Introduction

The p53 tumor suppressor has been termed ‘guardian of the genome’ [1] because of its nodal position linking many different pathways that safeguard the integrity of genetic information in response to various genotoxic and non-genotoxic insults [2]. Pivotal to the tumor-suppressor activity of p53 is its ability to activate apoptosis via multiple different pathways [3]. Since the most-studied function of p53 is its role as a transcription factor that can activate transcription of an ever-increasing number of target genes, its transcriptional activation of pro-apoptotic genes, as well as its transcriptional repression of anti-apoptotic genes, has been widely analyzed [3,4]. However, although a large number of genes regulated by p53 during induction of apoptosis are known [3,4], no single target gene has been identified whose altered expression alone can sufficiently explain p53 apoptosis, and whose genetic deficiency phenocopies p53 deficiency in vivo.

As an additional mode of p53’s pro-apoptotic activity, recent studies have placed non-transcriptional pro-apoptotic activities of p53 at the center of an active debate that aims to establish a comprehensive understanding of p53-mediated apoptosis.

Here we summarize the most important advances that have been made during the last two years and have substantially improved the knowledge regarding transcription-independent pro-apoptotic functions of p53. We thereby focus on mechanistic aspects by which p53 influences the intrinsic, mitochondrial pathway of apoptosis and discuss how its different modes of apoptosis induction may converge.

Non-transcriptional p53-mediated apoptosis – a long known puzzle

Despite the prominence of its transcriptional activities, the contribution of non-transcriptional activities to the pro-apoptotic effects of p53 is receiving increasing attention. The initial suggestion that p53 might promote transcription-independent apoptosis was made quite a while ago in p53-deficient cells stably expressing a temperature-sensitive mutant p53 protein (Val135). In these cells, induction of apoptosis by DNA damage strictly depends on p53 function. Upon shift to the permissive temperature, p53Val135 transcriptionally upregulated p53 targets and induced apoptosis, as expected [5]. However, when these cells were treated with drugs that block transcription or translation, upregulation of p53 targets was abolished while apoptosis still ensued [5]. Usage of such drugs has been a powerful tool to study transcription-independent activities of p53 since activation of p53 largely relies on post-translational effects, i.e. stabilization and modification of the protein, and thereby is not impaired by inhibitors of transcription or translation in common experimental settings. Further seminal studies then confirmed the occurrence of non-transcriptional p53-mediated apoptosis in other experimental settings and showed that even certain transcriptionally inactive mutant p53 proteins can induce apoptosis under specific conditions [6–8]. However, for a long time the idea of non-transcriptional functions of p53 in apoptosis was received by the scientific community with caution, mainly because of the lack of any mechanistic insight.

A major breakthrough has come from recent studies linking the transcription-independent apoptotic activity of p53 to the intrinsic mitochondrial apoptotic pathway.
Interestingly, two independent yet converging mechanisms have been described by which the p53 protein directly induces permeabilization of the outer mitochondrial membrane (OMM), thereby enabling cytochrome c release and effector caspase activation.

**Mitochondrial p53 and apoptosis induction**

The initial observation was that a fraction of induced p53 rapidly translocates to the mitochondria upon apoptotic stimuli [9,10]. The generality of this finding was widely confirmed and laid the foundation for a direct mitochondrial p53 death program [11**–14**,15–20]. In response to a broad spectrum of apoptotic stimuli, a fraction of wild-type p53 translocates to mitochondria in cell lines, in primary cells and in vivo in mice [9,10,11**]. P53 mitochondrial translocation occurs very rapidly, and precedes cytochrome c release, the collapse of the mitochondrial membrane potential and caspase-3 activation [9]. The majority of mitochondrial p53 localizes to the outer membrane [10], although a subfraction is found in complex with the major mitochondrial import proteins mt hsp70 and mt hsp60 in the mitochondrial matrix [9,12**]. The evidence for a direct apoptogenic role of p53 at mitochondria was then extended. Targeting p53 to mitochondria by fusing it with mitochondrial address peptides, thereby bypassing the nucleus, is sufficient to launch acute apoptosis and chronic suppression directly from the mitochondrial platform [9,11**]. In accordance with this model, the different apoptotic potentials of the two frequently occurring polymorphic variants (codon 72, Arg/Pro) of human p53 perfectly correlated with their different abilities to localize to mitochondria [12**]. Furthermore, the differences in the apoptotic potentials of the Arg/Pro p53 variants could be eliminated when these proteins were coupled to a leader sequence that directed both p53 variants equally well to mitochondria [12**].

