Mechanisms of p53-dependent apoptosis

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Abstract

Cellular stresses, such as growth factor deprivation, DNA damage or oncogene expression, lead to stabilization and activation of the p53 tumour suppressor protein. Depending on the cellular context, this results in one of two different outcomes: cell cycle arrest or apoptotic cell death. Cell death induced through the p53 pathway is executed by the caspase proteinases, which, by cleaving their substrates, lead to the characteristic apoptotic phenotype. Caspase activation by p53 occurs through the release of apoptogenic factors from the mitochondria, including cytochrome *c* and Smac/DIABLO. Released cytochrome *c* allows the formation of a high-molecular weight complex, the apoptosome, which consists of the adapter protein Apaf-1 and caspase 9, which is activated following recruitment into the apoptosome. Active caspase 9 then cleaves and activates the effector caspases, such as caspases-3 and -7, which execute the death program. Released Smac/DIABLO facilitates caspase activation through repression of the IAP caspase inhibitor proteins. The release of mitochondrial apoptogenic factors is regulated by the pro- and anti-apoptotic Bcl-2 family proteins, which either induce or prevent the permeabilization of the outer mitochondrial membrane. The mechanism by which p53 signals to the Bcl-2 family proteins is unclear. It was shown that some of the pro-apoptotic family members, such as Bax, Noxa or PUMA, are transcriptional targets of p53. In addition, transcription-independent, pro-apoptotic activities of p53 have been described. The elucidation of the p53-dependent pathway, resulting in mitochondrial outer membrane permeabilization through the pro-apoptotic Bcl-2 family proteins, is a key to unveiling the mechanism of stress-induced apoptosis.

p53-mediated caspase activation

Activation of the caspase proteinases is the central event in the effector phase of apoptosis [1]. The inhibition of caspase activity prevents the apoptotic phenotype, but under some circumstances still allows the progression to non-apoptotic cell death [2]. From current understanding there are two principal pathways that lead to the activation of effector caspases. The extrinsic pathway is initiated through ligation of the death receptor family receptors by their respective ligands. Amongst others this family includes the tumour necrosis factor receptors, CD95/Fas/APO-1 and the TRAIL receptors [3]. Receptor ligation is followed by the formation of the death inducing signalling complex (DISC), which is composed of the adapter molecule FADD and caspase 8 [4,5]. Recruitment to DISC activates caspase 8, which in turn either directly cleaves and activates the effector caspases, or indirectly activates the downstream caspases through cleavage of the BH3 protein Bid, leading to engagement of the intrinsic pathway of apoptosis [6–9]. This intrinsic pathway of caspase activation is regulated by the pro- and anti-apoptotic Bcl-2 family proteins. These proteins induce or prevent the release of apoptogenic factors, such as cytochrome *c* or Smac/DIABLO, from the mitochondrial intermembrane space into the cytosol [10–13]. The intrinsic pathway is triggered by stress stimuli, including growth factor deprivation and DNA damage. The release of mitochondrial cytochrome *c* facilitates the formation of the apoptosome complex (composed of the adapter molecule Apaf-1 and caspase 9 [14,15]), which then cleaves and activates the effector caspases.

Although the execution of DNA damage-induced or p53-mediated apoptosis clearly proceeds through the effector caspases [16–18], their mechanism of activation appears to depend on the experimental context. It was shown that DNA damage-induced apoptosis can proceed through death receptor signalling [19,20], and that p53 can transcriptionally and non-transcriptionally regulate the membrane expression of the death receptors such as CD95/Fas/APO-1 and TRAIL receptor 2/KILLER/DR5 [21–24]. These reports

Key words: caspases, cell death, mitochondria.
Abbreviations used: DISC, death inducing signalling complex; ROS, reactive oxygen species.
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would argue for a contribution of death receptor signalling to p53-mediated apoptosis.

