Apoptosis – the p53 network

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Summary

Exposure to cellular stress can trigger the p53 tumor suppressor, a sequence-specific transcription factor, to induce cell growth arrest or apoptosis. The choice between these cellular responses is influenced by many factors, including the type of cell and stress, and the action of p53 co-activators. p53 stimulates a wide network of signals that act through two major apoptotic pathways. The extrinsic, death receptor pathway triggers the activation of a caspase cascade, and the intrinsic, mitochondrial pathway shifts the balance in the Bcl-2 family towards the pro-apoptotic members, promoting the formation of the apoptosome, and consequently caspase-mediated apoptosis. The impact of these two apoptotic pathways may be enhanced when they converge through Bid, which is a p53 target. The majority of these apoptotic effects are mediated through the induction of specific apoptotic target genes. However, p53 can also promote apoptosis by a transcription-independent mechanism under certain conditions. Thus, a multitude of mechanisms are employed by p53 to ensure efficient induction of apoptosis in a stage-, tissue- and stress-signal-specific manner. Manipulation of the apoptotic functions of p53 constitutes an attractive target for cancer therapy.

Key words: p53, Apoptosis, Caspase, Mitochondria, Transcriptional activation

Introduction

The prevention of cancer is profoundly dependent on the p53 tumor suppressor protein. The ability of p53 to eliminate excess, damaged or infected cells by apoptosis (Kerr et al., 1972) is vital for the proper regulation of cell proliferation in multi-cellular organisms (Huang and Strasser, 2000). p53 is activated by external and internal stress signals that promote its nuclear accumulation in an active form. In turn, p53 induces either viable cell growth arrest or apoptosis. The latter activity is crucial for tumor suppression. The growth inhibitory activities of p53 prevent the proliferation of cells with damaged DNA or with a potential for neoplastic transformation. In addition, p53 contributes to cellular processes such as differentiation, DNA repair and angiogenesis, which also appear to be vital for tumor suppression (reviewed by Vogt Sionov and Haupt, 1999).

Being a key player in the cellular response to stress, p53 serves as the major obstruction for tumorigenesis. This obstacle has to be removed in order to allow tumor development. Indeed, approximately 50% of human cancers bear *p53* gene mutations; in the majority of the remaining cancer cases, p53 activity is compromised by alternative mechanisms (Vogelstein et al., 2000). These involve elevation in the expression levels of p53 inhibitors, such as Mdm2 or the E6 protein of HPV, or silencing of key p53 co-activators, such as ARF (Vogelstein et al., 2000; Vogt Sionov et al., 2001).

Under normal conditions p53 is a short-lived protein. The p53 inhibitor Mdm2 (Hdm2 in humans) is largely responsible for keeping p53 in this state. Mdm2 inhibits the transcriptional activity of p53 and, more importantly, promotes its degradation by the proteasome. However, the status of p53 is drastically altered when cells are exposed to stress, including DNA damage, untimely expression of oncogenes, hypoxia and nucleotide depletion (reviewed by Giaccia and Kastan, 1998). p53 activation involves stabilization of the protein, and enhancement of its DNA binding and transcriptional activity. These changes in p53 are mediated by extensive post-translational modifications of p53 and protein-protein interactions with cooperating factors. Ultimately, the activation of p53 leads to cell growth arrest, senescence or apoptosis, the choice of which depends on the summation of the incoming signals and the cellular context (see below). Because the apoptotic function of p53 is critical for tumor suppression, reconstitution of inactive p53-dependent apoptotic pathways is an attractive approach currently being explored for anti-cancer treatment. Here, we review recent developments in our understanding of p53-mediated apoptosis. References to relevant exhaustive reviews on this subject are made in the appropriate sections.

Growth inhibition by p53: cell cycle arrest or apoptosis?

p53 is a transcription factor that activates vital damage-containment procedures to restrict aberrant cell growth in response to DNA damage, oncogene activation, hypoxia and the loss of normal cell contacts (Giaccia and Kastan, 1998; Lohrum and Vousden, 1999). It restricts cellular growth by inducing senescence, cell cycle arrest (at G1 and/or G2 phase) or apoptosis (Jin and Levine, 2001). The exact criteria that influence p53 to stimulate cell cycle arrest or apoptosis are only partially understood and are the subject of intense study. Several general factors that influence this decision include p53 expression levels, the type of stress signal, the cell type and the cellular context at the time of exposure to stress (reviewed by Balint and Vousden, 2001; Vogt Sionov and Haupt, 1999).

Several intriguing observations have recently provided insight into the apparent intricacies of such cell fate determination. The examples described below involve the binding of p53 to its canonical binding sequence in target genes (el-Deiry et al., 1992). Note, however, that p53 can also activate target genes through a non-canonical sequence. The first such example is in the p53-induced gene 3 (*PIG3*), which has been implicated in the accumulation of reactive oxygen species and apoptosis induction (Polyak et al., 1997). *PIG3* can be induced by p53 through a microsatellite sequence within its untranslated region (Contente et al., 2002). Another recently described example is the gene encoding the pro-apoptotic phosphatase PAC1, which is induced through binding of p53 to a novel palindromic binding site (Yin et al., 2003). This might represent a new mechanism for transcriptional regulation of apoptotic genes by p53, which differs from that already described (see below).

