A*) p53-null mice are resistant to doses of TBI that induce hematopoietic (HP) but are hypersensitive to gastrointestinal (GI) acute radiation syndrome (ARS). (*B*) Complete destruction of the epithelium of small intestine in p53-null mice by day 5 after 15 Gy of TBI follows continuous proliferation and lack of early apoptosis observed on day 1 (8 h) post TBI. Growth arrest (determined by BrdU incorporation) and massive apoptosis in the crypts of p53 wild-type mice following TBI are associated with better preserved organ structure 5 d later. (*C*) Schematic interpretation of the earlier described results, which attributes radioresistance of p53 wild type small intestine to growth arrest function of p53.

obviously provide any selective advantage to the organism. However, it likely represents an exaggerated performance of the mechanism that normally plays a useful role by protecting the organism from the long-term pathological consequences of mutagenicity associated with sporadically occurring, less extreme, genotoxic stress. There is obvious "engineering sense," presumably supported by evolution, in having some tissues be more prone to p53-mediated apoptosis than others. The apoptotic response induced by activated p53 is characteristic of those cell populations (such as HP cells) that (1) are technically capable of rapid clonal expansion, (2) can be replenished through proliferation of stem cells (it is noteworthy that there is growing body of evidence indicating that pluripotent self-renewing HP stem cells respond to irradiation by growth arrest rather than apoptosis (Wang et al. 2006)), and (3) do not perform functions essential for tissue structure or integrity. Connective tissue and epithelial cells, which do perform essential tissue structure functions, typically respond to p53 activation with either reversible or permanent growth arrest rather than apoptosis. Growth arrest is an ideal response in these cases because it allows the cells to continue exerting their structural function without propagating possible genetic damage through proliferation.

This paradigm of tissue-specific p53 responsiveness is supported by the experimental data described earlier demonstrating that p53-dependent apoptosis is a critical determinant of the radiosensitivity of HP cells (as well as intestinal crypt cells, hair follicles, and other cell types), whereas p53-dependent growth arrest may be the prevailing response in cells such as vascular endothelial cells (as well as connective tissue and epithelial cells). The rapid development of lethal malignancies in p53-deficient mice leaves no time to appreciate the role of p53 (specifically p53-dependent apoptosis) in prevention of radiation-induced tumors. However, this role is well illustrated by the expedited development of tumors (predominantly lymphomas and sarcomas) in p53+/− mice treated with sublethal doses of γ-radiation (Kemp et al. 1994; French et al. 2001; Mitchel et al. 2003). On the other hand, the predominant involvement of the growth arrest function of p53 in irradiated connective tissue cells is shown by the finding that genotoxic stress-induced fibrosis develops much more quickly and is more severe in the lungs (E.A.K. and A.V.G., in preparation) and livers (Krizhanovsky et al. 2008) of p53-knockout mice than those of wild-type mice. In addition to the lack of growth arrest, deregulated cytokine secretion (because of alterations in nuclear factor κB [NF-κB] responses stemming from the lack of p53) may also contribute to this pathology (Komarova et al. 2005).

p53 AND PATHOLOGIES ASSOCIATED WITH ACUTE ISCHEMIA

p53 is a major regulator of developmental and DNA damage-induced apoptosis during embryogenesis (Armstrong et al. 1995; Sah et al. 1995; Komarova et al. 1997; Frenkel et al. 1999) and in neural precursor cells of the developing central nervous system (CNS) (Akhtar et al. 2006; Geng et al. 2007). In the adult brain, p53 controls self-renewal of adult neural stem cells (Meletis et al. 2006; Gil-Perotin et al. 2006), and loss of p53 leads to expansion of the stem cell/progenitor compartment (Gil-Perotin et al. 2006). Many specific death-inducible signals for neurons, including the excitotoxic agents glutamate and kainic acid (an analog of glutamate) and dopamine (a neurotransmitter) can induce p53-dependent apoptosis (Blum et al. 1997; Hughes et al. 1997; Sakhi et al. 1997; Uberti et al. 1998; Cregan et al. 1999; Daily et al. 1999; Inamura et al. 2000). DNA-damaging factors such as γ-radiation, treatment with anticancer drugs (camptothecin, etoposide), hypoxia, and oxidative stress can also activate p53-dependent apoptosis in neurons (Jordan et al. 1997; Banasiak and Haddad 1998; Johnson et al. 1998; Capranico et al. 1999; Takimoto et al. 1999). Neurons from p53-null mice are resistant to these stresses both in vitro and in vivo, and p53 overexpression induces apoptosis in them (Hughes et al. 1997). p53-associated apoptosis might be a common mechanism of cell loss in several neurological disorders including

Alzheimer's disease (Mattson et al. 1993; de la Monte et al. 1998), Parkinson's disease (Jenner and Olanow 1998) and stroke (Crumrine et al. 1994; Li et al. 1994; Li et al. 1997; Watanabe et al. 1999).

Ischemia-induced p53-dependent apoptosis of neurons plays an important role in the pathologies of cortical infarction and brain stroke (Covini et al. 1999; Tomasevic et al. 1999a; Tomasevic et al. 1999b; Watanabe et al. 1999; Zhao et al. 2001). The involvement of p53 in ischemic neuron loss is supported by the reduction of infarct volumes measured in p53 knockout mice and the increased neuronal p53 expression that temporally precedes neuronal death in the ischemic brain (Crumrine et al. 1994; Li et al. 1994). Ischemic preconditioning led to decreased p53 levels and resistance of neurons to subsequent ischemia (Tomasevic et al. 1999b; Maulik et al. 2000), presumably via NF-κB-mediated p53 suppression (Culmsee et al. 2003).

