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Review

The p53 response to DNA damage

David W. Meek*

Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK

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Abstract

The p53 tumour suppressor protein is a highly potent transcription factor which, under normal circumstances, is maintained at low levels through the action of MDM2, an E3 ubiquitin ligase which directs p53 ubiquitylation and degradation. Expression of the *mdm2* gene is stimulated by p53 and this reciprocal relationship forms the basis of a negative feedback loop. Both genotoxic and non-genotoxic stresses that induce p53 focus principally on interruption of the p53-MDM2 loop with the consequence that p53 becomes stabilised, leading to changes in the expression of p53-responsive genes. The biological outcome of inducing this pathway can be either growth arrest or apoptosis: factors affecting the functioning of the loop, the biochemical activity of p53 itself and the cellular environment govern the choice between these outcomes in a cell type- and stress-specific manner.

Keywords: p53; MDM2; Growth arrest; Apoptosis

1. The p53 pathway

The p53 tumour suppressor protein plays a pivotal role in the cellular response to a range of environmental and intracellular stresses including agents which cause DNA strand breaks, ultraviolet radiation, hyper-proliferation and hypoxia (for reviews see [1–4]). p53 acts as a node or hub for incoming stress signals which are then transduced, mainly through the ability of p53 to act as a transcription factor that binds to specific sites in the regulatory regions of p53-responsive genes. The biological end-points of p53 induction are growth arrest, which can be transient or permanent (senescence or differentiation), or apoptosis.

The number of physiological p53 responsive genes is likely to run into hundreds, but intensive studies have so far established direct roles for only a small group of players in mediating the p53 response. Principal among those involved in growth arrest are the CDK-inhibitor protein $p21^{WAF1}$ which mediates G1/S arrest by blocking cyclin E-Cdk2-mediated phosphorylation of Rb, and 14-3-3σ which may assist in the inactivation of Cdc25C. Expression of a host of apoptotic genes is stimulated by p53, particularly those involved in the mitochondrial apoptotic pathway such as BAX, NOXA, PUMA and APAF1. In addition, p53 stimulates expression of genes involved in the death receptor pathway including KILLER/DR5, FAS and PIDD (reviewed in [3]). While these genes play roles in the downstream series of events orchestrated by p53, no single gene product can mediate the effects of p53 in full. It is therefore envisioned that their expression and individual functions contribute to a coordinated series of events and that additional key contributions from other (less well characterised) responsive genes are likely to be important. There are also believed to be other, p53-transactivation-independent mechanisms that contribute to apoptosis including the ability of p53 to repress gene expression.

2. The p53-MDM2 feedback loop

Under normal circumstances, p53 is tightly regulated through its interaction with MDM2, a negative regulatory partner. MDM2 is an E3 ubiquitin ligase which, together with the p300 “transcriptional co-activator” protein (acting as an E4 polyubiquitin ligase), mediates both the ubiquitylation and proteasome-dependent degradation of p53 [2]. The binding of MDM2 to the transactivation domain within the N-terminus of p53 plays an additional role of blocking the interaction of p53 with the transcriptional apparatus. MDM2 can also mediate translocation of p53 to the cytoplasm thereby removing it from its site of action, and can

* Tel.: +44-1382-660111x22517; fax: +44-1382-669993.
E-mail address: david.meek@cancer.org.uk (D.W. Meek).

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recruit the histone deacetylase HDAC1 to deacetylate key lysine residues in the C-terminus of p53 thus making them available for ubiquitylation. The *mdm2* gene is itself a p53 target and the two proteins therefore function within an autoregulatory loop in which p53 positively regulates MDM2 expression while MDM2 negatively regulates p53 levels and activity [5].