Redox determination of p53 gene regulation

The nucleotide sequence of the binding site for p53 within the promoters of its target genes is a critical determinant in the response to stress (UV light or γ-irradiation exposure). In response to DNA damage, the binding affinity of p53 for the promoter of the cell-cycle-regulating gene $p21^{WAF/CIP1}$ (*p21*) is unchanged, whereas binding to the promoter of the DNA-repair-associated gene *Gadd45* is reduced. This is due, primarily, to oxidation of Cys277, residing within human p53, which contacts the third base (the first pyrimidine residue) in the p53-binding consensus pentamer A/TGPyPyPy: where the p53-binding element of p21 is 5′-GAACATGTCCcAACA-TGTTg-3′ and that of GADD45 is 5′-GAACATGTCTAAG-CATGCTg-3′ (and where the critical residues that determine the binding to Cys277 are indicated in red, and consensus-fitting bases are in uppercase). Although the mechanism of p53 Cys277 oxidation is unclear, it may be associated with the production of oxygen radicals that are induced in response to exposure to high-dose UV light (Buzek et al., 2002).

Only under reducing conditions is the affinity of p53 for the *Gadd45* promoter increased, which suggests that the reduction of Cys277 is necessary to enable binding of p53 to C-rich binding sequences, such as that of *Gadd45*. Intriguingly, Seo et al. found that reduction of residues Cys275 and Cys277 by selenomethionine (the major dietary source of selenium) caused p53 to recruit the p53-binding redox factor Ref1 and activate DNA-repair machinery through the induction of *Gadd45*, without affecting cell growth (Seo et al., 2002). Thus, the redox state of p53 Cys277 appears to serve as a switch for activating the DNA repair machinery. This selective activation of p53-dependent DNA repair activity has been proposed as a novel approach to cancer prevention (Gudkov, 2002).

p53 co-activators

The interaction between p53 and transcriptional co-activators also influences its affinity for promoters. It is therefore plausible that the specific co-factors expressed in a particular cellular context determine the repertoire of p53-target genes induced, and consequently whether the cell undergoes growth arrest or apoptosis, or even a particular apoptotic pathway (Fig. 1), may be subject to the availability of co-activators (Rozenfeld-Granot et al., 2002).

The Myc protein has been implicated as an important determinant of the choice between p53-induced growth arrest or apoptosis. Myc inhibits growth arrest in response to UV light, γ-irradiation and DNA damage inflicted by reactive oxygen species (Sheen and Dickson, 2002; Vafa et al., 2002). In the absence of Myc, cells that are exposed to UV light arrest in a p53- and Miz-1 (DNA-binding Myc-interacting zinc-finger 1)-dependent manner through activation of *p21*. However, when Myc is present, exposure to UV triggers its recruitment by Miz-1 to the proximal promoter region of *p21*. This interaction effectively represses *p21* induction by p53 and other activators (Herold et al., 2002; Seoane et al., 2002). Intriguingly, this repression appears to be specific for *p21*, because other p53-target genes, such as p53 upregulated modulator of apoptosis (*PUMA*) and *PIG3*, are induced. This block in *p21* induction shifts the balance away from growth arrest towards apoptosis (Seoane et al., 2002). It should be noted, however, that arrested cells are not necessarily protected from apoptosis. For example, normal thymocytes and mature lymphocytes undergo p53-mediated apoptosis under certain stress conditions (Strasser et al., 1994). Interaction of p53 with several other proteins specifically enhances the induction of apoptotic target genes. The apoptosis stimulating proteins of p53 (ASPP), for example, increases the DNA binding and transactivation activity of p53 on the promoters of apoptotic genes (e.g. *Bax* and *PIG3*), while failing to promote binding to the *p21* promoter by a mechanism that remains to be defined (Samuels-Lev et al., 2001).

A novel insight into the interplay between p53 and its family members, p63 and p73, in the induction of apoptosis has been recently revealed by Flores et al. (Flores et al., 2002). Their study of the effect of p63 and p73 on p53 transcriptional activity, using a selection of knockout mouse embryo fibroblasts (MEFs), defined two distinct classes of target gene. Whereas p53 alone is sufficient for the induction of *p21* and *Mdm2*, the induction of the apoptotic genes *PERP*, *Bax* and *Noxa* requires p53 together with p63 and p73. This finding demonstrates an essential role for both p63 and p73 in the efficient induction of apoptotic target genes by p53. The mechanism of this cooperation is currently unknown, but it may involve an enhanced binding to and/or stabilization of the transcription complex on the promoters of p53 apoptotic target genes by the cooperative action of all three members (Urist and Prives, 2002). In addition to the contribution of p63 and p73 to the apoptotic function of p53, they play an important role in the precise control of cell death during normal mouse development. p73 also plays a role in the induction of cell death in response to DNA damage, a process involving cooperation between the Abl tyrosine kinase and p73 (reviewed by Shaul, 2000).