Heart ischemia is another common pathology associated with hypoxia. It can result in the apoptotic death of cardiomyocytes and is one of the frequent causes of fatal heart failure. Data are accumulating that implicate p53 in the regulation of cardiomyocyte death. The accumulation of p53 protein and occurrence of apoptosis was shown in reperfused ventricular heart tissue after coronary occlusion (Xie et al. 2000). p53-dependent apoptosis plays an important role in cardiac remodeling after myocardial infarction because p53+/− mice have significantly higher survival rate and lower incidence of left ventricular rupture after ligating the left coronary artery (Matsusaka et al. 2006) suggesting that this pathology may be suitable for treatment with p53 inhibitors (Mocanu and Yellon 2003).

p53 has also been shown to play a crucial role in mediating apoptotic cell death in renal ischemia-reperfusion injury (Wada et al. 2005; Wada et al. 2008), a common cause of acute kidney injury that is characterized by widespread tubular and microvascular apoptosis (Dagher 2004; Hochegger et al. 2007; Sutton et al. 2008; Singaravelu et al. 2009; Molitoris et al. 2009). It was suggested that an apoptosis-associated p53 transcriptional target PERP can play a key role in p53-mediated apoptotic pathways (Singaravelu et al. 2009).

In summary, p53 activity can contribute to pathologies originating from a variety of acute ischemia scenarios by strengthening the degree of tissue damage via broadening hypoxia-induced apoptotic zones.

OTHER POTENTIAL AREAS OF p53 PATHOLOGIES: p53 IN THE REGULATION OF INFLAMMATION AND AGING

There is a growing body of evidence indicating that p53 is a negative regulator of inflammation. Manifestations of autoimmune diseases including collagen-induced arthritis (Yamanishi et al. 2002) and experimental autoimmune encephalitis (Okuda et al. 2003) were found to be more severe in p53-deficient mice than wild-type mice. In addition, inflammatory infiltration of the lung and subsequent disruption of alveolar architecture caused by chronic exposure to the DNA damaging agent bleomycin was markedly increased in p53-null mice and transgenic mice expressing mutant p53 in the lung as compared with wild-type mice (Davis et al. 2000; Ghosh et al. 2002). Accelerated growth of atherosclerotic plaques was observed in p53−/−/apoE−/− mice as compared with p53+/+/apoE−/− mice and in LDL receptor-knockout mice that were lethally irradiated and transplanted with bone marrow from p53−/− mice as compared with the same mice transplanted with p53-wild-type bone marrow (Guevara et al. 1999; van Vlijmen et al. 2001; von der Thusen et al. 2002; Merched et al. 2003). The observed acceleration in plaque growth was associated with increased invasion of activated macrophages into the plaques. It was also shown that IR induces faster and stronger invasion of inflammatory cells and fibroblasts into damaged tissues in p53-null mice than in wild-type mice (Komarova et al. 2004). Finally, a significant proportion of p53-null mice (25%) die before tumor development from unresolved infections that result in abscesses, gastroenteritis, or myocarditis (Donehower et al. 1992), suggesting that the innate immune system is defective in these mice.

Consistent with the observations described earlier, we found that p53 is a general inhibitor of inflammation and that this activity is because of its antagonism of NF-κB (Komarova et al. 2005). This was first suggested by our observation of striking similarities in the global gene expression profiles of human LNCaP prostate cancer cells transduced with a p53-inhibitory genetic element or treated with TNF. This data suggested that p53 inhibits transcription of TNF-inducible genes, many of which are regulated by NF-κB. In support of this, ectopically expressed p53 was shown to inhibit transcription from NF-κB-dependent promoters. Furthermore, suppression of inflammatory responses by p53 was observed in vivo by comparing wild-type and p53-null mice at molecular, cellular, and organismal levels. For example, p53-deficient mice were significantly more susceptible to septic shock-inducing treatments than wild-type mice (Komarova et al. 2005). Recently, similar results were described in the work of Liu et al. (Liu et al. 2009). These observations indicate that p53, acting through suppression of NF-κB, plays the role of a general "buffer" of innate immune responses in vivo. This role is consistent with both the tumor suppressor function of p53 (because inflammation is frequently associated with tumorigenesis [Karin et al. 2006]) and the constitutive activation of NF-κB that is commonly observed in tumors, the majority of which are p53-deficient.

It has been well shown that p53 and NF-κB are involved in mutual negative regulation. For example, pro-inflammatory NF-κB-induced cytokines (such as IL-6 and MIF) can suppress p53 transcriptional activity (Yonish-Rouach et al. 1991; Hudson et al. 1999) and drugs suppressing NF-κB cause activation of p53 (Gurova et al. 2005). Nevertheless, many mechanistic questions about the role of p53–NF-κB interaction in regulation of inflammation remain to be resolved.

To date, the immunosuppressive function of p53 has been primarily considered in the context of its positive anti-inflammatory function. However, one can presume that there may be circumstances in which this p53 activity could be disadvantageous to the organism. This could happen, for example, if p53 weakened the immune response to a pathogen. An extreme example of this type of scenario would be complete disarmament of the immune system by p53 following from its activation by systemic genotoxic stress. This could result in death from infections. Such possibilities should be kept in mind when considering possible clinical applications of pharmacological inhibitors of p53, which in addition to their other properties, are expected to act as immunostimulators.

Regulation of inflammation by p53 is conceptually linked to another systemic pathology: aging. Although p53 has been implicated as a potential regulator of longevity and aging (Campisi 2002; Donehower 2002), the data currently available on its role in these processes are somewhat controversial. At the cellular level, p53 has been shown to mediate the irreversible growth arrest that occurs in fibroblasts undergoing replicative senescence (Gottlieb and Oren 1996). Gradual accumulation of senescent cells characterized by the so-called "pathological secretion phenotype" is considered one of the important systemic pathological alterations accompanying and possibly contributing to aging (Coppe et al. 2008). At the same time, negative regulation of AKT signaling by p53, which mechanistically mimics calorie restriction, provides further support for a putative antiaging role of p53 (Feng et al. 2008).