Endogenous mitochondrial p53 physically interacts with the Bcl-2 family member proteins Bcl-xL and Bcl-2 and antagonizes their anti-apoptotic stabilization of the outer mitochondrial membrane [11**,17**,21**]. For example, pro-apoptotic Bcl-2 family members Bid and Bax that have been sequestered by Bcl-xL in a preformed complex are liberated by recombinant p53 protein in vitro [21**]. Using computational and mutational structure/function studies, it was determined that the p53 DNA interface and the BH14 domain of Bcl-xL are involved in the formation of the p53–Bcl-xL complex. This result was confirmed by a structural NMR study [22**]. Conversely, tumor-derived transactivation-deficient missense mutants concomitantly lose or compromise their ability to interact with Bcl-xL and to promote cytochrome c release [11**]; UMN Moll, unpublished). Likewise, in vitro, purified wild-type p53 protein induces rapid and robust outer mitochondrial membrane permeabilization (MOMP) and promotes cytochrome c release [11**,13**,14**]. This activity is dependent upon p53/Bcl-xL complex formation, since p53 mutants with impaired Bcl-xL binding concomitantly lose their ability to promote cytochrome c release. Thus, tumor-derived p53 mutations may represent ‘double hits’, eliminating the transcriptional as well as the direct mitochondrial functions of p53. Therefore, one mechanism of mitochondrial p53-mediated permeabilization of the outer mitochondrial membrane and rapid induction of cytochrome c release involves BH3-like actions of derepression, in other words the inhibition of the anti-apoptotic Bcl-2 family members. Mitochondrial p53 also directly promotes the pro-apoptotic activities of Bak [11**,13**,14**] and directly induces Bak oligomerization [11**,13**]. After camptothecin treatment or ultraviolet irradiation stress, mitochondrial p53 physically interacts with the outer membrane resident protein Bak, thereby liberating Bak from its complex with the anti-apoptotic Bcl-2 family member Mcl-1 [13**]. Hence, mitochondrial p53 has a dual action of neutralising anti-apoptotic members as well as activating pro-apoptotic members of Bcl protein MOMP regulators.

The first compelling in vivo evidence that mitochondrial p53 contributes to the induction of apoptosis was provided by a recent study monitoring the kinetics of p53 translocation to mitochondria upon DNA damage in mice (resulting from 5 Gy γ-irradiation or intravenous etoposide of a single clinical dose) [14**]. Mitochondrial p53 accumulation occurred in radiosensitive organs like the thymus, spleen, and testis, but not in radioresistant organs like the liver or kidney. In this context, radiosensitivity is defined as the tissue response to irradiation that is mediated by p53 induction and induces apoptosis [23,24], which is but one measure of the response to radiation. Importantly, the apoptotic response in the radiosensitive tissues occurred in two waves. Upon γ-irradiation, p53 rapidly translocated to mitochondria (within 30 min in thymus and spleen) and triggered a first wave of caspase-3 activation. Notably, the transcriptional p53 response, as monitored by upregulation of p53 targets, occurred significantly later and was followed by a second wave of caspase-3 activation [14**]. Similar biphasic kinetics of mitochondrial versus nuclear p53 action and apoptosis were seen in cultured human cells [14**]. This result was further extended using isogenic PUMA−/− and PUMA−/+ animals. Although PUMA is one of the earliest and most important transcriptional effectors of p53 in γ-irradiation-induced apoptosis, the direct p53 mitochondrial pathway triggered an early apoptotic wave in a sub-population of cells in the thymus, spleen, jejunum, ileum and rectum of PUMA−/− mice (UM Moll, unpublished). This indicates that the direct mitochondrial p53 death pathway is sufficient for the initiation and completion of irradiation-induced apoptosis in a subpopulation of cells in vivo.

Moreover, in a rat model of acute ischemic injury to the kidney, p53 translocation to the mitochondria occurred during apoptosis and contributed to the apoptotic cell...
death program in tubular epithelial cells [25]. Also, after oxidative stress, primary rat cortical astrocytes underwent apoptosis characterized by p53 mitochondrial targeting followed by cytochrome c release and nucleosomal fragmentation [26]. p53 levels are elevated in the distal synapses of cortical neurons after DNA damage and in isolated cortical synaptosomes after oxidative and excitotoxic stress [27]. Neuronal apoptosis triggered by oxidative stress has been implicated in the pathogenesis of neurodegenerative diseases, traumatic brain injuries and stroke [28].