The physiological relevance of the p53-MDM2 loop is underscored by several observations. For example, knock-out of the *mdm2* gene in mice gives rise to an embryonic lethal phenotype, and this can be rescued in a *p53*-null background [6–8]. At the cellular level, disruption of the p53-MDM2 interaction with synthetic competitive inhibitors is sufficient to induce a p53 response in cultured cells [9–13]. Of particular significance, however, is the observation that the p53-MDM2 loop is the focal point of many different types of stress that activate the p53 pathway [14]. These stress signals act by interrupting the p53-MDM2 interaction, leading to a rapid rise in p53 levels, and operate through several mechanisms depending upon the type and magnitude of the initiating stress [15]. For example, DNA strand breaks and UV radiation initiate a series of phosphorylation events that perturb the p53-MDM2 interaction and inhibit the ability of MDM2 to mediate p53 degradation. Other stresses such as hypoxia and high, but now low, doses of UV are thought to act on the loop by repressing *mdm2* expression. Hyper-proliferation, induced by dominant oncogenes, operates through a different route, that of inducing expression of $p14^{ARF}$, one of two products encoded by overlapping reading frames within the INK4A gene, which binds to MDM2 and blocks its ability to mediate ubiquitylation and degradation of p53. Similarly, inhibition of MDM2-mediated p53 degradation and nuclear export by HIF1α may contribute to the induction of p53 by hypoxia [16]. Thus different stresses, and indeed different levels of stress, can act through different but potentially overlapping routes but with the same principal outcome, i.e. the interruption of the p53-MDM2 loop leading to the induction of the p53 protein and p53-dependent changes in gene expression.

3. The p53 response following DNA strand breaks

Of the various cellular stresses that initiate a p53 response, the molecular mechanisms by which p53 is activated following DNA double strand breaks are perhaps the most comprehensively understood. Activation of p53 by DNA damage occurs at two levels: the stabilisation of the p53 protein, leading to its accumulation in the nucleus, and activation of biochemical functions encompassed within the p53 protein.

Stabilisation of p53 occurs through inhibition of its degradation by MDM2 and is mediated mainly by multi-site phosphorylation of both the p53 and MDM2 proteins (comprehensively reviewed by [17]; other mechanisms can impinge on the p53-MDM2 loop to modulate the DNA damage response). p53 is modified initially through the phosphorylation of ser15 by the ATM protein kinase. (Ser15 is also phosphorylated in response to UV radiation but through the action of ATR. In the DNA strand break response, the transiently-activated ATM mediates the initial phase of ser15 phosphorylation while the more slowly activated ATR maintains modification of this residue over several hours.) Ser15 phosphorylation is thought to nucleate a series of subsequent post-translational modifications on p53 that contribute to both its stablisation and biochemical activation (as many as 17 sites in p53 undergo phosphorylation or acetylation [17]). For example, ser15 phosphorylation primes the subsequent phosphorylation of thr18 (possibly by the protein kinase CK1) and positively influences phosphorylation of ser9 and ser20 by other protein kinases [18]. Phosphorylation of thr18 and/or ser20, both of which lie within close proximity of the MDM2 binding site, are thought to disrupt p53-MDM2 binding independently, thereby attenuating, at least in part, the inhibitory actions of MDM2 on p53. DNA damage-induced changes in the phosphorylation of MDM2 are also thought to contribute to blocking the degradation of p53 (reviewed in [19]). Phosphorylation of MDM2 itself at serine 395 by ATM, and at the adjacent tyrosine 394 by the c-Abl protein kinase (also a downstrean effector of ATM), inhibit, independently of each other, the ability of MDM2 to mediate the degradation of p53 and, in the case of ser395 phosphorylation, block MDM2-mediated nuclear export of p53. (This coordinate regulation of proteins by the ATM protein kinase provides a means, not only for regulating different components within the p53 pathway simultaneously, but in synchronising p53 control with the regulation of other cellular events, especially DNA repair.) These post-translational events in MDM2 are coupled with DNA damage-induced dephosphorylation of a cluster of phosphoserine residues in the central domain, but it is not yet clear whether this involves inhibition of a protein kinase(s), activation of a protein phosphatase(s), or both of these mechanisms. Interestingly, dephosphorylation of these residues does not affect the ability of MDM2 to ubiquitylate p53, but inhibits p53 degradation, strongly indicating that ubiquitylation and degradation of p53 by MDM2 are separate and consecutive events. Other post-translational modifications of MDM2, including regulatory events mediated through the PI3-kinase/Akt survival pathway, are likely to have a bearing on the control of the p53-MDM2 loop and are currently the focus of much attention [19]. Clearly, however, molecular events inpinging on both MDM2 and p53 can independently regulate p53 stabilisation following DNA damage, and are likely to operate in a cooperative or integrated manner thereby conferring significant sensitivity on the control of the p53-MDM2 loop.

