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Commentary

Chemoprotection from p53-dependent apoptosis: potential clinical applications of the p53 inhibitors

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Abstract

The p53 tumor suppressor pathway is a key mediator of stress response that protects the organism from accumulating genetically altered and potentially cancerous cells by inducing growth arrest or apoptosis in damaged cells. However, under certain stressful conditions, p53 activity can result in massive apoptosis in sensitive tissues, leading to severe pathological consequences for the organism. One such situation is anticancer therapy that is often associated with general genotoxic stress, leading to p53-dependent apoptosis in the epithelia of the digestive tract and in the hematopoietic system. A chemical inhibitor of p53, capable of suppressing p53-mediated apoptosis, was shown to protect mice from lethal doses of gamma-radiation, making pharmacological suppression of p53 a perspective therapeutic approach to reduce the side-effects of cancer treatment. There are other situations, besides anti-cancer therapy, when humans are exposed to stressful conditions known to involve p53 activation, which, in extreme cases, could result in the development of life-threatening diseases. Here we review the experimental evidence on the role of p53 in tissue injuries associated with hypoxia (heart and brain ischemias) and hyperthermia (fever and burns), comparing these pathologies with the consequences of genotoxic stress of cancer treatment. The accumulated information points to the involvement of p53 in the generation of the pathological outcome of the above stresses, making them potential targets for the therapeutic application of p53 inhibitors.

Keywords: p53 Inhibitors; Apoptosis; Ischemia; Cancer therapy; Hyperthermia

1. Introduction

p53 is a key mediator of cell response to a variety of stresses, including DNA damage and abnormal growth regulation. It is encoded by a tumor suppressor gene that is functionally inactivated in the majority of cancers. p53 accumulates in the cells shortly after stress and acts as a nuclear transcription factor that modulates the expression of numerous p53-responsive genes. Activated p53 initiates a cascade of events that result in either growth arrest at one of the cell cycle checkpoints or apoptosis leading to the elimination of genetically altered cells, thus exerting its tumor suppressor function. p53 deficiency, in both mice and humans, is associated with genomic instability and the high frequency of cancer development [1–3].

However, under certain circumstances of severe stress, this generally important and useful function of p53 could lead to massive apoptosis in sensitive tissues that may result in severe negative consequences for the organism. One such situation is anticancer therapy that usually involves strong genotoxic stress (gamma-radiation, cytotoxic drugs) that cannot be specifically targeted against the tumor and, therefore, affects both normal and cancerous tissues. Cancer treatment is associated with the induction of p53-dependent apoptosis in normal cells of several sensitive tissues, such as the hematopoietic system and epithelia, resulting in severe side-effects. This led us to consider p53 as a potential target for therapeutic suppression to reduce the side-effects of cancer treatment, an idea that received experimental support in our recent work on the isolation and characterization of p53 inhibitors, which protected mice from lethal doses of irradiation. This result encouraged us to look for other clinical situations where temporary suppression of p53-mediated apoptosis by specific inhibitors could provide a therapeutic benefit.

Among the numerous stresses to which the human or-

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Abbreviations: CP, cyclophosphamide; PFT, pifithrin-α or p53 inhibitor; HT, hyperthermia; HS, heat shock; HO, hypoxia; and HIF-1, hypoxia inducible factor.

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ganism could be exposed, some are known to be capable of inducing a p53-mediated response in cultured cells and *in vivo*. Besides anti-cancer therapy with drugs and radiation, these conditions include hypoxia (such as heart and brain ischemias), hyperthermia (fever, burns), or UV irradiation. All of them in extreme cases could lead to severe consequences including the development of life-threatening diseases. In this commentary, we have made an attempt to review the available experimental data to estimate the likelihood of p53 involvement in the pathology resulting from the above stresses in order to determine whether treatment with p53 inhibitors could have therapeutic benefit.