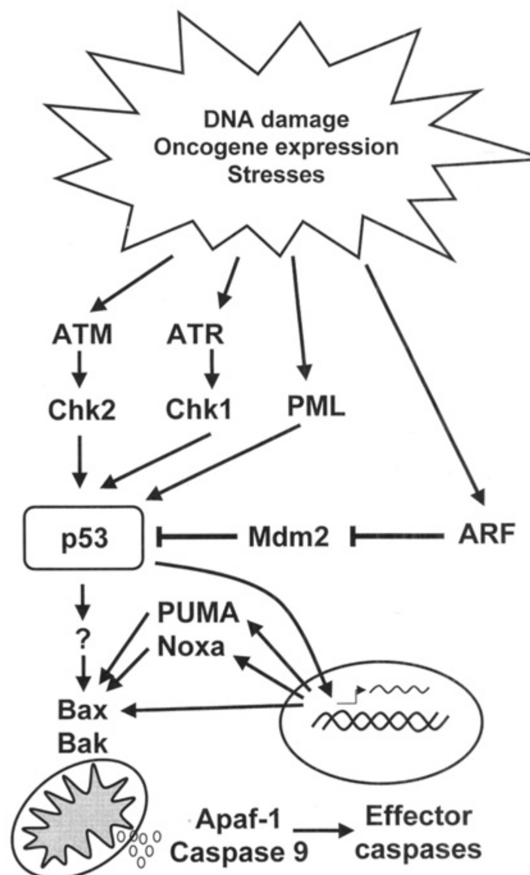
In contrast, thymocytes from *gld* and *lpr* mice, which have defective Fas ligands or receptors, show no resistance to radiation-induced apoptosis, and DNA damage-induced cell death is not prevented by the expression of a dominant inhibitor of DISC formation, but by Bcl-2 instead [25]. The release of mitochondrial cytochrome *c* can be triggered through p53-induced activation of Bax in a caspase-independent manner [26], and cells from mice deficient in caspase 9 or Apaf-1 were resistant to DNA damage-induced apoptosis and to p53-dependent death stimuli [27–31]. Moreover, it was confirmed by single-cell analysis that the p53-induced activation of Bax and the release of mitochondrial cytochrome *c* proceed independently and, therefore, upstream of caspase activation, which is the apical event in death receptor signalling (Schuler, M. and Green, D. R., unpublished data). This argues that in most, if not all, cell types the intrinsic, 'mitochondrial', pathway is essential for stress-induced and p53-dependent caspase activation, whereas death receptor signalling seems to be dispensable in this context.

In addition, alternative pathways of p53-dependent caspase activation such as by generation of reactive oxygen species (ROS) have been postulated [32,33]. However, using experimental systems described above, we found that ROS inhibitors failed to protect against p53-mediated apoptosis [26], and the generation of ROS appeared to occur downstream of p53-induced caspase activation (Schuler, M. and Green, D. R., unpublished data). In conclusion, the intrinsic apoptotic pathway (Figure 1) is both necessary and sufficient for stress-induced and p53-dependent caspase activation *in vitro* and *in vivo*. Death receptor signalling and alternative pathways may contribute to the full development of apoptosis.

Figure 1

Summary of the pathways of p53-dependent apoptosis

The inactivation of p53 by Mdm2 is counteracted by mechanisms involving post-translational modification of p53, or the Mdm2 inhbibitor ARF. Through its activity as a sequence-specific transcriptional activator, p53 increases the expression of pro-apoptotic Bcl-2 family proteins. Potential transcription-independent functions of p53 co-operate with the Bcl-2 protein to induce the release of apoptogenic factors (depicted as circles) from the mitochondrial outer membrane. Released apoptogenic factors facilitate the activation of the effector caspases through the Apaf-1–caspase 9-apoptosome. ATM, ataxia telangiectasia mutated protein; ATR, ataxia telangiectasia-related; PML, promyelocytic leukaemia protein; ARF, alternative reading frame of p16 protein.



Engagement of the mitochondria through p53

While the requirement of the events downstream of mitochondrial cytochrome *c* release for p53-dependent apoptosis has been confirmed *in vitro* and *in vivo*, it is less clear how the intrinsic apoptotic pathway is engaged. The involvement of the pro-apoptotic Bcl-2 family proteins in this process, however, is more than likely. Some cell types from Bax-deficient mice exhibit an attenuated p53-dependent apoptotic response and Bax contributes significantly to p53-dependent tumour suppression and apoptosis *in vivo* [34–37]. The combined genetic ablation of Bax and the pro-apoptotic Bak protein results in severe developmental defects, and cells from these mice are highly protected against stress-induced apoptosis [38,39]. This suggests that p53 more than likely signals to the mitochondria through more than one of the redundant pro-apoptotic Bcl-2 family proteins (Figure 1).

Many functions of p53 are exerted by its activity as a sequence-specific transcriptional

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activator [40,41]. Accordingly, p53 response elements were found in the promoters of the pro-apoptotic Bcl-2 protein Bax and the BH3-only proteins Noxa and PUMA, which are upregulated during p53-induced cell death [42–45]. As these proteins have the ability to induce mitochondrial cytochrome *c* release [12,45], transactivation of their promoters through p53 might be a way of triggering the intrinsic pathway of caspase activation. Interestingly, a p53-induced upregulation of Bax or PUMA was also observed under conditions leading to p53-dependent cell-cycle arrest rather than apoptosis [45,46]. Thus, it seems unlikely that p53 can signal to caspase activation solely by transactivating pro-apoptotic Bcl-2 proteins.

A transactivation-independent, pro-apoptotic activity of p53 has been described in several experimental systems [47–50]. These studies, however, provide no explanation as to how p53 could induce caspase activation independently of its function as a transcriptional activator. Recently, potential mechanisms have been introduced, including the shuttling of preformed Fas receptors to the cell membrane [23], a direct import of p53 into the mitochondrial matrix to act on ROS generation [51], or FADD-independent cytosolic activation of caspase 8 [52]. We have found that the activation of cytosolic p53 can induce mitochondrial cytochrome *c* release in cytoplasts and in a cell-free system through a mechanism requiring its intact N-terminus (Schuler, M. and Green, D. R., unpublished data). It is possible that transcription-independent functions of p53 co-operate with its transcription-dependent functions in the induction of apoptosis.