In vivo, a misbalance in p53 activity can result in different effects on longevity. For example, increasing potential p53 activity, by adding an extra copy of the p53 gene in transgenic "super p53" mice significantly protected them from cancer and did not show premature aging (Garcia-Cao et al. 2002). "Super Ink4a/Arf" mice carrying a transgenic copy of the entire Ink4a/Arf locus show higher resistance to cancer than wild-type mice and have normal aging and lifespan as well. Thus, modest increases in the activity of the Ink4a/Arf tumor suppressor result in a beneficial cancer-resistant phenotype without affecting normal viability or aging (Matheu et al. 2004). Also, mice in which Mdm2 expression was genetically reduced had normal lifespan and were resistant to tumor development (Mendrysa et al. 2003; Mendrysa

et al. 2006). However, mice in which overexpression of p53 was accompanied by an imbalance in the normal ratios of different p53 isoforms showed an alarming premature aging phenotype (Maier et al. 2004). Also, several knockout and transgenic mouse lines that showed increased p53 activity had premature aging phenotypes (Chin et al. 1999; Lim et al. 2000; Tyner et al. 2002; Cao et al. 2003; Wong et al. 2003; Varela et al. 2005). In some cases, these aging phenotypes were partially rescued by reduction of the p53 dosage (Purdie et al. 1994; Chin et al. 1999; Donehower 2002; Bauer et al. 2005).

In summary, despite some remaining controversies, the existing evidence shows a clear role for p53 in the negative regulation of inflammation and a possible role for p53 in the regulation of aging. Although p53-dependent pathologies related to these functions of the protein have not been described as such, they may arise from conditions of insufficient p53 function, such as the decline in p53 function observed in old organisms (Feng et al. 2007). In addition, as mentioned earlier, both hyperactivation and inactivation of p53 have the potential to alter inflammatory/immune responses in ways that may be unfavorable to the organism.

CAN FUNCTIONAL p53 BE USEFUL FOR TUMORS?

p53 is inactivated (directly or indirectly) in the vast majority of human tumors (Levine 1997; Soussi et al. 2000), and loss of p53 in tumors is associated with an unfavorable prognosis in many forms of cancer. This has led the p53 field to predominantly focus on the possibility of restoring p53 activity as a way to induce cancer cell death (Hupp et al. 1995; Bottger et al. 1997; Selivanova et al. 1997; Foster et al. 1999; Midgley et al. 2000; Bykov et al. 2002; Issaeva et al. 2004; Vassilev et al. 2004). The validity of this approach is supported by numerous demonstrations of the antitumor effect of p53 activated either by genetic manipulation (Ventura et al. 2007; Xue et al. 2007) or by small molecules (Selivanova et al. 1997; Vassilev 2005; Kravchenko et al. 2008) including drugs that inhibit the interaction between p53 and Mdm2, such as RITA (Issaeva et al. 2004) and Nutlin-3 (Vassilev 2005). The potential power of therapeutic activation of wild-type p53 was recently strengthened by works in which mouse tumor models were engineered to conditionally express p53. Three papers (Martins et al. 2006; Ventura et al. 2007; Xue et al. 2007) reported that restoration of p53 function in established tumors (including lymphomas, sarcomas, and hepatocellular carcinomas) caused regression of the tumors in vivo and could, therefore, represent an effective approach to treating cancer. In addition, a recent paper reported that treatment of a tumor in a patient with Li-Fraumeni syndrome with a replication-deficient adenoviral vector containing a human p53 cDNA under the control of the cytomegalovirus promoter (Advexin) was strikingly successful (Senzer et al. 2007).

Despite the demonstrations of p53's antitumor effect described earlier, there are also indications that p53 activity may not always be a beneficial factor in preventing tumor progression. Clinical studies indicate that death of tumor cells by apoptosis in response to treatment is characteristic of tumors that originate from tissues that are naturally prone to apoptosis (Kemp et al. 2001), such as HP and reproductive tissues. In most other cancers, treatment-induced apoptosis did not correlate with a decrease in clonogenic survival (Brown and Wouters 2001) or with a favorable prognosis (Nieder et al. 2000). Interestingly, the effect of p53 activation on tumor clearance was also shown to be tumor-type dependent. p53-dependent apoptosis was important for clearance of lymphomas (Martins et al. 2006; Ventura et al. 2007), whereas p53-dependent senescence was required for clearance of sarcomas (Ventura et al. 2007) and liver carcinomas (Xue et al. 2007).

p53 is inactivated by mutations in more than half of human malignancies and is not functional in the remainder because of alterations in other components of the p53 pathways (Johnstone et al. 2002). What is the role of p53 in those tumors that lack the p53-dependent

apoptotic pathway? Because p53 is responsible for the prolonged cell cycle arrest after IR treatment, it is expected to promote DNA repair in the absence of an apoptotic response. Therefore, tumors that inactivate p53 during progression should be less capable of DNA repair and more sensitive to DNA damage-induced mitotic catastrophe. Hence, in the absence of apoptosis, p53 can act as a survival factor. A remarkable example of the counterintuitive role played by p53 in determining sensitivity of some tumors to anticancer treatment is seen in human glioblastoma tumors (Kim et al. 2009). Glioma cells with wild-type p53 show higher resistance to cytotoxic treatments used in clinical practice than glioma cells with transcriptionally inactive mutant p53 (Hermisson et al. 2006; Batista et al. 2007; Roos et al. 2007). Inhibition of endogenous wild-type p53 augmented apoptosis in glioma cells in response to temozolomide and the chloroethylating nitrosoureas ACNU and BCNU (Batista et al. 2007). It was shown that wild-type p53 activities associated with DNA repair contribute to the overall survival potential and drug resistance of glioma cells. Clearly, in this case, the prosurvival DNA repair activity of p53 succeeds in overruling the apoptosis-inducing activity of p53. The possibility of p53 acting as a survival factor in some tumor cells is supported by a number of studies demonstrating enhanced cytotoxic chemotherapeutic responses in association with p53 inhibition in vitro and in different types of cancer (Pellegata et al. 1996; Peller and Rotter 2003; Sak et al. 2003; Scott et al. 2003).