2. p53 and the side-effects of cancer treatment

2.1. Negative effect of anticancer therapy on normal tissues

Cancer treatment with radiation and cytostatic drugs is associated with severe acute side-effects resulting from the damage caused by exposure of certain sensitive tissues, including the hematopoietic and immune system, the gastrointestinal (GI) tract, and the skin to these therapies [4–11]. Besides these general side-effects that are common for radiation and drugs, almost each type of treatment has its own set of specific pathological consequences. For example, anthracyclines (doxorubicine and others), besides their effects on the GI tract, myelosuppression, and alopecia, cause both acute and remote cardiotoxic effects [12]. The kidney is known to be the most vulnerable organ for ifosfamide and CP treatment [13], while methotrexate and cisplatin induce neurotoxicity [14,15]. The use of cisplatin is also often accompanied by complications in hearing [16]. The most usual delayed side-effect of gamma-radiation treatment is fibrosis, developing in treated organs [17] and in skin [18] and resulting from a replacement of functional epithelial tissues by abnormally proliferating connective tissue. The mechanisms for treatment-specific side-effects in most cases remain unclear.

2.2. p53 as a determinant of the toxicity of anticancer treatment

Cancer treatment with radiation and cytostatic drugs is also associated with the induction of genotoxic stress resulting from either direct DNA damage (radiation, anti-topoisomerase drugs, nucleotide analogs) or inability to undergo normal mitosis (anti-microtubule agents: e.g. Vinca alkaloids and taxol). Cell reaction to genotoxic stress *in vitro* involves activation of p53, which initiates a cascade of events leading to growth arrest or apoptosis. Transgenic mouse models have been used to determine the impact of p53 in tissue reaction to genotoxic stress *in vivo*. By analyzing mice expressing the *lacZ* reporter gene from p53-responsive promoters [19–21] it was found that whole-body gamma-irradiation or treatment with high dosages of DNA-damaging chemotherapeutic drugs led to a pronounced activation of the transgene, indicative of p53 activity, in the spleen, thymus, small intestine, and tissues of early embryos. Remarkably, p53-mediated transgene activation coincided with the most obvious areas of radiation or drug-induced apoptosis that did not develop in p53-deficient mice [22–28]. These areas, in turn, coincided with the sites known to be affected by anticancer treatment, suggesting p53 involvement in the treatment-induced damage of sensitive tissues. Interestingly, the level of expression of the *p53* gene in tissues appeared to correlate with their relative sensitivity to radiation. All of these facts indicate that p53 plays a key role in radiation and chemo-sensitivity of tissues, thus determining general radiosensitivity of the organism. In fact, p53-deficient mice can survive doses of radiation that are lethal for wild-type animals [29].

The side-effects of chemotherapy are not restricted to injuries of the inner organs but also include alopecia (massive hair loss), occurring shortly after treatment with different drugs. In a mouse model for chemotherapy-induced hair loss, mice with a synchronized hair cycle were treated with CP. This treatment resulted in complete alopecia followed by hair regrowth, imitating hair follicle response and histopathology seen in human chemotherapy-induced hair loss [30]. The drug treatment induced dystrophic changes in growing hair follicles, or in more severely damaged follicles—premature regression as a result of massive apoptosis in the entire proximal hair bulb, leading to subsequent hair loss [31]. By comparing the effects of CP on actively growing hair follicles in *p53* knockout and wild-type mice, we found that chemotherapy-induced apoptosis in hair follicles and subsequent hair loss was p53 dependent in wild-type mice, whereas neither apoptosis nor hair loss was seen in the CP-treated *p53* knockout mice [32]. Considering the similarity of chemotherapy-induced alterations in hair follicles of humans and mice, we presume that p53 is likely to play a similar role in hair loss in cancer patients during chemotherapy.

2.3. p53 suppression may reduce the side-effects of cancer treatment

Since p53-mediated apoptosis is likely to be one of the major determinants of the side-effects of cancer treatment, we presumed that therapeutic suppression of p53 could reduce the damage to normal tissues [33]. Obviously, this approach could be applicable only for those tumors that lack functional p53 and which themselves could not benefit from p53 suppression.