Cytosolic p53 and apoptosis induction

In a different series of studies, the ability of p53 to initiate permeabilization of the OMM in a transcription-independent manner could be assigned to activation of Bax. In contrast to Bak, which constitutively resides at the OMM, Bax is largely present in the cytosol in non-stressed cells [29,30]. Upon activation, Bax undergoes conformational changes, homo-oligomerizes and inserts into the OMM, thereby inducing membrane permeabilization, cytochrome c release and caspase-3 activation [29]. Elegant studies showed that cytosolic p53 can directly activate Bax and thereby induce apoptosis [21,31], although the exact mechanism by which p53 activates Bax is still an open question. In initial experiments, the apoptosis-inducing potential of cytoplasm (i.e. enucleated cells) from p53-overexpressing cells was demonstrated by adding them to mitochondria derived from p53-deficient cells [32]. Notably, p53-dependent cytochrome c release from isolated mitochondria was abrogated when Bax was immune-depleted from the reaction mix, indicating that the apoptotic potential of p53-containing cytoplasm required Bax [32]. Conversely, activation of Bax in the context of UV-induced apoptosis was demonstrated to be strictly p53-dependent in mouse fibroblasts [33]. Taken together, these studies indicate the importance of a functional crosstalk between Bax and p53 for cytosolic p53-mediated apoptosis.

Compelling evidence that p53-mediated activation of Bax is indeed transcription-independent was provided in different experimental settings. In cells expressing p53 as a fusion protein coupled to a pharmacologically activatable hormone receptor, Bax-mediated apoptosis could also be induced when nuclei were removed before p53 activation [34]. Thus the physical presence of the p53 protein in the cytoplasm and not its transcriptional activity appears to be responsible for the apoptotic effect. Further proof of such a direct transcription-independent activation of Bax by p53 was obtained in a simplified in vitro system. Addition of purified p53 to isolated mitochondria or synthetic liposomes led to oligomerization of recombinant Bax and triggered permeabilization of the organelles or the artificial membranes [21]. Hence, it became clear that at least in vitro p53 is capable of directly activating Bax without active transcription. The assignment of the Bax-activating capacity of p53 to the cytosolic fraction of the tumor suppressor was based on studies with wheat germ agglutinin, a drug that inhibits nuclear import of p53 in mouse embryo fibroblasts [21]. As expected, such treatment impaired the upregulation of p53 targets. However, the p53 protein that accumulated in the cytosol efficiently induced Bax-mediated apoptosis [21]. In vivo evidence demonstrating the physiological relevance of this mechanism was recently provided by Speidel et al. [33]. In this study, mouse fibroblasts were treated with different doses of UV- and γ-irradiation. These treatments selectively induced different p53-mediated responses (temporary cell cycle arrest, terminal growth arrest or apoptosis), allowing the analysis of the contribution of transcription-dependent and -independent p53 activities to the outcome of the stress responses. Importantly, induction of apoptosis could not be correlated with an apoptosis-specific transcriptional program of p53, since p53-mediated induction of Bax, Puma and PIG-3 was observed in damaged cells regardless of whether the cells underwent apoptosis or not. These results suggest that in mouse fibroblasts upregulation of pro-apoptotic p53 targets is not sufficient to initiate apoptosis but that an additional, transcription-independent p53 activity is required. Such an activity can be provided by the high amounts of activated p53 that accumulate in the cytosol specifically after high-dose UV irradiation, but not after non-apoptotic irradiation treatments [33]. In agreement with in vitro data [21], accumulation of stress-induced endogenous p53 in the cytosol was specifically coupled to Bax activation in vivo, with both events significantly preceding the executioner phase of the apoptotic program [33]. Also, in this in vivo system, p53-dependent Bax activation was not impaired by inhibitors of transcription and thus was transcription-independent. As Bax activation could also be triggered by an endogenous, transcriptionally inactive mutant p53 protein upon genotoxic stress [33], Bax-mediated apoptosis is not strictly coupled to upregulation of Bax. Transcriptional upregulation of the Bax gene and apoptosis-specific non-transcriptional activation of the Bax protein are thus independent, p53-regulated processes.