By comparing tumor models differing in the p53 status of their stromas, we showed that tumors with p53-deficient stroma were significantly more sensitive to experimental chemo- and radio-therapy than tumors with p53-wild-type stroma (Burdelya et al. 2006). A similar effect was achieved when anticancer treatment was combined with pharmacological suppression of p53 by PFTα (see later). Potentiation of the anticancer effect of chemo- and radiotherapy by p53 suppression in the tumor stroma is likely because of increased sensitivity of p53-deficient endothelial cells to genotoxic stress, as shown both in cell culture and in experimental tumors.

In summary, tumors that retain wild-type p53 (which is usually not fully functional because of deficiencies in other components of p53 pathways) can benefit from the prosurvival functions of p53 either directly, by finding "smart" solutions to avoid its proapoptotic functions, or indirectly, through increased resistance of tumor vascular endothelial cells to anticancer treatment. Thus, reversible pharmacological suppression of p53 may be a viable approach to improving anticancer treatment via enhancement of the antiangiogenic effect of chemo- and radiotherapy.

POTENTIAL APPLICATIONS OF p53 INHIBITORS

The experimental evidence described earlier shows that p53-mediated apoptosis can significantly increase the severity of tissue damage under conditions of systemic or local genotoxic or ischemic stresses such as those associated with radio- and chemotherapy of cancer patients or present in patients with heart, brain or renal ischemia. Theoretically, in all of these circumstances, the severity of tissue damage could be reduced by pharmacological suppression of the p53 response. Although this was first described as only a theoretical possibility (Komarova and Gudkov 1998), we soon provided experimental evidence supporting this idea by isolating a small molecule inhibitor of p53 named pifithrin-α(PFTα) with radioprotective properties (Komarov et al. 1999). PFTα undergoes spontaneous circularization in solution forming a circular derivative named PFTβ which has similar properties in terms of p53 inhibition and radioprotection, but is more stable and less toxic than PFTα. Injection of either PFTα or PFTβ shortly before TBI rescued mice from doses of radiation that induce lethal HP ARS, but failed to protect against doses that induce GI ARS. This pattern of radioprotection is exactly as expected for a p53 inhibitor based on the different roles played by p53 in HP and GI radiosensitivity (see earlier).

We isolated small molecule p53 inhibitors with the expectation that they might eventually be developed into radio- and chemotherapy adjuvants capable of reducing the severity of adverse side effects of cancer treatment (Komarov et al. 1999; Komarova and Gudkov 2001; Gudkov and Komarova 2005). We presumed that the cytoprotective effect of p53 inhibitors would be limited to normal tissues (and not alter tumor cell killing), in cases in which the tumor has mutations inactivating the p53 pathway (Fig. 5). In reality, situation appeared to be even better: We were not able to identify any experimental tumor model, regardless of its p53 status, in which treatment with PFTα or PFTβ was beneficial (in terms of tumor escape from irradiation or chemotherapeutic drugs) possibly because p53 activity is universally impaired in tumors, even in those that retain wild-type p53. Furthermore, PFTβ administration was shown to increase the radio- and chemo-sensitivity of tumor vasculature (Burdelya et al. 2006), which is consistent with the earlier-described prosurvival role of p53 in endothelial cells (Gudkov and Komarova 2003).

Since its discovery in 1999, PFTα, as well as PFTβ and several more potent structural analogs of these compounds (Zhu et al. 2002; Pietrancosta et al. 2005; Pietrancosta et al. 2006), has been widely used as a tool to assess possible therapeutic applications of p53 inhibitors in animal models. In addition to studies on prevention of toxicity associated with radio- and chemotherapy (discussed earlier and reviewed in (Gudkov and Komarova 2005)), such studies have been performed in a variety of animal models mimicking different acute stresses that involve induction of p53-dependent apoptosis (e.g., ischemic heart, brain, and kidney injury) (Crumrine et al. 1994; Li et al. 1994; Li et al. 1997; Watanabe et al. 1999) or human disorders in which p53-associated apoptosis is suspected as a mechanism of cell loss (e.g., Alzheimer's and Parkinson's diseases) (Mattson et al. 1993; de la Monte et al. 1998; Jenner and Olanow 1998). The accumulated data show a protective effect of PFTα in different apoptosis-dependent pathologies. Here we briefly review these results.

PFTα and Neuropathology

Ischemia-induced p53-dependent apoptosis of neurons plays an important role in the pathologies of cortical infarction and brain stroke (Covini et al. 1999; Tomasevic et al. 1999a; Tomasevic et al. 1999b; Watanabe et al. 1999; Zhao et al. 2001). The involvement of p53 in ischemic neuron loss is supported by observations of reduced infarct volumes in p53 knockout mice and increased expression of p53 in neurons before their death in the ischemic brain (Crumrine et al. 1994; Li et al. 1994). Moreover, ischemic preconditioning led to decreased p53

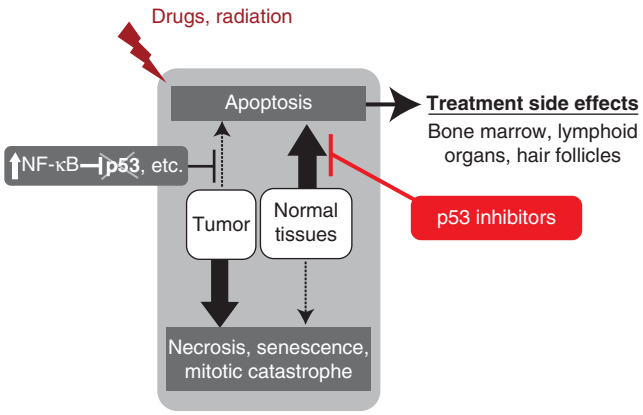

Figure 5. Schematic description of the strategy of pharmacological inhibition of p53 to protect mammalian organism from tissue specific toxicities associated with systemic genotoxic stresses.