Coupled with the stabilisation of p53, phosphorylation of ser15, thr18 and ser20 also stimulates recruitment of transcription factors including p300, CBP (both transcriptional co-activators and histone acetyl-transferases [HATs]) and P/CAF (a p300/CBP-associated HAT). These factors not only stimulate transcription from p53-responsive promoters

but also promote acetylation of a cluster of C-terminal lysine residues in p53 that are normally targets for ubiquitylation [20]. Acetylation of these residues is therefore thought to contribute to p53 stabilisation by impairing ubiquitylation. Furthermore, in addition to acting as a binding site for MDM2 and transcription factors, the N-terminus of p53 is thought to contain a nuclear export sequence (amino acids 11–27). DNA damage-induced phosphorylation of ser15 and ser20 may also block export of p53 mediated by this NES thereby maintaining p53 within the nucleus [21]. The phosphorylation-dependent changes in partner interactions occurring within this region of p53 can therefore concurrently regulate stabilisation, transactivation function and subcellular localisation of p53.

DNA damage-induced post-translational modification of p53 also extends to other residues (Fig. 1) [17]. Serines 6, 9, 33, 37 and 46 within the N-terminus of p53 have also been reported to occur following strands breaks. At the C-terminus, phosphorylation of ser315 also occurs and can regulate promoter selectivity, while dephosphorylation of ser376 by an ATM-regulated phosphatase allows 14-3-3 binding to phosphorylated ser378 with consequent effects on site-specific DNA binding. The modifications of p53 are therefore highly complex and inter-dependent. However, insight into a compelling degree of specificity arising from this complexity is emerging in several ways. Firstly, different stresses, and indeed different levels of stress, lead to the differential modification, both qualititative and quanititative, of many of these and other sites in p53 [18]. (For example, in addition to the aforementioned sites of strand break-induced modification, significant phosphorylation of ser33, ser37, ser392 and acetylation of lys320 are stiumulated by UV radiation.) The second observation is that modifications, such as ser46 phosphorylation that occurs under specific circumstances, can significantly influence the outcome of inducing the p53 pathway in favour of apoptosis [22]. Thirdly, MDM2 is also under complex regulation and is acutely sensitive to changes in the cellular environment, indicating that both the activity of p53 itself, and its ability to be induced through targeting of the p53-MDM2 loop, may be crucial factors in determining whether cells undergo arrest or apoptosis. Collectively, therefore, published evidence supports a model in which differential post-translational modification may form the basis of a code that programmes the response of the p53 pathway to a given stress encountered under a given set of conditions.