Mitochondrial and cytosolic versus nuclear p53 in apoptosis induction

Mechanistically, apoptosis induced by mitochondrial p53 differs from apoptosis induced by cytosolic p53. Given the complexity of the p53 regulatory network it seems plausible that the pathways are differently regulated and operate under different stress conditions and in different cellular environments. Nevertheless, common to both pathways is the transcription-independent, direct or indirect activation of pro-apoptotic Bcl-2 family members, leading to the permeabilization of the OMM, release of cytochrome c and activation of caspase 3. In addition, both pathways require extra-nuclear p53. It is an attractive model that non-transcriptional apoptosis is triggered by a
specific modification of p53 that directs the tumor suppressor out of the nucleus, where it accumulates upon genotoxic stress to exert its transcriptional activity. So far, however, comparative analysis of the phosphorylation and acetylation pattern of nuclear versus mitochondrial p53 failed to detect any substantial difference between the two protein fractions [16], although it cannot be excluded that another, not yet analyzed modification is responsible for the specific localization of the tumor suppressor. Ubiquitination, which was also implicated in this respect [12**,35], eventually might be such a localization signal. A stringent connection between mono- or poly-ubiquinated p53, its cellular localization and its transcription-independent pro-apoptotic activity, however, remains to be elucidated. In an alternative model, accumulation of p53 in the cytosol and/or mitochondria is simply the consequence of its normal intra-cellular transport. Given balanced nucleo-cytoplasmic shuttling of p53 and/or stabilization of pre-existing cytoplasmic p53, a threshold level of activated cytosolic or mitochondrial p53 required for Bax and Bak activation can be reached when the total amount of cellular p53 reaches a certain level. In agreement with this model, stress-induced p53 levels differed dramatically in NIH3T3 cells when different irradiation schemes were applied. Significantly higher total p53 levels were selectively generated upon apoptosis-inducing high-dose UV-irradiation, leading to enhanced levels of cytosolic p53 and to Bax activation [33**]. In contrast, high-dose γ-irradiation led to only moderate p53 accumulation, thereby directing the p53 response to a terminal growth arrest and protection against p53-independent apoptosis [33**]. Of note, higher p53 levels were not coupled to a stronger upregulation of target genes [33**], supporting the idea that high intracellular p53 levels constitute a separate signal that provides the switch towards activation of non-transcriptional apoptosis. The question of why high-dose UV-irradiation, but not high-dose γ-irradiation, led to the generation of high p53 levels and thus to accumulation of cytosolic p53 and apoptosis might be therapeutically relevant. In the study by Speidel et al. [33**], accumulation of p53 upon UV-irradiation correlated with significantly delayed upregulation of Mdm2, an E3 ubiquitin-ligase and major p53 antagonist that targets p53 for proteasomal degradation [36]. Delayed upregulation of Mdm2 might be due to a particular feature of the mdm2-promoter, namely that supercoiling of the promoter-DNA prevents its activation [37]. Clearly, double-strand breaks induced by γ-irradiation will promote local DNA relaxation and thus support transcription from the mdm2-promoter, whereas DNA cross-linking agents such as cisplatin or high-dose UV-irradiation might impair or slow down this process. Low levels of Mdm2 might further lead to mono- instead of poly-ubiquitination of p53, which was reported to support nuclear export of p53 rather than its degradation [35]. Modulation of Mdm2 levels or blocking of the interaction of Mdm2 with p53 by interfering molecules [38**,39,40] might thus be a therapeutic option to activate non-transcriptional apoptosis in tumors expressing a functionally wild-type p53.

**Conclusions**

On the basis of the studies summarized above, it appears that transcription-independent activities of p53 play an important role in the ability of p53 to activate the mitochondrial pathway in many circumstance. It will be the challenge of future efforts to dissect the signaling network that coordinates and couples the transcriptional and non-transcriptional pro-apoptotic activities of p53. It is clear, however, that both activities cooperate to ultimately cause cell death (Figure 1).

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Transcription-independent pro-apoptotic functions of p53 Moll, Wolff, Speidel and Deppert

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest


Cell division, growth and death

damage, and oxidative and excitotoxic insults. Neuromolecular Med 2003, 3:159-172.


By analysis of differently irradiated mouse fibroblasts this study provides the first in vivo evidence for transcription-independent activation of Bax by p53 and its specificity for p53-dependent apoptosis. Moreover, it is shown that an endogenous, transcription-inactive mutant p53 utilizes this mechanism and activates Bax upon DNA damage.


Using a pharmacologically activatable p53 fusion protein, this paper demonstrates that p53-dependent and transcription-independent apoptosis proceeds via activation of Bax. This is the first report coupling a non-transcriptional pro-apoptotic activity of p53 to activation of Bax.


This work is the first to successfully identify potent, selective small-molecule antagonists of MDM2 as a potentially novel strategy for treating wild-type p53 cancers. The Nutlin compounds displace p53 from its binding pocket in Mdm2. This activates the p53 pathway in cancer cells, leading to cell cycle arrest, apoptosis, and inhibition of human tumor xenografts in nude mice.