p53-mediated apoptosis

Exacting discrimination between p53 arrest and apoptotic functions has been critical to the identification of the importance of the latter in tumor suppression. A strong link between the apoptotic function of p53 and tumor suppression has been demonstrated using transgenic mice bearing an SV40 large T antigen (LT) mutant, which inhibited pRb function without directly compromising p53 activity. p53-mediated growth arrest is however impaired in these mice owing to pRb

loss of function, but the apoptotic activity is functional. These mice develop choroid plexus tumors, but at a slow rate owing to continuous p53-dependent apoptosis. Elimination of p53 from these mice, by crossbreeding with p53-null mice, resulted in aggressive tumor development. This finding suggested that tumor suppression is primarily due to p53-mediated apoptosis **(**reviewed by Vogt Sionov and Haupt, 1999). The most compelling insight into this fundamental question came from a recent study using the Eμ-*myc*-mediated lymphoma mouse model. The apoptotic pathway of the lymphoma cells was blocked either by retroviral expression of *bcl-2* or a dominant negative *caspase-9*. The effect of this block on the growth of these lymphomas was then tested in recipient mice. In the apoptosis-impaired cells there was no selection for p53 mutations, in contrast to cells that had intact apoptotic pathways. Remarkably, in the apoptosis-impaired lymphoma cells expressing functional p53, the integrity of the genome and the cell cycle checkpoints were maintained. Taken together these studies support the notion that apoptosis is the critical function of p53 in tumor suppression.

How p53 mediates apoptosis has been a matter of intensive study since this was first demonstrated (Yonish-Rouach et al., 1991). Numerous publications have recently described the importance of p53 transcriptional regulation of components of both the extrinsic and intrinsic pathways. However, few target gene products have been unequivocally established to be essential to p53-dependent apoptosis induction; we discuss the supporting evidence below. p53 is also able to promote apoptosis through transcription-independent apoptotic mechanisms. Under certain conditions, p53 induces apoptosis in the absence of transcription or protein synthesis (e.g. Caelles et al., 1994). Moreover, transcriptionally inactive mutants of p53 can induce apoptosis in certain cell types (Haupt et al., 1995), and PIASγ (protein inhibitor of activated STAT), which blocks binding of p53 to DNA, does not inhibit p53-mediated apoptosis (Nelson et al., 2001). In general, the transcription-independent apoptotic activities of p53 have been demonstrated in transformed cells rather than in normal cells (e.g. lymphocytes or fibroblasts). Presumably, these activities of p53 require cooperation with other apoptotic factors – for instance E2F-1 (a transcription factor in the retinoblastoma protein pathway) (reviewed by Vogt Sionov and Haupt, 1999). Experimental cell transformation may mimic various stages of tumor development, where the apoptotic function of p53 is being activated and becomes critical for the suppression of tumor progression. These apoptotic activities of p53 may not be sufficient to induce apoptosis in non-transformed cells, such as normal thymocytes. Whereas the transcription-dependent and -independent apoptotic functions of p53 are often described separately, they appear to complement each other. We therefore discuss their contributions together in the context of the extrinsic and intrinsic apoptotic pathways.

Extrinsic and intrinsic apoptotic pathways

p53 is implicated in the induction of what had until recently been understood to be two distinct apoptotic signaling pathways that lead to the activation of the aspartate-specific cysteine proteases (caspases) that mediate apoptosis (Fig. 1). The extrinsic pathway involves engagement of particular 'death' receptors that belong to the tumor necrosis factor receptor (TNF-R) family and, through the formation of the death-inducing-signaling-complex (DISC) (Ashkenazi and Dixit, 1998), leads to a cascade of activation of caspases, including caspase-8 and caspase-3, which in turn induce apoptosis. The intrinsic pathway is triggered in response to DNA damage and is associated with mitochondrial depolarization and release of cytochrome c from the mitochondrial intermembrane space into the cytoplasm. Cytochrome c, apoptotic protease-activating factor 1 (APAF-1) and procaspase-9 then form a complex termed the apoptosome, in which caspase-9 is activated and promotes activation of caspase-3, caspase-6 and caspase-7 (reviewed by Nicholson and Thornberry, 2003). Recent studies, however, link the extrinsic and intrinsic pathways, lending support to the idea of converging rather than distinct pathways (Gross et al., 1999; Li et al., 1998).

The extrinsic pathway

p53 can activate the extrinsic apoptotic pathway through the induction of genes encoding three transmembranes proteins: Fas, DR5 and PERP. The cell-surface receptor Fas (CD95/Apo-1), a member of the TNF-R family of receptors, is a key component of the extrinsic death pathway (Nagata and Golstein, 1995). Fas is activated by binding of its ligand, FasL, which is expressed predominantly by T cells (Muzio, 1998). p53 induces *Fas* mRNA expression by binding to elements found in the promoter and first intron of the *Fas* gene (Muller et al., 1998). This induction occurs in response to γ-irradiation, and it appears to be strictly tissue specific (Bouvard et al., 2000). p53-dependent *Fas* mRNA induction has been demonstrated in the spleen, thymus, kidney and lung, but not in the heart and liver (Bouvard et al., 2000). However, at least in lymphocytes, *Fas* appears to be dispensable for p53-dependent apoptosis (Fuchs et al., 1997; O'Connor et al., 2000). The importance of *Fas* as a p53 target in other cell types remains to be elucidated.