levels and resistance of neurons to subsequent ischemia (Tomasevic et al. 1999b; Maulik et al. 2000). Using different animal models of transient global cerebral ischemia, it was shown that PFTα had a protective effect on hippocampal neurons as judged by the outcome of histological, motor, and behavioral assays (Culmsee et al. 2001; Leker et al. 2004; Endo et al. 2006; Gupta et al. 2007; Niizuma et al. 2009). Remarkably, even delayed application of PFTα (6–9 d after cerebral artery occlusion) enhanced the survival, proliferation, and migration to the subventricular zone of neural progenitor cells which died after ischemia-induced stroke in control animals (Luo et al. 2009). Thus, delayed treatment with PFTα is able to modify stroke-induced endogenous neurogenesis and improve functional recovery in stroke-affected animals. Because drug administration after the event is the only realistic scenario for use of supportive care drugs in stroke patients, the feasibility of using PFTα as a human treatment will largely depend on how long after stroke the drug can be administered to see protective effects.

In addition to its neuroprotective effects under ischemic conditions, PFTα (and a set of its novel functionally active structural analogs) protected primary hippocampal neurons against death induced by DNA-damaging drugs such as camptothecin and etoposide (Culmsee et al. 2001; Zhu et al. 2002; Martin et al. 2009). Culmsee et al. reported that the mechanism underlying this protective effect involves PFTα-mediated inhibition of the interaction of p53 with the transcriptional activator p300, whereas NF-κB binding to p300 was enhanced. Thus, PFTα suppressed p53 activity and preserved NF-κB activity, with a net effect of protecting neurons against apoptosis. A similar protective mechanism was also observed in neurons following glucose deprivation and in ischemic brain tissue (Culmsee et al. 2003).

In Alzheimer's disease, an association was proposed between p53 and amyloid β-peptide (ABP)-related neuronal death (LaFerla et al. 1996). PFTα protected against ABP-induced cell death in cortical primary neurons and differentiated neurons (Culmsee et al. 2001; Tamagno et al. 2003; Strosznajder et al. 2005; Uberti et al. 2007). The lysosomal branch of the apoptotic cascade (destabilization of the lysosomal membrane and concomitant increase in cytosolic cathepsin-L activity), which contributes to ABP-mediated neurodegeneration, was abolished by PFTα (Fogarty et al. 2008). Remarkably, in cultured cortical neurons, PFTα prevented cell death mediated by Delta9-THC (the psychoactive ingredient of marijuana) by the same mechanism of decreasing lysosomal permeability (Downer et al. 2007; Gowran and Campbell 2008). Inhibition of caspase activity by PFTα was also detected in neurons overexpressing presenilins, which modulate cell apoptotic responses during early onset Alzheimer's disease (Alvarez-Salas et al. 1999; Alves da Costa et al. 2003).

In mouse models of Parkinson's disease, both p53 knockout mice and mice pretreated with PFTα were protected from death of dopamine neurons in the substantia nigra pars compacta region of the brain (Trimmer et al. 1996; Duan et al. 2002; Nakaso et al. 2004; Perier et al. 2007; Karunakaran et al. 2008). PFTα was also shown to protect against neuron degeneration induced by 6-Hydroxydopamine (Biswas et al. 2005; Liang et al. 2007), proteasome inhibitors (Nair et al. 2006), and the herbicide paraquat (a suspected etiologic factor in the development of Parkinson's disease) (Yang and Tiffany-Castiglioni 2008). Moreover, PFTα inhibited glutamate- and kainite (an analog of glutamate)-induced p53-mediated neuronal death (Morrison et al. 1996; Xiang et al. 1998; Liu and Zhu 1999; Culmsee et al. 2001; Neema et al. 2005) and protected mouse synaptosomes against excitotoxic injuries (Gilman et al. 2003).

A role for p53 was also shown in the mitochondria-associated cellular dysfunction of Huntington's disease (HD). In neuronal cultures, the mutant huntingtin protein (mHtt) that is associated with HD binds to p53 and up-regulates its levels in the nucleus and its transcriptional activity. Perturbation of p53 function by PFTα, RNA interference, or genetic deletion prevents cytotoxicity in HD cells and mHtt-transgenic mice (Bae et al. 2005).

Finally, use of PFTα has shown a role for p53 in spinal cord cell death. PFTα reduced the toxic

effect of $FeSO_4$ applied to organotypic slice cultures of mouse spinal cord as a model of amyotrophic lateral sclerosis associated with spinal cord cell loss (Eve et al. 2007).

Thus, the results of numerous studies using PFTα and its analogs in mouse models of different neuropathologies clearly indicate that p53–mediated apoptosis plays an important role in these diseases and that pharmacological suppression of p53 presents a possible strategy for their treatment.