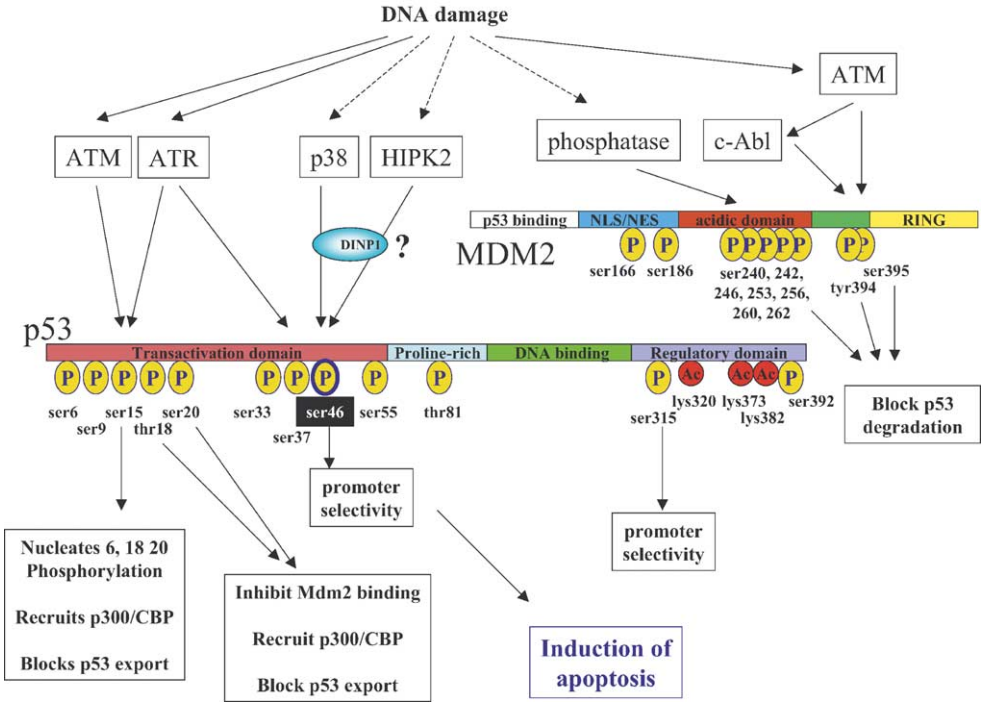

Fig. 1. DNA damage-induced post-translational modification of p53 and MDM2. The p53 and MDM2 proteins are shown schematically (not to scale). The transactivation-, proline-rich-, DNA binding- and C-terminal regulatory domains of p53 are highlighted, as well as the p53-binding-, nucleo-cytoplasmic shuttling- (NLS/NES), acidic- and RING-finger domains of MDM2. Phosphorylation and acetylation sites are indicated by ellipses containing, respectively, the letters P and Ac. In each case the target residue is stated underneath the appropriate symbol. Boxes containing text summarise the biological consequences of each of the relevant modification sites. Signalling enzymes are also indicated, where appropriate. Broken lines indicate signalling enzymes that are likely to be activated indirectly by DNA damage. The ser46 phosphorylation of p53 is highlighted because this modification is induced by high levels of stress and is thought to lead to the induction of apoptosis [22]. The p53DINP1 protein stimulates ser46 phosphorylation but its mechanism of action is not yet understood. This figure only highlights signalling events relevant to the text and for a fully comprehensive review of all of the known modifications in p53, the reader is referred elsewhere [17]. Current understanding of the post-translational modification of MDM2 has been summarised recently in a separate review [19].

4. Growth arrest or apoptosis?

Different types of cells often show different biological responses to a given stress [1]. For example, thymocytes and splenocytes undergo p53-dependent apoptosis in vivo in response to low levels of ionising radiation. In contrast, fibroblasts arrest in a p53-dependent manner following DNA damage but will nevertheless undergo p53-dependent apoptosis following a different type of stress such as oncogenic transformation. These different cell types may therefore exhibit low and high thresholds, respectively, for the onset of apoptosis. In many other cell types, however, the outcome of DNA damage can be either growth arrest or apoptosis, depending on the circumstances. The ability of a cell to execute a choice between life and death suggests that the p53 pathway is able to sense whether or not recovery from DNA damage has been successful (DNA repair) thus permitting the p53 response to be attenuated, or, assuming the initiating signal is still present, maintained or promoted to deliver an irreversible outcome.

The currently accepted model for the choice between arrest and apoptosis involves the complex interplay of a number of factors (summarised in Fig. 2), but is still incomplete. This model is based principally on the idea that p53 is able to differentially transactivate promoters of "growth arrest" and "apoptosis" genes. Built into this idea are factors that influence the extent to which p53 can favour a given class of promoters, including the presence of cellular pro-apoptotic proteins that govern promoter selectivity, and the concerted action of apoptosis-related pathways that shift the balance of p53 induction in favour of cell death, either by influencing the cellular environment or by impinging directly on the ability of stress signals to induce a p53 response [1,3].