In addition to stimulating *Fas* transcription, overexpressed p53 may enhance levels of Fas at the cell surface by promoting trafficking of the Fas receptor from the Golgi (Bennett et al., 1998). This may allow p53 to rapidly sensitize cells to Fas-induced apoptosis before the transcription-dependent effect operates. How p53 promotes Fas trafficking is not understood.

The second member of this receptor family that is induced by p53 is *DR5/KILLER*, the death-domain-containing receptor for TNF-related apoptosis-inducing ligand (TRAIL). *DR5* is induced by p53 in response to DNA damage (Wu et al., 1997) and in turn promotes cell death through caspase-8 (reviewed by Ashkenazi and Dixit, 1998). *DR5* induction is cell type specific. Whole body γ-irradiation induces *DR5* expression in the spleen, small intestine and thymus (Burns et al., 2001), which is consistent with DR5 participating in the p53-mediated response to DNA damage in these tissues. Strikingly, in MEFs exposed to DNA damage (by doxorubicin), similar levels of DR5 were identified in cells undergoing G1 arrest and apoptosis (Attardi et al., 2000). Thus, the contribution of DR5 to these different p53-determined cell fates remains to be clarified.

Another apoptotic gene, *PERP*, is induced in MEFs in response to DNA-damage in cells transduced with either E2F-1 or with the adenoviral E1A protein, which targets pRb,