Prevention of Renal-, Cardio-, Liver-, and Oto-Toxicity by PFTα

p53 response can complicate a variety of renal pathologies. Cisplatin, a widely used chemotherapeutic drug, can induce acute kidney injury, which sets limitations on its clinical use. Cisplatin-induced nephrotoxicity is mediated by p53, as indicated by its absence in p53-deficient mice (Wei et al. 2007; Jiang et al. 2006). In addition, cisplatin-induced apoptosis of renal tubular cells was suppressed by PFTα both in vitro and in vivo (Jiang et al. 2004; Jiang et al. 2006; Wei et al. 2007; Yano et al. 2007). This protective effect of PFTα coincided with inhibition of expression of proapoptotic genes (Seth et al. 2005; Jiang et al. 2006; Tsuruya et al. 2008). Similarly, PFTα abrogated puromycin aminonucleoside-induced apoptosis of kidney cells (podocytes) (Wada et al. 2005; Wada et al. 2008). p53 also plays a role in ischemia-reperfusion injury, a common cause of acute kidney injury that is characterized by widespread tubular and microvascular apoptosis. Short-term inhibition of p53 with PFTα provided significant renal protection in mouse models of kidney ischemia-reperfusion (Dagher 2004; Hochegger et al. 2007; Sutton et al. 2008). In this scenario, PFTα-mediated protection correlated with modulation of HIF-1α and Von Hippel-Lindau protein expression.

Apoptosis was shown to be involved in cardiac damage following myocardium infarction (Xie et al. 2000; Mocanu and Yellon 2003). Consistent with this, PFTα significantly improved cardiac function in animals following myocardial ischemia/reperfusion which induces apoptosis in cardiac myocytes (Mocanu and Yellon 2003; Liu et al. 2006; Liu et al. 2008b). Doxorubicin is also known to cause both acute and long-term cardiotoxicity (in addition to general side effects that are common to all genotoxic drugs) (Horenstein et al. 2000; Kemp et al. 2001). PFTα injection inhibited doxorubicin-induced apoptosis in rat cardiac myoblasts in vitro and in mouse hearts in vivo (Liu et al. 2004; Chua et al. 2006; Liu et al. 2008a; Venkatakrishnan et al. 2008). Thus, PFTα could be a prototype for a novel cardioprotective drug that might be especially useful for treatment of cancer patients with preexisting heart conditions.

PFTα also protected normal hepatocytes against death induced by the genotoxic agent arsenic trioxide, ammonia, diterpenoids (oridonin), alkaloids (arecoline), mycotoxins, or LPS both in vitro and in vivo (Begum et al. 2002; Schafer et al. 2003; Farah et al. 2007; Bouaziz et al. 2008; Chou et al. 2008; Huang et al. 2008; Panickar et al. 2009). Leukocyte recruitment and microvascular dysfunction in the liver were also reduced in PFTα-pretreated animals exposed to these drugs. PFTα lowered the ratio of nuclear-to-cytoplasmic p53 and reduced activation of both NF-κB and caspase 3 (Schafer et al. 2003). PFTα effectively attenuated spontaneous apoptosis in liver grafts (rat liver transplantation model) as well (El-Gibaly et al. 2004).

In addition to its general genotoxicity and induction of renal toxicity, cisplatin also frequently causes complications in hearing (Rybak et al. 2007). Addition of PFTα to cisplatin-treated cochlear and utricular cultures resulted in an increase in hair cell survival (Zhang et al. 2003). This result suggests that p53 inhibition could be a feasible approach for reducing the ototoxic, vestibulotoxic and neurotoxic side effects of cisplatin, a strategy, which is currently being clinically tested.

PFTα can Rescue Mice from Developmentally Lethal Gene Defects

Pax-3 is essential for brain development and Pax-3 gene knockout is embryonically lethal.

The embryonic lethality observed in Pax-3 knockout mice results from a neural tube closure defect and increased apoptosis in neural tissue. This defect is not seen on a p53-null background, suggesting that excessive p53-dependent apoptosis is the cause of embryonic death. Remarkably, injection of pregnant Pax-3-deficient females with PFTα during a critical period of gestation prevented the neural tube defect in the embryos (Pani et al. 2002). Administration of PFTα also prevented the defective cardiac neural crest cell migration and apoptosis observed in Pax3-deficient embryos and restored proper development of cardiac outflow tracts (Morgan et al. 2008). Thus, PFTα provides a rare example of pharmacological rescue of embryonic lethality caused by a congenital defect.

PFTα and Autophagy

Autophagy is a cellular catabolic process that involves sequestration of portions of the cytoplasm in vesicles that then fuse with lysosomes to allow degradation of their contents. This process is important for normal cell growth and homeostasis and can promote cell survival under some stress conditions, for example by allowing starving cells to reallocate limited nutrients to essential cellular components and processes or to eliminate damaged cytoplasmic organelles. Thus, the increase in autophagy that frequently accompanies cell stress constitutes an attempt to adapt to and survive the stress (Levine and Kroemer 2008). It was shown that physiological levels of p53 inhibit autophagy (Tasdemir et al. 2008a), which may represent another mechanism by which p53 eliminates damaged (and potentially "dangerous") cells from the organism. The role of p53 as a negative regulator of autophagy is supported by work showing that deletion, depletion or PFTα-mediated inhibition of p53 induced autophagy in human, mouse and nematode cells (Tasdemir et al. 2008b). PFTα triggered autophagy in cytoplasts as well, thereby demonstrating that p53 inhibits autophagy through a cytoplasmic (nonnuclear) effect. Systemic administration of PFTα in mice also induced autophagy in such tissues as the cerebellum and heart. These studies suggest that induction of cytoprotective autophagy by p53 inhibitors could have potential therapeutic applications.

p53-Independent Effects of PFTα

Although most of the effects of pifithrins that have been published are consistent with their p53 inhibitory activity, it is clear that these compounds also have some p53-independent effects. For example, it was shown that PFTα is a potent agonist of the aryl hydrocarbon receptor (AhR) and that this activity of PFTα is not related to its p53 inhibitory properties (Hoagland et al. 2005). Consistent with this, PFTα abolished cardiac and neural crest-derived jaw and brachial cartilage defects induced in zebra fish by the environmental mutagen polychlorinated biphenyl, which exerts its effects through AhR (Grimes et al. 2008). In addition, it was shown that PFTα directly inhibits the catalytic activity of recombinant isoforms of the human cytochrome P450 superfamily of enzymes, CYP1A1, CYP1A2, and CYP1B1. Although it has yet to be examined in vivo, this observation provides a plausible explanation for the protective effects of PFTα against chemical carcinogens (Sparfel et al. 2006). Finally, at least in some cell systems, PFTα is capable of suppressing heat shock response and inhibiting glucocorticoid receptor signaling (Komarova et al. 2003; Murphy et al. 2004). These examples show that it is essential to use appropriate controls (e.g., comparison of PFTα effects in isogenic p53-null and p53-wild-type models) to determine whether experimental data can be interpreted as a p53-specific effect of the compound.