Originally, the idea that p53 could discriminate between promoters of "growth arrest" and "apoptosis" genes was built on the suggestion that promoters of growth arrest genes encompassed high-affinity p53 binding sites (e.g. p21, GADD45, MDM2), whereas the promoters of apoptotic genes contained low-affinity p53 binding sites (e.g. BAX, IGF-BP3). This model explained the observations that: (i) increased levels or activity of p53 (governed by the type and/or intensity of the signal) can lead to the onset of apoptosis (presumably by achieving a certain threshold level); and (ii) that p53 mutants with marginally altered conformations retain sufficient activity to induce arrest, but not apoptosis (presumably because they can still interact with high affinity sites) [23–25]. When analysing the recruitment of p53 to responsive promoters in a cellular and chromatin-relevant

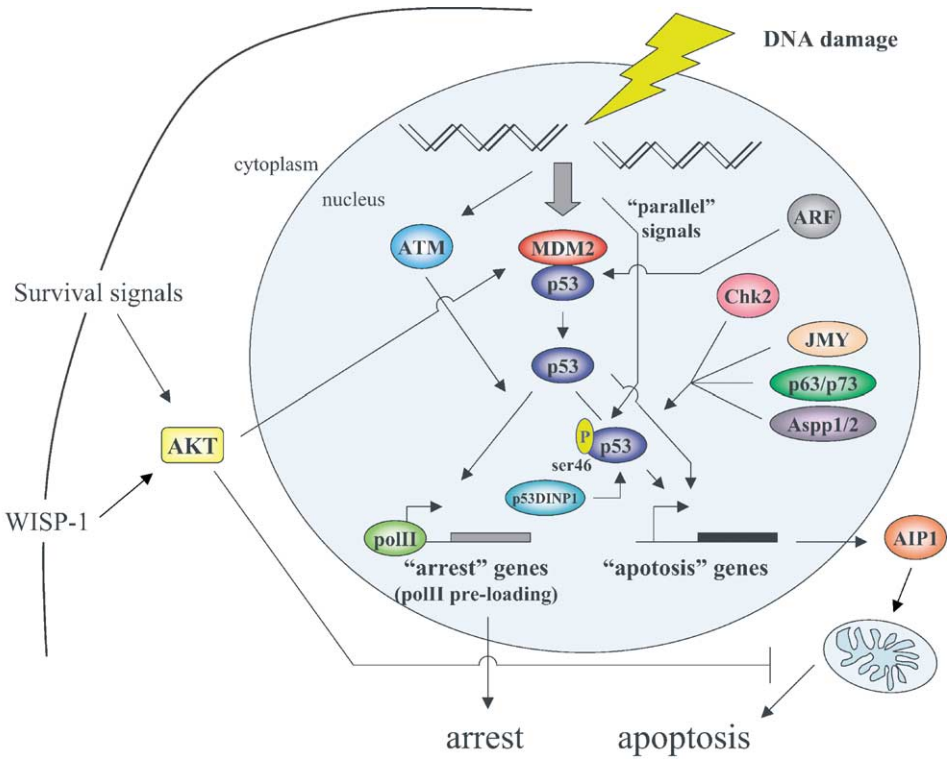

Fig. 2. Factors influencing the choice between p53-dependent growth arrest or apoptosis. The figure shows induction of p53 by DNA damage leading to the release from MDM2 control and the induction of "growth arrest" genes or "apoptosis" genes. Chk2, JMY, p63/p73 and ASPP1/2 are indicated as favouring the apoptosis route whereas ATM is indicated as favouring transcription of arrest genes. ARF and AKT, factors which affect the operation of the p53-MDM2 loop, are shown. The AKT pathway responsive to WISP-1 signalling and targeting the mitochondrial apoptosis pathway is also indicated. The preloading of polII complexes on arrest genes is shown. Also indicated are "parallel signals" such as the HIPK2 and p38 pathways that phosphorylate ser46 of p53 and lead to the expression of AIP1 and subsequent apoptosis by the mitochondrial pathway. The p53DINP1 protein stimulates ser46 phosphorylation but its mechanism of action is not yet understood.