To explore this possibility, we isolated a chemical inhibitor of p53 and characterized its effects *in vitro* and *in vivo* [34]. This synthetic compound, named pifithrin-α (PFT, an abbreviation for "p-fifty-three inhibitor"), saved E1a+ras-

transformed mouse fibroblasts from apoptosis induced by UV and gamma-radiation, and different anticancer drugs. Suppression of p53-dependent apoptosis by PFT correlated with an increase in long-term cell survival, as judged by the results of colony growth assays. It was shown that although PFT can efficiently inhibit p53-dependent apoptosis, its effects are reversed in the absence of the drug.

Application of PFT *in vivo* demonstrated that a single i.p. injection of the compound rescued C57BL/6 and Balb/c mice from 60% killing doses of gamma-irradiation. Significant protection was also seen at higher doses of irradiation that were lethal for control animals. The extensive apoptosis observed in the intestine was reduced in mice treated with PFT before irradiation. No tumors or any other pathological lesions were found within one year of observation in the group of mice rescued from a lethal dose of gamma-irradiation [34]. Thus, temporary suppression of p53 appears to differ from complete p53 deficiency in terms of cancer predisposition (*p53*-null mice developed tumors within 6 months).

One of the most important conditions for the use of p53 inhibitors in cancer therapy was to confirm that they did not have a protective effect on p53-deficient tumors. In fact, injection of PFT did not jeopardize the efficacy of the anticancer treatment as the response of mouse experimental tumors to gamma-radiation and CP treatment was not affected (Komarova & Gudkov, in preparation).

All of these results formed the basis for potential therapeutic applications of PFT and other p53 inhibitors to reduce the side-effects of anticancer therapy, and encouraged us to consider the use of a similar approach to other pathologies that might involve p53 activation.

3. p53 and hyperthermia (HT)

3.1. HS can induce p53

HT is one of the most frequent naturally occurring stress factors. In the form of fever, HT accompanies any inflammation, including viral and bacterial infections. HT (artificially induced) is also used in anticancer therapy (alone or in combination with radiation and drug treatment) [35,36]. As opposed to long-term HT, leading to necrosis, short-term HT leads to the injury of cells by inducing apoptosis in them [37]. *In vitro* experiments demonstrated that in response to short-term HT (heat shock) in normal [38–41] and tumor [42,43] cells, p53 is stabilized and translocated to the nucleus, resembling the cellular response to DNA damage. p53 accumulation correlated with the increase of the level of HS proteins (hsp70, hsp72) [44] and the induction of p53-dependent apoptosis [37,42,45–48]. Experiments with HT induction *in vivo* demonstrated that it could induce apoptosis in the cells of certain tissues, including those of the intestine and hematopoietic systems [49]. This is similar to the p-53-dependent response observed in these tissues following genotoxic stress (see above).

The accumulation of p53 induced by HS as well as gamma-irradiation was found to be dependent upon the cell type. Analysis of a series of human tumor cell lines showed that accumulation of p53 in response to HS was most pronounced in those cells that exhibited the highest levels of radiation-induced p53, suggesting the involvement of common mechanisms of p53 stabilization after these stress factors [50]. Moreover, it was shown that both thermosensitivity and radiosensitivity were dependent upon the p53 status of the cells [19,51]. Furthermore, thermosensitivity of *p53*-null fibroblasts can be increased by transfection with wild-type *p53*. Consistently, the induction of apoptosis by HS could be inhibited by transfection of a dominant negative *p53* mutant [46,48,51,52].

The mechanics of p53 interaction with the HS response machinery and the role of HS factors in p53-dependent apoptosis remain poorly understood. HS proteins act as chaperones that, like p53, can be induced by a variety of stress factors. HS proteins were found in association with p53 that forms hetero-oligomers with hsp70, hsp90, cyclophilin 40, and hsp23 [53–58]. Cytoplasmic complexes are most likely formed to protect p53 from proteolysis and are probably involved in translocation of activated p53 from the cytoplasm to the nucleus for transactivation of other cell cycle control genes [53–58]. Nuclear translocation of wild-type p53 could be inhibited by transfection with an expression plasmid encoding the hsp70 family gene *mot-2* [52].