POTENTIAL SAFETY ISSUES ASSOCIATED WITH THE USE OF p53 INHIBITORS

The increased frequency of cancer that is observed in p53 deficient mice and men (Donehower et al. 1992; Jacks et al. 1994; Varley et al. 1997) presents the theoretical risk that use of p53 inhibitors to treat the various p53-dependent pathologies described earlier could have

carcinogenic side effects. However, accumulating experimental evidence argues against this. For example, there was no increase in cancer development in wild-type mice rescued from lethal irradiation by PFTα injection (Komarov et al. 1999). We also tested the effect of PFTβ on radiation-induced carcinogenicity in cancer-prone p53+/− mice, 100% of which develop tumors (mostly lymphomas and sarcomas) within 1 yr after sublethal total body irradiation (4 Gy) (Kemp et al. 2001; Mitchel et al. 2003; Perez-Losada et al. 2005). We found that treatment with PFTβ just before irradiation (the optimal regimen for improved survival) does not affect the timing, frequency, and spectrum of tumors that develop in the p53+/− mice (A.V.G. and E.A.K., in preparation). These findings indicate that temporary pharmacological inhibition of p53 does not increase the risk of tumor development.

The most convincing evidence showing that p53 does not need to be at a constantly high level to exert its tumor suppressor function was provided by a recent study from Gerard Evan's laboratory (Christophorou et al. 2006). They created a transgenic mouse model allowing for pharmacological switching between functional and nonfunctional forms of p53 by substituting the coding regions of p53 with an artificial construct encoding chimeric p53 fused to part of the estrogen receptor. Thus, hydroxytamoxifen treatment was used to switch phenotypically p53-deficient animals into phenotypically p53-wild-type animals. Using this elegant model, it was shown that switching off p53 function immediately after DNA damage protects animals from development of HP ARS but leads to subsequent rapid tumor development, as expected for p53-deficient animals. However, even transient restoration of p53 activity at remote time points after irradiation was sufficient to suppress tumor development, presumably because of elimination (through apoptosis or growth arrest) of cells with damaged DNA and heightened tumorigenic potential. An important conclusion that can be drawn from this work is that some responses to genotoxic stress involving p53-dependent apoptosis, such as widespread apoptosis in lymphoid organs and the intestinal epithelium, are not necessary for tumor suppression by p53 and are detrimental to the organism. Thus, both pharmacological and genetic evidence indicates that temporary and reversible suppression of p53 function does not significantly alter its tumor suppression function. This provides additional support for the potential usefulness of pharmacological suppression of p53 as the way to prevent and treat p53-related pathologies.

Another theoretical risk associated with use of p53 inhibitors stems from the fact that p53 acts as a survival factor in some specific tissues under conditions of genotoxic stress. For example, as mentioned earlier, studies using p53-knockout mice showed that lack of p53 dramatically increases the susceptibility of the small intestine to γ-radiation (Komarova et al. 2004). This property of p53-deficient animals was found to be due, at least in part, to increased sensitivity of their vascular endothelium to genotoxic stress (Burdelya et al. 2006), which was previously defined as the primary target of IR in the GI system (Paris et al. 2001; Maj et al. 2003). The mechanisms underlying the protective role of p53 in endothelial cells are not completely understood, but may involve the ability of p53 to protect cells from mitotic catastrophe by inducing cell cycle arrest (Gudkov and Komarova 2003; Komarova et al. 2004) and/or its ability to activate DNA repair (Gudkov and Komarova 2003).

Thus, the dual role of p53 in defining tissue and organismal survival provides an additional challenge for the use of p53 inhibitors. An ideal solution would be if one could separately target the tissue-damaging (apoptosis induction) and tissue-protecting (cell cycle arrest, DNA repair) functions of p53. We explored this possibility by attempting to identify small molecules capable of suppressing the proapoptotic activity of p53 whereas having no effect on other (mostly pro-survival) p53 functions. Our approach was based on findings from Uta Moll's lab demonstrating that p53 can induce apoptosis through a mechanism that does not depend on transactivation, but rather involves translocation of p53 to mitochondria (Marchenko et al. 2000; Mihara et al. 2003). In contrast, other p53

functions (growth arrest, DNA repair, etc.) are known to be exerted through the transactivation function of p53 (Prives and Hall 1999). This bifurcation of the p53 signaling pathway provides support for the possibility of pharmacological dissection of p53 functions by a small molecule. Using p53-mediated apoptosis as a cell-based readout, we screened libraries of chemicals and isolated a small molecule named pifithrin-μ (PFTμ). PFTμ inhibits p53 binding to mitochondria by reducing its affinity to the antiapoptotic proteins Bcl-xL and Bcl-2, but has no effect on p53-dependent transactivation. PFTμ rescues primary mouse thymocytes from p53-mediated apoptosis induced by radiation and protects mice from doses of radiation that cause lethal HP syndrome (Strom et al. 2006). These results indicate that selective inhibition of the mitochondrial branch of the p53 pathway is sufficient for radioprotection in vivo (at least for doses of radiation producing HP ARS) and show the feasibility of developing safe tissue-protective p53 inhibitors that preserve the pro-survival functions of p53 (Fig. 6).