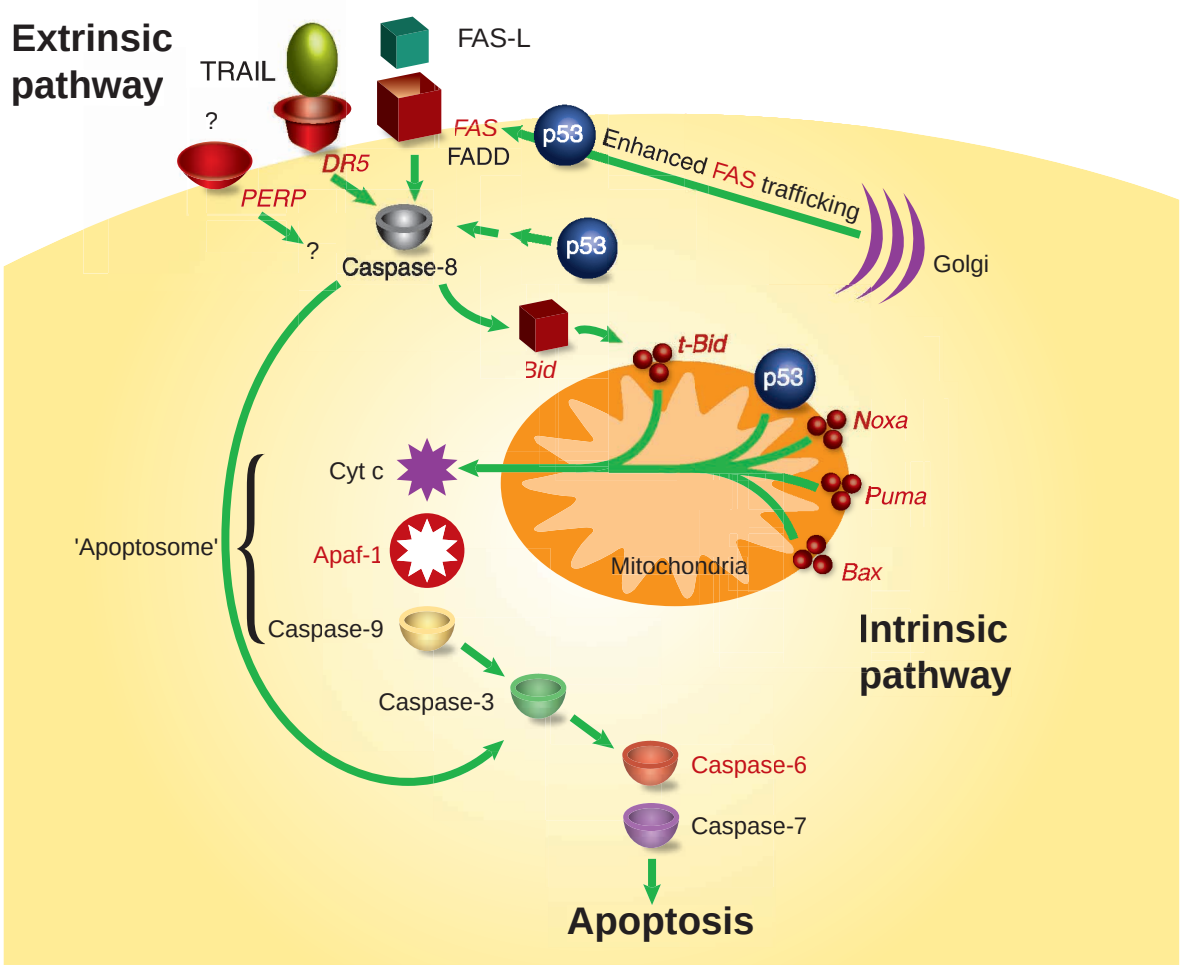

Fig. 1. A model for p53-mediated apoptosis. This model depicts the involvement of p53 in the extrinsic and intrinsic apoptotic pathways. p53 target genes are shown in red. The convergence of the two pathways through Bid is shown.

thereby releasing active E2F-1. In this context, PERP probably cooperates with E2F-1 to induce apoptosis. PERP is a putative tetraspan transmembrane protein that represents a new member of the PMP-22/gas family of proteins implicated in cell growth regulation. The kinetics of *PERP* induction in response to DNA damage and the presence of a p53-responsive element in the *PERP* promoter support the notion that it is a direct p53 target. A role for PERP in apoptosis is suggested by the significantly higher levels of *PERP* mRNA in cells undergoing apoptosis than in arresting cells. However, the mechanism by which PERP contributes to p53-mediated apoptosis is yet to be defined (Attardi et al., 2000).

The intrinsic pathway

The intrinsic apoptotic pathway is dominated by the Bcl-2 family of proteins, which governs the release of cytochrome c from the mitochondria (Cory and Adams, 2002; Kuwana et al., 2002). The Bcl-2 family comprises anti-apoptotic (pro-survival) and pro-apoptotic members. Family members are classified on the basis of structural similarity to the Bcl-2 homology (BH) domains (BH1, BH2, BH3 and BH4), and a transmembrane domain. The BH3 domain, which is present in all members and is essential for heterodimerization among members, is the minimum domain required for the pro-apoptotic function (Kelekar and Thompson, 1998; Yu et al., 2001). The Bcl-2 family is divisible into three classes: pro-survival proteins, whose members are most structurally similar to Bcl-2, such as Bcl-X_L; pro-apoptotic proteins, Bax and Bak, which are structurally similar to Bcl-2 and Bcl-X_L and antagonize their pro-survival functions; and the pro-apoptotic 'BH3-only' proteins (Bouillet and Strasser, 2002). Intriguingly, a key subset of the Bcl-2 family genes are p53 targets, including *Bax*, *Noxa*, *PUMA* and the most recently identified, *Bid*.

Bax was the first member of this group shown to be induced by p53, but p53-responsive elements have only recently been unequivocally identified in the *Bax* gene (Thornborrow et al., 2002). In response to stress activation, Bax forms a homodimer and releases cytochrome c from the mitochondria (Skulachev, 1998), which results in caspase-9 activation (reviewed by Adams and Cory, 1998). The requirement for Bax in p53-mediated apoptosis appears to be cell-type dependent. Bax is required for the apoptotic response of the developing nervous system to γ-irradiation (Chong et al., 2000) and contributes to chemotherapy-induced killing of E1A-expressing fibroblasts (McCurrach et al., 1997).

In contrast, equivalent levels of *Bax* induced in MEFs

undergoing either arrest or apoptosis had been understood to indicate that Bax does not dictate cellular fate in these cells (Attardi et al., 2000). In addition, in colonic epithelia undergoing apoptosis in response to γ-irradiation, Bax did not appear to be essential (Pritchard et al., 1999).

A fascinating explanation for the apparent enigmatic role of Bax in apoptosis induction has recently been offered in the context of PUMA. The *PUMA* gene is also directly induced by p53 in response to DNA damage, through p53-responsive elements within the first intron of *PUMA*. In humans, *PUMA* encodes two BH3-domain-containing proteins, PUMA-α and PUMA-β (Nakano and Vousden, 2001; Yu et al., 2001). A vital balance between PUMA and p21 has been identified to determine the onset of arrest, or death, in response to exogenous p53 expression and also hypoxia in human colorectal cancer cells. Growth arrest through activation of *p21* is the normal response to p53 expression in these cells. If *p21* is disrupted the cells die through apoptosis; if, however, *PUMA* is disrupted, apoptosis is prevented. Bax is absolutely required for *PUMA*-mediated apoptosis. *PUMA* expression promotes mitochondrial translocation and mulitmerization of Bax, culminating in apoptosis induction (Yu et al., 2003). Thus, although p53 can bind to the *Bax* promoter, the affinity is weak in contrast to *p21* and *PUMA* binding (Kaeser and Iggo, 2002). Bax thus participates in the death response as an indirect target of p53 through *PUMA* (Yu et al., 2003).