Similar to p53, HS proteins can be induced by a variety of stresses, not limited to HT, suggesting that chaperones might be common factors of different apoptotic pathways, including the cellular response to gamma- and UV-radiation, HS, and glucocorticoids. Exposure of tumor [58] and normal [59] cells to sublethal HS or ectopic overexpression of hsp70 can protect cells from gamma- or UV-radiation-induced apoptosis [58]. Moreover, introduction of an hsp70 antisense oligonucleotide prior to heating reversed the protective effect of hsp70. It has been suggested that hsp70 induction protects cells (thymocytes) from radiation-induced apoptosis by down-regulating p53 expression [59].

Geldanamycin, a selective hsp90-binding agent that alters the chaperone associations and regulates the function of steroid receptors, affected the stabilization of p53 and translocation to the nucleus [55–58,60–62]. Furthermore, the flavonoid quercetin, a suppressor of HS response, could also regulate p53 accumulation and induction of apoptosis in different cell types [63–66], indicating possible mutual regulation of p53 and HS responses. Bone marrow myeloid progenitor cells from p53-deficient mice are more resistant to both radiation-induced apoptosis and HS [24].

All these findings support the idea of p53 involvement in heat-induced cell death and make it worthwhile to consider p53 inhibition as an approach to reducing the pathological consequences of heat-induced damage.

3.2. Maternal fever as a risk factor for embryonic defects

One of the most dangerous consequences of elevated temperature associated with fever is its effect on developing embryos. Maternal HT is a proven teratogen in all mammalian species studied, including humans. HT often results in the appearance of different organ anomalies or spontaneous abortions [67]. An embryo must absorb a threshold 'dose' of heat if defects are to be caused, the dose being the product of the level and the duration of elevation above the normal maternal temperature. The lowest elevation causing damage is 2 to 2.5°C. The period of high susceptibility coincides with the time of organ induction. Studies with induced HT in pregnant animals defined the prevalent defects that were produced. It was shown that elevations of 2° and greater sustained over early rat organogenesis cause defects mainly in the developing central nervous system and include open neural tube, microencephaly, and microphthalmia [68]. Interestingly, a significant proportion of p53-deficient mouse embryos showed opposite pathological abnormalities in the same organs: they failed to undergo the normal process of neural tube closure, resulting in exencephaly [69] and ocular abnormalities [69,70] due to decreased apoptosis in the developing tissues.

Mammalian embryos are highly susceptible to stress caused by another physical factor, gamma-irradiation. It was found recently that the well-known phenomenon of high radiosensitivity of mammalian embryos is likely to be determined by the activity of p53. Strong correlation between embryonic radiosensitivity and p53 activity was found in mouse embryos in different stages of development [19,33,71,72]. Unlike wild-type mouse embryos, p53-deficient embryos do not develop massive apoptosis after gamma-radiation [19] and are characterized by a higher survival rate. However, increased viability of p53-deficient embryos after irradiation is accompanied by the development of malformations apparently originating from genetically damaged cells that remained alive because of the absence of p53 [73]. These observations determined a uniquely important role of p53 in embryonic development, allowing Hall and Lane to call p53 a "guardian of babies" [74].

Considering that HS and DNA damage are efficient inducers of p53 *in vitro,* that mammalian embryos are highly sensitive to both factors, and that radiosensitivity of embryos is p53 dependent, we can speculate that p53 might also be a determinant of embryonic sensitivity to HT.

3.3. HT in cancer therapy

Artificial HT is used as a treatment of certain types of tumors, including prostate, rectal, and breast tumors [75–79]. It is usually applied in combination with drugs and radiation therapy to increase the efficacy of the treatment [80,81]. It was shown that, aside from enhancing apoptosis of tumor cells, HT also leads to an increased apoptotic effect in normal tissues (thymus, ileum, hematopoietic cells) [82–84]. If HT-induced apoptosis *in vivo* appears to be p53 dependent, as was demonstrated for *p53* wild-type cells *in vitro,* p53 inhibitors could provide a useful approach to reducing HT-associated side-effects in normal tissues during treatment of p53-deficient tumors.