TRANSLATION OF EXPERIMENTAL p53 SUPPRESSION INTO CLINICAL USE

The accumulated information described earlier provides numerous indications of potential clinical use of p53 inhibitors to prevent or treat p53-mediated pathologies. Such potential therapeutic uses include reduction of adverse toxicity associated with genotoxic cancer treatment regimens (radio- or chemotherapy), protection from or mitigation of acute radiation syndrome in biodefense scenarios (nuclear accidents or warfare), reduction of tissue damage in acute ischemic conditions (especially brain and renal ischemia, in which the role of p53 is well established), and treatment of neurodegenerative diseases. However, despite the significant experimental data supporting their therapeutic potential, there are currently no p53 inhibitors approved for any clinical use. Both PFTα-like "general" inhibitors of p53 and PFTμ-like inhibitors specifically targeting the pro-apoptotic branch of p53 function seem to be attractive prototype tissue-protecting agents and will hopefully be developed into drugs by the relevant intellectual property holders (see http://www.quarkpharma.com/qbi-en/newslist/us_patent_on_p53_inhibitor/).

The most clinically advanced version of a p53-inhibitory approach involves use of siRNA technology for in vivo p53 gene knockdown. The candidate p53-inhibitory drug QPI-1002 (a formulation of anti-p53 siRNA) is being assessed in ongoing Phase I/II clinical trials for prevention of kidney ischemia-reperfusion injury (in renal transplantation and cardiac surgery, see http://www.quarkpharma.com/qbi-en/products/qpi-1002/). The expectation is that p53 inhibition will reduce the frequency

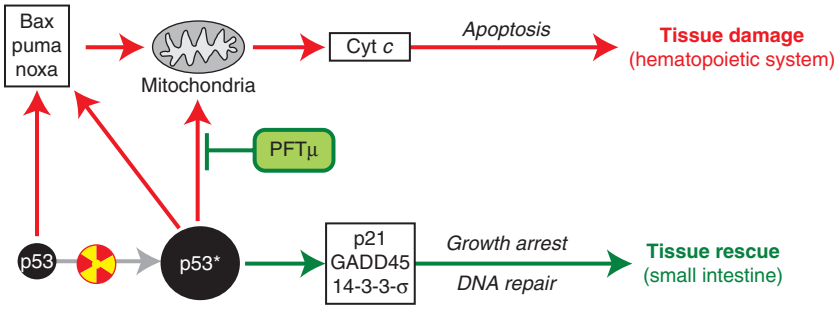

Figure 6. Existence of two branches in the p53 pathway allows pharmacological dissection of different p53 activities. By selective blocking of the mitochondrial branch of the pathway (by PFTμ) can protect from lethal hematopoietic radiation syndrome without affecting transactivation-mediated functions of p53 (growth arrest at cell cycle checkpoints, DNA repair, etc.), thus preserving tissue protective functions of p53. For details, see Strom et al. (2006).

of delayed graft function, which is a result of ischemia-reperfusion injury and is one of the most common complications during the immediate postoperative period in renal transplantation.

CONCLUDING REMARKS

We have reviewed here a series of situations in which the activity of wild-type p53 creates the risk of development of a serious—sometimes life threatening—pathology. Most of these situations are related to stresses associated with medical treatment of diseases (radiotherapy and chemotherapy of cancer, various types of surgery), whereas some reflect naturally occurring, acute, typically age-related pathologies (ischemias, neurodegenerative diseases). Research demonstrating that p53 inhibitors can reduce the severity of these pathologies underscores the practical importance of understanding the role played by p53 in these situations and presents a new strategy for treatment of conditions that, in many cases, are currently poorly treatable. Although development of these inhibitors into drugs has been delayed (predominantly because of theoretical safety concerns), recent advances in our understanding of the mechanisms underlying the cancer preventive role of p53 and experiments directly testing the carcinogenicity of p53 inhibitors have practically resolved these concerns. Based on the accumulated data illustrating both the efficacy and safety of prototype small molecule p53 inhibitors, we expect that development of clinically useful inhibitors will now be expedited.

Although the existence of p53-mediated pathologies initially came as a surprise, there is a clear conceptual similarity between this situation and another essential emergency response mechanism—the immune response. Similar to p53, the activity of the immune system is critical for protection of the organism from a number of extrinsic and intrinsic stresses, in this case through recognition and elimination of "foreign" components of endogenous (mutant cells) and exogenous (infectious agents) origin using a combination of innate and adaptive responses. And, as in the case of p53, overreaction of the immune system, can lead to disastrous outcomes (e.g., autoimmune disease, septic shock, etc.). Indeed, the potential danger associated with mechanisms responsible for emergency responses can be seen in virtually any social system, regardless of whether it is a society of cells or organisms.

Exploration of the concept of using temporary pharmacological suppression of p53 for tissue protection led us to consider the possibility of achieving similar effects through pharmacological activation of another stress response factor, NF-κB. Constitutive activation of NF-κB, like inactivation of p53, is frequently acquired by tumor cells as part of their survival strategy. Thus, we predicted that compounds capable of activating NF-κB in normal tissues would promote cell survival under stress conditions. Consistent with this prediction, we recently showed that bacterial flagellin, the natural agonist of Toll-like receptor 5 which activates NF-κB signaling in TLR5-expressing tissues, protects such tissues from radiation damage. Importantly, TLR5 is expressed in the critical cell types affected in both of the major components of ARS (HP and GI). Moreover, unlike p53 inhibitors which were found to increase the radiosensitivity of the GI tract, flagellin-based NF-κB activators were found to protect against radiation doses producing both GI and HP toxicity (Burdelya et al. 2006). This indicates that NF-κB-activating agents will be superior as compared with p53 inhibitors for tissue protection in the context of radiation toxicity; however, this will not necessarily be applicable to other pathologies and by no means downplays the potential clinical importance of p53 inhibitors.

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