Another p53 target gene, *Noxa*, contains a single p53-responsive element in its promoter and is induced in response to X-ray irradiation (Oda et al., 2000). *Noxa* encodes a BH3-only protein and hence is likely to contribute to p53-mediated apoptosis in a similar manner to PUMA and Bax, although this is yet to be demonstrated. Thus, it appears that, in response to DNA damage, p53 activates the intrinsic mitochondrial apoptotic pathway by inducing the expression of at least three Bcl-2 pro-apoptotic family members, shifting the balance towards pro-apoptotic effects.

Apoptosome activation by p53

The formation of the apoptosome requires the release of cytochrome c and APAF-1 from mitochondria and their formation of a complex with pro-caspase-9 (Adams and Cory, 2002). p53 promotes cytochrome c release through the induction of target genes encoding BH3-only proteins. Importantly, p53 also induces *APAF-1* expression through a response element within the *APAF-1* promoter (Kannan et al., 2001; Moroni et al., 2001; Robles et al., 2001; Rozenfeld-Granot et al., 2002). This induction by p53 is boosted by E2F-1, which induces *APAF-1* expression, and activates p53 in an ARF-dependent manner (Moroni et al., 2001). *APAF-1*, is required for stress-induced p53-dependent apoptosis of fibroblasts and also for the induction of apoptosis by Myc, in which caspase-9 is an essential downstream component (Soengas et al., 1999). In another study the response to IR of thymocytes from Apaf-1/caspase-9 null mice was compared with that of normal mice. This comparison revealed an impaired response, but neither protection from apoptosis nor normal sensitivity to IR-induced death (Marsden et al., 2002). This apparent difference may represent a different role for the apoptosome in Myc-expressing fibroblasts versus normal thymocytes. It may also suggest that the apoptosome can contribute to p53-mediated apoptosis, but perhaps is not essential for it to occur, at least in thymocytes.

Caspase activation

Caspase-9 and caspase-2 respond to changes in mitochondrial potential, whereas caspase-8 and caspase-10 sense activation of death receptors. These initiator caspases cleave the pro-enzyme forms of the effector caspases, caspase-3, caspase-6 and caspase-7, allowing digestion of essential targets that affect cell viability (Fig. 1) (MacLachlan and El-Deiry, 2002). Intriguingly, p53 boosts the activation of the caspase cascade by both transcription-dependent and -independent mechanisms. In response to γ-irradiation of nucleus-depleted S100 cell-free extracts, p53 can activate caspase-8 (Ding et al., 1998). Depletion or inactivation of caspase-8 in cell-free extracts completely prevents this effect and significantly attenuates overall apoptosis induced by wild-type p53. However, etoposide- and UV-mediated death of fibroblasts derived from caspase-8-deficient mice is not impaired (Varfolomeev et al., 1998). Thus, caspase-8 can contribute to, although is not always essential for, DNA-damaged induced death.

p53 stimulates caspase-6 through a more conventional mechanism. In response to DNA damage, p53 directly induces *caspase-6* expression through a response element within the third intron of the gene (MacLachlan and El-Deiry, 2002). Caspase-6 cleaves the nuclear envelope protein lamin A and several transcription factors (Galande et al., 2001). Caspase-6 plays an important role in p53-induced neuronal cell death and is the major protein involved in the cleavage of the amyloid precursor protein (LeBlanc et al., 1999).

p53 localization to the mitochondria

p53 also participates in apoptosis induction by acting directly at mitochondria. Localization of p53 to the mitochondria occurs in response to apoptotic signals and precedes cytochrome c release and procaspase-3 activation. Importantly, redirecting p53 to mitochondria by using mitochondrial-import leader peptides is sufficient to induce apoptosis in p53-deficient Saos-2 cells (Marchenko et al., 2000). Recently Mihara et al. also extended this finding to show that p53 promotes permeabilization of the outer mitochondrial membrane by forming complexes with the protective Bcl-X_L and Bcl-2 proteins. Interestingly, p53 binds through its DNA-binding domain to Bcl-X_L. Tumor-derived transactivation-deficient mutants of p53 cannot interact with Bcl-X_L and hence do not promote mitochondrial apoptotic events, even though they localize to the mitochondria (Mihara et al., 2003). Separating these two activities of p53 may shed light on the biological relevance of p53 localization to the mitochondria. Since p53 can mediate apoptosis without its DNA-binding domain (Haupt et al., 1995) it is likely that the mitochondrial localization of p53 is not the only transcription-independent mechanism by which p53 promotes apoptosis.