3.4. Does p53 contribute to the pathological consequences of severe burns?

Local and systemic effects of severe burns caused by heat or by UV irradiation are likely to involve a p53-mediated response. It was shown, for example, that the healing of a full-thickness burn wound in guinea-pig skin was correlated with increased expression of p53 protein and induction of apoptosis detected in the peripheral zone of the heat-injured skin [85]. A second-degree burn on the skin of rats induced apoptosis in the cells of the hair follicles [86]. Moreover, Wolf *et al.* [87] have shown that cutaneous burn damage is followed by injuries (apoptosis) in tissues distant to the place of burn damage, suggesting the involvement of secreted factors capable of inducing this effect. Interestingly, the affected tissues were the same ones that suffered from massive p53-dependent apoptosis after genotoxic stress, including thymus, spleen, gut, and skin [87,88]. There were no injuries detected in the lungs and liver, organs that express low levels of p53, which are also known to be relatively radiation resistant. These facts allow us to hypothesize that p53-mediated apoptosis can be involved in the determination of both local and distant pathological consequences resulting from burn wounds, opening the possibility for the use of p53 inhibitors in the reduction of the complications from burn-induced injuries.

4. p53 in ischemic diseases

4.1. Involvement of p53 in cell reaction to hypoxia (HO)

HO is one the most frequent natural stresses that can lead to the activation of p53. It was shown that exposure of different types of normal (trophoblasts, cardiomyocytes, neurons, endothelial and human diploid fibroblasts) and tumor (breast and colon carcinoma, glioblastoma) cells to HO or HO-mimetic drugs induced apoptosis, accompanied by increased expression of p53 [89–94]. Human tumor cell lines harboring a mutated p53 allele(s) exhibit reduced apoptosis under hypoxic conditions. Restoration of p53 function in these lines often re-establishes apoptotic potential [95]. Changes in p53 protein levels correlated with the induction of apoptosis in hypoxic endothelial cells. Overexpression of p53 by adenoviral transduction was sufficient to initiate apoptosis in endothelial cells. Proteasome inhibition, which increases p53 protein levels, also accelerated cell death in hypoxic endothelial cells [91]. Tumor cells can

undergo apoptosis in hypoxic areas of tumors that were shown to be p53 dependent [96,97].

Here we focus on the involvement of p53 in the HO-induced death of neurons and cardiomyocytes during brain and cardiac ischemia (HO), both causing substantial mortality during the later decades of life.

4.2. p53 and brain ischemia

4.2.1. Role of p53 in developmental, disease-associated, and stress-induced apoptosis in neurons

p53 plays an active role in neuronal development and regulation. Many specific death-inducible signals for neurons, including the excitotoxic agent glutamate and kainic acid, an analogue of glutamate [98–100], *N*-methyl-d-aspartate (NMDA) [100], quinolinic acid (an NMDA receptor agonist) [101], and dopamine (a neurotransmitter) [102], may induce p53-dependent apoptosis. Besides them, DNA-damaging factors such as gamma-irradiation [103–105] and treatment with the anticancer drugs bleomycin, ara-C, and etoposide [98,103,106–108] can also activate p53-dependent apoptosis. Serum deprivation [109] and HO [110] are also among the factors that can induce p53-dependent apoptosis. Neurons derived from *p53*-null mice are resistant to excitotoxicity and DNA-damaging agents both *in vitro* and *in vivo*, and p53 overexpression induces neuronal apoptosis in them [98].