context, however, exceptions to this proposed relationship between promoter binding and the induction of apoptosis were found. (For example, the pro-apoptotic gene PUMA shows high p53 promoter occupancy in vivo while other apoptotic genes such as PIG3 show delayed kinetics of induction and p53 binding that is barely distinguishable from that of non-p53-responsive genes [26,27].) Therefore, while the respective presence of high- and low-affinity p53 binding sites in growth arrest and apoposis promoters may contribute to apoptosis in the presence of high levels of active p53, other factors are likely to play an essential role in governing the choice between arrest and apoptosis.

Established cellular factors which influence p53-dependent apoptosis include the JMY protein which interacts with p300 to enhance, selectively, the ability of p53 to induce expression of apoptotic genes such as BAX [28]. Similarly, ASPP-1 and -2 proteins can discriminate in favour of the interaction of p53 with the promoters of apoptotic genes [29]. The p53 family members p63 and p73 are also thought to favour selective binding of p53 to apoptotic promoters, but the precise mechanism through which this enhancement occurs is still uncertain [30]. One can therefore envisage that the presence or indeed the levels of such proteins in a given cell type may influence or even "hard-wire" the type of response that this given cell will undertake following activation of the p53 pathway. The absence of cellular factors can also influence growth arrest versus apoptosis significantly. For example, in the absence of functional RB, uncontrolled E2F proteins drive hyper-proliferation which subsequently leads to apoptosis through p53-dependent and -independent mechanisms (reviewed in [31]). Therefore changes in the availability of cellular proteins, as for example, might occur during tumour pathogenesis, can also influence the p53 pathway.

Given, however, that high- and low-affinity p53 binding sites, or the presence of pro-apoptotic factors, may only contribute in part to the choice between growth arrest and apoptosis, what other mechanisms may govern this decision? A recent study has provided compelling evidence that the composition of transcription initiation complexes at the core promoters of p53-responsive genes may profoundly influence this decision [32]. The emerging model proposes that basal levels of p53, binding to cognate sites upstream of the core promoter region, mediate assembly of a poised RNA polymerase II initiation complex on the core promoters of genes involved in the growth arrest/DNA repair response (including p21, GADD45, PCNA and 14-3-3σ). Following DNA damage, the paused polII complex is permitted to engage in the elongation phase of transcription. This pre-loading of the transcriptional machinery provides sensitivity by lowering the threshold required for a p53 response with the result that small changes in p53 levels or activity give rise to a rapid activation of target gene expression. (At the same time there is increased binding of p53 to its responsive elements, further stimulating the recruitment of histone acetylases.) In contrast, the mechanism of transcription of pro-apoptotic genes differs such that there is a significantly lower level of bound initiation complex compared with the growth arrest genes prior to a stress stimulus. Following the stress stimulus, induction of gene expression presumably requires a higher level or degree of activation of p53 and proceeds with much slower kinetics. One could therefore envisage in this model the possibility that failure to terminate the p53 response would provide time for the accumulation of sufficient levels of the appropriate apoptotic gene products to initiate an apoptotic response. This would also be consistent with the idea that the duration of the p53 response (which could be prolonged, for example, through persistent stimulation such as extensive or unrepaired DNA damage) may influence whether growth arrest or apoptosis prevails.

The intensity of the stress can also give rise to apoptosis by directly influencing the activity of p53. High, but not low, levels of UV radiation induce the phosphorylation of p53 on ser46 which promotes expression of p53AIP1, a novel pro-apoptotic factor that dissipates the electrochemical gradient across the mitochondrial inner membrane [22]. An additional novel p53-responsive gene, p53DINP1, is thought to mediate the stimulation of ser46 phosphorylation and apoptosis, possibly by a mechanism involving interaction of the p53DINP1 protein with a ser46 kinase [33]. These particular studies underscore the principle that cellular mechanisms that are differentially sensitive to the levels of a particular stress signal can have a direct and selective bearing on the activity of p53. They also reinforces the idea that different p53 responsive genes can be differentially regulated according to the intensity of the incoming stress signal.