BID: a link between the extrinsic and intrinsic apoptotic pathways

The pro-apoptotic Bid is distinguished by its unique ability to

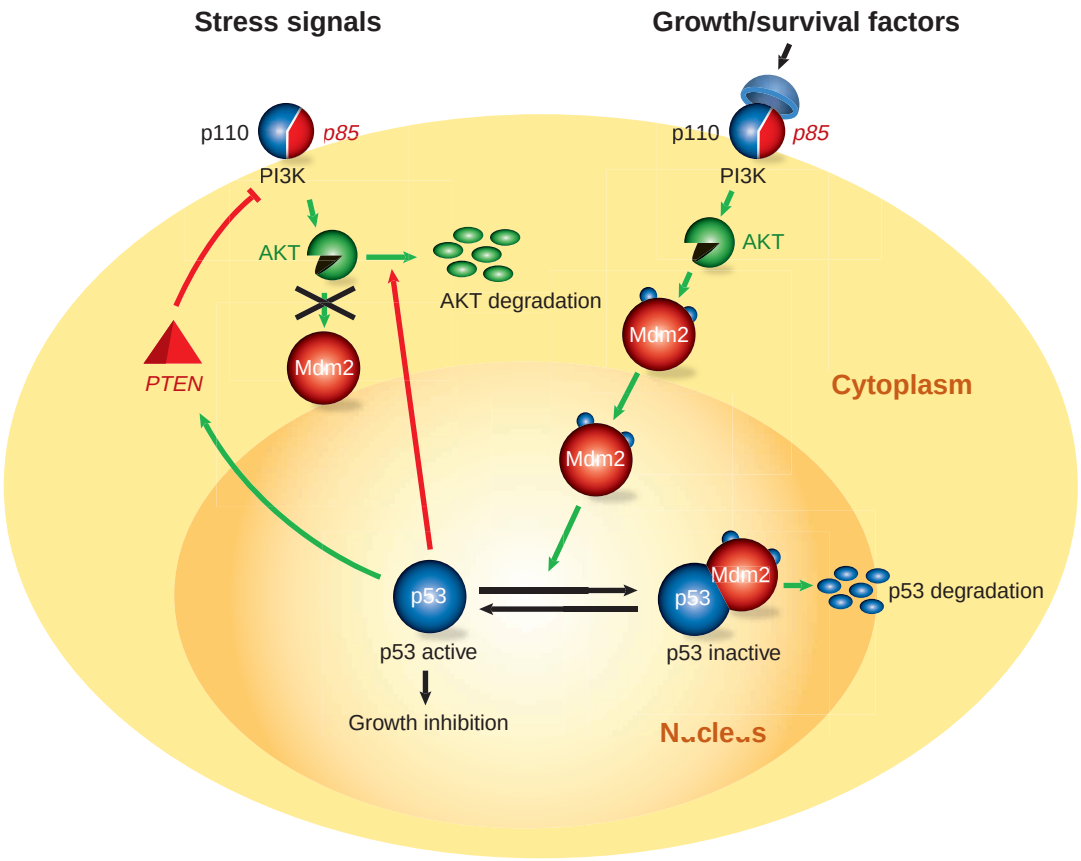

Fig. 2. A model for the regulation of p53 by the AKT pathway under growth/survival conditions and under stress signals. The negative regulation of p53 by AKT is induced in response to survival signals from Mdm2. The activation of this pathway leads to the inhibition and destruction of p53. Under stress conditions this pathways is blocked through the cleavage and degradation of AKT, and the inhibition of PI3K through *PTEN*. Both of these activities are induced by p53. In this model survival is achieved by inhibition of p53 by AKT, whereas apoptosis is achieved by counteracting AKT by p53. p53 target genes are shown in red. Green arrows represent activation, whereas red arrows represent inhibition.

connect activation of the extrinsic death receptor pathway to activation of the mitochondrial-disruption processes associated with the intrinsic pathway. Activation of Bid involves cleavage of cytoplasmic Bid by caspase-8 to expose a new N-terminal glycine residue, which undergoes post-translational myristoylation. Myristoylated Bid translocates to the mitochondria, inserts into the membrane and activates BAX and BAK to initiate mitochondrial events leading to apoptosome formation. The *Bid* gene is transcriptionally regulated by p53 in response to γ-irradiation through response elements in the first intron of the human gene or in the promoter of the mouse gene. *Bid* mRNA increases in a p53-dependent manner in the splenic red pulp and the colonic epithelium; however, a correlation with an increase in Bid protein levels needs to be shown. Cellular chemosensitivity to the DNA-damaging agents adriamycin and 5-fluorouracil appears to be critically dependent on the presence of wild-type p53 and Bid, *Bid*-null cells being resistant to the effects of these drugs (Sax et al., 2002). p53 therefore appears to promote the convergence of the intrinsic and extrinsic pathways through *Bid* regulation.