Recent reports demonstrate the involvement of p53 in neuronal apoptosis in the normal development of the nervous system. p53-dependent apoptosis naturally occurs, for example, in the development of sympathetic neurons and is the result of two apoptotic signaling events: one normally suppressed by the nerve growth factor (NGF) survival signal and a second activated by the p75 neurotrophin receptor. In cultural neonatal sympathetic neurons, p53 protein levels are elevated in response to both NGF withdrawal and low-affinity nerve growth factor receptor (p75 NTR) activation. When p53 levels are reduced or absent (in $p53^{+/-}$ or $p53^{-/-}$ mice, respectively) naturally occurring sympathetic neuron death is inhibited [111]. It was shown that one of the primary regulatory steps in inducing apoptosis in ganglion cells is the activation of p53. Increased apoptotic death of ganglion cells in the optic nerve suggests the involvement of p53 in such diseases as glaucoma [112].

p53-associated apoptosis might be a common mechanism of cell loss in several important neurodegenerative diseases, for example, Alzheimer's disease or multiple system atrophy [113]. Catts and Catts [114] suggested that *p53* should be considered a candidate susceptibility gene in schizophrenia because increased apoptosis might account for neurodevelopmental abnormalities. All of the above indicates an important role for p53 in neuronal development and regulation of response to regulatory and stress signaling.

4.2.2. Mechanism of p53 involvement in HO-induced neuronal death

Recent data implicate the coordinate activities of p53 and a basic helix-loop-helix transcription factor, HIF-1, in driving ischemia-induced neuronal death [115–117]. Exposure to HO or HO-mimetic drugs has been shown to stabilize p53 and HIF-1α protein in immortalized cell lines. Furthermore, co-immunoprecipitation studies have suggested that HIF-1α stabilizes p53 through the formation of a hypoxic complex, which in turn enhances the transcription of known p53 targets [116,117].

It was found that HO could induce p53-dependent apoptosis in cortical neurons [110]. Experiments with HIF-1α-null embryonic stem cells and primary cortical cultures from newborn mouse brains, where HIF-1α activity was inhibited by overexpression of a dominant negative form of HIF-1α, revealed reduction of cell death in response to oxygen deprivation [115,118]. These results are consistent with a model in which HO-induced HIF-1α associates with and prevents the degradation of p53 protein [116]. Unlike the effect of ionizing radiation, HO induces p53 nuclear accumulation through Mdm2 down-regulation [119]. Ravi *et al.* [93] reported that p53 promotes Mdm2-mediated ubiquitination and proteasomal degradation of the HIF-1α. Loss of p53 in tumor and normal cells enhances HIF-1α levels and augments HIF-1-dependent transcriptional activation of the vascular endothelial growth factor gene (*VEGF*) in response to HO.

The molecular mechanisms of sensing and signal transduction by which HO results in changes in HIF-1 activity are not understood, but recent data suggest that the HO signal is converted to a redox signal [92,120] that may trigger a kinase cascade and/or regulate HIF-1 directly [121,122]. The identification of the HIF-1/p53-mediated signaling pathway in neurons highlights a novel target toward which anti-ischemic neuroprotective drug discovery can be applied.

4.2.3. Involvement of p53 in animal models of brain ischemia

An increase in p53 protein expression and the occurrence of DNA fragmentation in cerebral neurons after ischemia (permanent occlusion of the middle cerebral artery) were observed in a model of stroke-prone spontaneously hypertensive rats [123]. It was shown that 48 hr after rats have undergone 8–12 min of forebrain ischemia most pyramidal cells undergo death by necrosis, whereas dentate granule cells undergo death by apoptosis [124–126]. In addition, the selective expression of proteins associated with DNA damage and cell cycle, including p53, in apoptotic granule cells suggests a role for these proteins in the induction of apoptosis. Covini *et al.* [127] suggested that p53 plays two roles in the post-ischemic brain. The primary role of p53 is to activate DNA repair processes, but if repair fails, apoptosis may be initiated.

Ischemia-induced apoptosis can be p53 dependent [123, 125,127,128]. It was shown that neural p53-dependent apoptosis occurs in pathophysiological states such as cortical infarction and brain stroke. The involvement of p53 in ischemic neuron loss is supported by the reduction of infarct volumes measured in *p53* knockout mice and by increased neuronal p53 expression, which temporally precedes neuronal death in the ischemic brain [124,129].