In addition, the cellular context determines whether or not the transactivation of pro-apoptotic genes through p53 results in an apoptotic response. The p53-dependent stress response of primary fibroblasts comprises cell-cycle arrest and proliferative senescence. The expression of oncogenes or oncogenic transformation, however, sensitizes these cells to stress-induced apoptosis, which involves signalling of p53 to the intrinsic apoptotic pathway [31,53–57]. Recently, a dominant inhibitory activity was postulated in untransformed fibroblasts, which prevented stress-induced apoptosis at the level of mitochondrial cytochrome *c* release. Oncogenic transformation extinguished this activity and resulted in a sensitization to apoptotic cell death [58]. Thus, the outcome of a p53-dependent upregulation of pro-apoptotic protein expression could be influenced by inhibitory factors. Their presence could result in cell-cycle arrest, and their absence could result in default apoptosis.

Future directions

The core machinery involved in stress-induced apoptosis has been identified in recent years [59], and the relevance of most of its components has been confirmed by the use of genetic *in vivo* models. The fine tuning of the interplay between Bcl-2 family proteins, mitochondrial apoptogenic factors, inhibitors and the caspases by opposing survival and death signals converging in the cell, however, still remains to be understood. Another key question is, "What is the actual mechanism by which the Bcl-2 proteins maintain or ablate the integrity of the outer mitochondrial membrane?"

Upstream of the mitochondria, the modulation of p53 stability and activity through phosphokinases, additional modifying enzymes, and ARF is being unravelled [60–63]. The actual link between p53 and the activation of pro-apoptotic Bcl-2 family proteins, however, is still missing. Transactivation by p53 of BH3-only proteins [43–45] or additional factors acting on Bax [64] or the mitochondria [65] certainly may contribute to the mechanism. As Bcl-2 family members and stress-induced apoptosis can be activated independently of transcription, the presence of alternative pathways of regulation of these key players upstream of the mitochondria are likely. In addition, survival signals from growth factor receptors, stress kinases, or oncogenes, as well as metabolic factors converging at the Bcl-2 family proteins and the mitochondria, participate in the regulation of mitochondrial homeostasis during apoptosis [66–71]. A complete picture of stress-induced and p53-dependent apoptosis will require the integration of all the regulatory signals acting upstream and downstream of p53, upstream of the mitochondria, and downstream of the release of mitochondrial apoptogenic factors.

This work is supported by grants from the Dr Mildred Scheel Stiftung für Krebsforschung (to M. S.) and the National Institutes of Health (to D. R. G.).

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Received 4 July 2001

UV stimulation induces nuclear factor κB (NF-κB) DNA-binding activity but not transcriptional activation

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Abstract

The cellular response to DNA-damaging agents is partly mediated by DNA-binding transcription factors such as p53 and nuclear factor κB (NF-κB). Typically NF-κB activation is associated with resistance to apoptosis. Following stimulation with UV light however, NF-κB activation has been shown to be required for programmed cell death. To study this effect further and to determine the relationship between NF-κB and p53 function, we have examined the effect of UV light on U2OS cells. UV stimulation resulted in the activation of NF-κB DNA-binding and the induction of p53. Surprisingly, and in contrast with tumour necrosis factor α stimulation, this UV-induced NF-κB was transcriptionally inert. These observations suggest a model in which the NF-κB switch from an anti-apoptotic to a pro-apoptotic role within the cell results from modulation of its ability to stimulate gene expression, possibly as a result of the ability of p53 to sequester transcriptional co-activator proteins such as p300/CREB (cAMP-response-element-binding protein)-binding protein.

Key words: p53, DNA damage, U2OS cell.

Abbreviations used: CAT, chloramphenicol acetyltransferase; CBP, CREB-binding protein; CREB, cAMP-response-element-binding protein; EMSA, electrophoretic mobility shift assay; NF-κB, nuclear factor κB; TNFα, tumour necrosis factor α.

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Introduction

The Rel/nuclear factor κB (NF-κB) complex consists of homodimers or heterodimers formed from a pool of five proteins, RelA, c-Rel, RelB, p50/p105 and p52/p100 [1,2]. Within their N-termini, these proteins all contain a Rel homology domain that mediates both their DNA binding and dimerization. NF-κB1 and NF-κB2 are both synthesized as precursor proteins and remain in the cytoplasm until proteolytic processing yields the DNA-binding isoforms termed p50 and p52 respectively. In contrast, RelA, RelB and c-Rel do not require proteolytic processing and contain non-homologous transactivation domains within their C-termini. Distinct NF-κB complexes have subtly different DNA-binding specificities, which results in them being targeted to different genes [1]. In most cell types, NF-κB complexes are held in an inactive form in the cytoplasm through interactions with one of a family proteins; inhibitory κB (IκB)-α, -β and -ε. Activation of NF-κB in response to cellular stimulation involves the rapid phosphorylation and degradation of IκB, allowing NF-κB to translocate to the nucleus [2]. The role of NF-κB in disease stems primarily from circumstances where it becomes inappro-

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