p53-mediated abrogation of survival signals: the AKT pathway

Binding of mitogens and cytokines to cell surface receptors including the insulin receptor, the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor receptor (PDGFR), and the actions of oncogenes such as Ras and Her2/Neu, is transduced by phosphoinositide 3-kinase (PI3K) activating signaling pathways that promote cell proliferation and viability (Fig. 2). PI3K comprises a p85 regulatory subunit, which interacts with phosphorylated receptor tyrosine kinases, and the 110 kDa subunit, which localizes to the membrane upon receptor binding. In response to a change in redox state caused by H_2O_2-induced oxidative stress, p85 is upregulated by p53. p85 is involved in the p53-dependent apoptotic response to H_2O_2 in MEFs, but its precise role in cell death is unclear. PI3K activates AKT, a serine/threonine kinase, through phosphorylation on Ser473 by the 3′-phosphoinositide-dependent kinase PDK1 (Lawlor and Alessi, 2001). In turn, AKT phosphorylates a range of targets that function to promote cell survival, including the major inhibitor of p53, Mdm2 (reviewed by Mayo and Donner, 2002). This phosphorylation enhances the nuclear accumulation of Mdm2, augments Mdm2 interaction with p300, and reduces the affinity of Mdm2 for p19ARF (reviewed by Testa and Bellacosa, 2001). Consequently, AKT augments the inhibition and destabilization of p53 by Mdm2 (Fig. 2). Interestingly, stress-induced activation of p53 counteracts the inhibitory effects of this survival pathway by multiple mechanisms (Fig. 2). First, p53 promotes caspase-mediated cleavage and subsequent degradation of the AKT protein itself (Gottlieb et al., 2002). Second, p53 induces the expression of the *PTEN* tumor suppressor gene, which encodes a phosphatase that de-phosphorylates PI3K, thereby impairing AKT activation (reviewed by Mayo and Donner, 2002). Third, p53 induces expression of *cyclin G*, which in turn recruits the phosphatase PP2AB' to the Mdm2-p53 complex, where it de-phosphorylates Mdm2 at the AKT phosphorylation sites. These

feedback loops determine the survival versus apoptotic outcome in the interplay between p53 and the AKT survival pathway (Fig. 2) (reviewed by Oren et al., 2002). This fine balance is often interrupted in cancer, either by mutations in *PTEN* or amplification of *Mdm2* (Mayo and Donner, 2002).

p53-mediated cancer therapy

Stimulation of disabled p53 pathways has been suggested as a potential mode of therapy for cancer: potential approaches include introducing wild-type p53 genetically, empowering aberrant p53 molecules to perform wild-type functions, or intervening to activate directly targets in the p53 apoptotic pathways. Gene therapy based on the introduction of wild-type p53 (reviewed by Wen et al., 2003) and elimination of mutant-p53-expressing cells (reviewed by Post, 2002) is undergoing clinical trials. Restoration of wild-type conformation to structurally contorted p53 DNA-binding mutants has been demonstrated, using peptide constructs and small molecular weight synthetic molecules (reviewed by Bullock and Fersht, 2001). Synthetic peptides derived from the C-terminus of p53 can induce p53-dependent apoptosis in tumor cells and restore the specific DNA-binding and transcription functions to mutant p53 in vitro (Abarzua et al., 1996; Selivanova et al., 1997; Selivanova et al., 1999). In vivo activity of these peptides has been associated with an increase in the levels of the Fas receptor on the cell surface, through a p53-dependent mechanism that is independent of transcription (Kim et al., 1999). Other small synthetic peptides derived from a p53-binding protein have been introduced to restore sequence-specific DNA-binding activity to mutant p53 (Friedler et al., 2002). The expense and complexity of synthesizing synthetic peptides and stability limitations have stimulated screening for small, easily synthesized molecules that have greater therapeutic potential. PRIMA (p53 reactivation and induction of massive apoptosis) is a small molecular weight molecule that provokes apoptosis in a transcription-dependent fashion through conformational manipulation of p53 mutants to restore sequence-specific DNA binding (Bykov et al., 2002). CP-31398 is another small synthetic molecule (Foster et al., 1999) with the capacity to restore wild-type p53 function (apoptosis or growth arrest induction) to mutants (Wang et al., 2003); however, exposure is also associated with the elevation of steady-state wild-type p53 to levels induced by DNA damage (Smith and Fornace, 2002; Takimoto et al., 2002). CP-31398 has been suggested to trigger apoptosis through the intrinsic Bax/mitochondrial/caspase-9 pathway (Luu et al., 2002), while additional responses involving increased DR5 cell surface exposure and reduction in p53-ubiquitination are also associated with this molecule (Wang et al., 2003).

Conclusion

Deconstruction of p53-mediated apoptosis reveals an extensive network of signaling pathways triggered by p53 to ensure an appropriate response to a given stress. Intriguingly, p53 can intervene at every major step in apoptotic pathways: from extrinsic death receptor signaling, through the convergent pathway component Bid, to the intrinsic mitochondria pathways involving apoptosome formation, and culminating in direct caspase activation. Many of these effects are mediated through the activation of specific p53-target genes. In addition, p53 is able to activate apoptotic pathways by transcription-independent mechanisms (including direct shuttling of p53 to the mitochondrial membrane), more of which are likely to be unraveled in the future. Why is this complex apoptotic network required? One possible reason may be to ensure a rapid response. Another is that specific sets of target genes might be activated under a given set of conditions, in a stage-, tissue- and stimulus-specific manner. Alternatively, active p53 may induce the same set of target genes under different conditions, the specificity being determined by other cellular factors. Currently there are examples to support each of these options. Defining the effect of these variables on p53 transcriptional activity using genome-wide expression analysis may help to answer some of these fundamental questions. Such analyses are likely to shed new light on the determinants of the cellular decision between growth arrest and apoptosis.

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