It is interesting that ischemic preconditioning (3-min period of global forebrain ischemia) in the rat confers resistance to neurons against a subsequent, 10-min, ischemia, which is normally lethal to these cells. The results demonstrate that activation of p53 occurs in the brain following nonconditioned lethal as well as non-lethal ischemic insults. The level of p53 expression was markedly diminished in 10-min ischemia compared with 3-min ischemic preconditioning [125]. This inhibition of p53 might probably play a role in decreasing apoptosis in the neurons.

Experiments on mice with different *p53*-genotypes have shown that attenuated p53 expression may have a protective effect after an ischemic event. *p53*-null and *p53*-heterozygous mice were subjected to middle cerebral artery occlusion and the results were compared with those from wild-type *p53* animals [129]. Both mutant groups had significantly less ischemic damage than the wild-type mice. Unexpectedly, the heterozygous group had the least amount of ischemic damage [129]. Thus, although the absence of p53 expression was protective, greater protection was afforded by reduced expression of p53. We can expect that instead of ischemic preconditioning, the application of p53 inhibitors may induce the same effect.

Neurons undergo p53-dependent apoptosis following different traumatic brain injuries [130,131], a condition that is likely to be caused by the blockade of local blood access, resulting in HO. This was found in animal models for pneumatic and fluid percussion, and also in brain contusion [132–134]. In an experimental rat model for traumatic brain injury by fluid percussion, p53 was induced 6 hr after injury in the damaged areas of the brain. p53 expression returned to sham levels in all animals by 24 hr post-injury [133,135]. After cortical contusion induced in Wistar rats by a pneumatic impactor device [132], apoptotic cells were observed primarily 2 hr after the impact in the cortex adjacent to the site of injury. Apoptotic cell death peaked at 2 days. A great majority of the apoptotic cells (>95%) were neurons.

Moreover, overexpression of p53 was demonstrated also in patients with contused brain tissue. Samples of 20 patients who underwent emergency craniotomy and removal of mass lesions were analyzed immunohistochemically [136]. Significant levels of Bax protein were noted in all samples, and p53 was overexpressed in a significant part of the samples, which suggests a p53-dependent mechanism of apoptosis in brain injury. Thus, the use of agents to inhibit apoptosis (and p53) may be beneficial to patients with head injuries.

4.3. HO-induced apoptosis in cardiomyocytes

Heart ischemia is another frequent pathology associated with HO. It may 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. It was demonstrated that exposure of neonatal rat cardiac myocytes to HO resulted in intranucleosomal cleavage of genomic DNA, accompanied by increased p53 transactivating activity and p53 protein accumulation [90,137]. Infection of normoxic cultures with a replication-defective adenovirus expressing wild-type human p53 resulted in massive apoptosis, accompanied by a decrease of the Bcl-2/Bax ratio and a strong increase in the secretion of angiotensin II, triggering apoptosis [138,139]. Ectopic expression of the antiapoptotic gene *bcl-2* was sufficient to prevent apoptosis provoked by p53 [139]. These results show that cardiomyocytes are susceptible to p53 activation and suggest that the intracellular signaling pathways activated by p53 may play a critical role in the regulation of hypoxia-induced apoptosis of cardiomyocytes [137]. It remains unclear though, whether hypoxia itself serves as a trigger of programmed cell death or whether apoptosis is induced by hypoxia-associated factors, such as acidosis [140] or the accumulation of reactive oxygen species [92].

In addition, p53 might contribute to cardiac myocyte death following exposure to other types of stressors. For example, it was found that stretching *in vivo* also could activate p53 and p53-dependent genes in myocytes, leading to the production of angiotensin II and apoptosis. Insulin-like growth factor (IGF1) inhibits apoptosis via the induction of Mdm2 and the formation of Mdm2–p53 complexes [141,142]. The activation of p53 and p53-dependent genes may be critical in the modulation of myocyte apoptosis in pacing-induced heart failure [143] and in congestive heart failure (CHF) of patients during cardiac transplantation [144].

In vivo, cardiomyocyte apoptosis, accompanied by enhanced expression of p53 and Bax proteins, was detected in the rat after pulmonary arterial banding [145]. Moreover, acute and remote cardiac toxicity is one of the most serious side-effects of such a broadly used chemotherapeutic drug as doxorubicin [12], which is known to be a potent activator of p53 [146].

In summary, although the evidence on the involvement of p53 in stress-induced apoptosis in cardiomyocytes is incomplete and somewhat controversial, we can conclude that p53 is likely to be a player in cardiac myocyte death under a variety of pathological conditions, opening a new arena for therapeutic applications of p53 inhibitors.

5. Concluding remarks

p53 has been traditionally viewed through the scope of its tumor suppressor function. In fact, it is frequently inac-

tivated in tumors, and tumor cells in most cases are extremely sensitive to p53 and can be efficiently killed by ectopic expression of this protein. These facts suggested that p53 could be used for cancer treatment by (i) direct delivery to cancer cells [147,148] or (ii) its activation with specific peptides [149,150] or small molecules [151]. With the accumulation of evidence regarding the role of p53 in normal physiologic processes, it became clear that the price for its tumor suppressor function is the hypersensitivity of certain normal rapidly proliferating tissues to a variety of stresses, including those associated with anticancer treatment [33]. These sensitive tissues define the overall radiosensitivity of the mammalian organism, which can survive much higher doses of radiation if p53 is inactivated or suppressed [29]. A new therapeutic concept of temporary pharmacological suppression of p53 to reduce the side-effects of cancer treatment was suggested, based upon these observations. This approach is possible because the majority of tumors lose the expression of p53 and, therefore, cannot benefit from p53 suppression. Rescue of mice from lethal doses of radiation by a chemical inhibitor of p53 became a proof of principle for this concept [34].

However, p53 can trigger apoptotic cell death in response to a variety of other stresses besides cancer therapy. In this review, we analyzed experimental evidence supporting the involvement of p53 in apoptosis induced by HT and HO. Unlike genotoxic stress, there is no unequivocal proof that p53 is the major determinant of tissue sensitivity to these factors. Additional experiments with p53-deficient mice and with p53 inhibitors are required before we can conclude that p53 suppression could really make a difference and reduce the rate of fatalities in, for example, ischemic diseases. Nevertheless, the collected information provides, in our opinion, a strong rationale for experimental testing of this possibility.

As in the case of cancer treatment, safety is an obvious issue in potential clinical applications of p53 inhibitors. Although we have demonstrated that radioprotection by a p53 inhibitor was not associated with a high increase in tumor frequency in mice, even short-term p53 suppression could result in the survival of genetically altered cells that otherwise would be eliminated by apoptosis. The risk/benefit ratio could be very different for different diseases. While such risk is probably worth taking in the case of life-threatening diseases in adults (cancer, stroke, severe burns), the use of a similar approach to protect embryos from maternal fever seems less attractive, considering the probable increased risk of developmental malformations. However, any conclusions would be premature at the current level of knowledge.

The use of p53 inhibitors, as opposed to general inhibitors of apoptosis, is well justified for the relief of the side-effects of cancer treatment. In this case, suppression of p53, unlike general inhibition of apoptosis, should provide selective survival benefits to normal tissues but not to p53-deficient cancer cells. Even if the critical role of p53-dependent apoptosis in the pathologies associated with HT or HO is confirmed, it does not necessarily mean that p53 inhibition would have advantages over general inhibitors of apoptosis (i.e. caspase inhibitors) that are already being tested in ischemic cells and animal models [152–155]. One potential benefit of p53 inhibitors is that they are expected to block not only apoptotic function of p53 but also its other activities, such as the induction of secretion of biologically active factors by stressed cells that might affect neighboring cells [156]. All of these issues should be experimentally tested.

Note added in proofs

While this review was in preparation, Culmsee et al. (J. Neurochem. 2001, 77, 220–228,) reported that a synthetic inhibitor of p53 (PFT) protects neurons against death induced by ischemic and excitotoxic insults, thus providing additional evidence in favor of the hypotheses described in this Commentary.

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