Other factors suggest a relationship between the intensity of the stress and the signals to which p53 is sensitive. Genetic analyses indicate that both the ATM protein kinase and checkpoint kinase 2 (Chk2) play a role in activating p53 following DNA damage. It is well established that ATM phosphorylates serine 15, but whether Chk2 phosphorylates p53 directly, and indeed whether Chk2 is itself phosphorylated by ATM, remains controversial. A recent investigation, based on the use of fibroblasts from $ATM^{-/-}$ and $Chk2^{-/-}$ mice, challenges current dogma by demonstrating that ATM is required for p53-mediated growth arrest but Chk2 is dispensible for this function [34]. In contrast Chk2, but not ATM, seems to be required to bring about p53-mediated apoptosis. This separation of function implies that different pathways arising from the same type of stress stimulus may play a role in tailoring the p53 response. However, it is not clear what the mechanism of this effect might be nor whether the extent of the DNA damage or the intensity of the signal can activate these pathways differentially.

Over and above events which influence the p53 response directly or cooperate with p53, factors which influence the p53-MDM2 loop are also likely to play a significant role in determining the outcome of inducing p53, possibly by setting thresholds for p53 induction and, consequently, apoptosis. For example, MDM2 is the target of the AKT/PKB protein kinase which mediates many of the

survival signals that are transduced through the PI3-kinase pathway [35–38]. AKT-mediated phosphorylation of two residues, ser166 and ser186 in MDM2, has been reported to stimulate nuclear import of MDM2 and MDM2-mediated p53 degradation. Withdrawal of survival signalling or inhibition of the PI3-K/AKT pathway blocks phosphorylation of ser166 and ser186 in MDM2, with the result that the basal level of p53 is increased thereby lowering of the threshold needed to initiate p53 induction in response to other factors. Under these circumstances, p53 induction becomes acutely sensitive to DNA damage-inducing drugs in a manner that favours apoptosis [38,39]. Mutation of the PTEN tumour suppressor gene, whose product impinges on this survival mechanism by inhibiting the activation of AKT, can lead to a constitutively high level of activity of MDM2 in tumour cells. This, in turn, can increase the threshold required to induce p53 making it more difficult to induce apoptosis [36].

A second key player in regulating the threshold level for p53 induction is $p14^{ARF}$ (ARF), the protein inhibitor of MDM2 that is induced by hyper-proliferative signals. Murine fibroblasts lacking expression of ARF show defects in the DNA damage response and show a significantly weaker induction of p53, and consequently p21, in response to DNA damage as compared with wild type cells [40,41]. These data are consistent with the idea that ARF may contribute to setting the threshold for p53 induction through its regulation of MDM2 in a manner that may reflect the proliferative status of the cell. Moreover, subsequent induction of ARF by the induced p53 may contribute to sustaining the p53 response [41], raising the possibility that these mechanisms could influence the choice between arrest and death in cells susceptible to undergo apoptosis.

Additional proteins may impact on the p53-MDM2 loop and influence the outcome of p53 induction. For example, the RB tumour suppressor has been demonstrated to form a trimeric complex with p53 and MDM2 with the outcome of inducing apoptosis [42]. p53 is able to repress the expression of several anti-apoptitic genes and, interestingly, the RB-p53-MDM2 complex retains p53-mediated repression function but is unable to induce expression of p53-responsive genes such as p21. These data highlight a further contributory factor to p53-dependent apoptosis but one which is likely to operate through a transactivation-independent mechanism. Other proteins such as MDMX and TSG101, which can regulate the action of MDM2, or transcription factors such as TAFII31 or p300/CBP which can compete with MDM2 for binding to p53, have a bearing on the function of the p53-MDM2 loop. Whether these proteins can potentially impact on the choice of cell fate remains to be firmly established.

Finally, in addition to factors that promote apoptosis by impinging directly on the p53 pathway itself, the operation of p53-independent, or parallel, pro-apoptotic pathways that contribute to apoptosis in their own right may also affect the outcome of inducing p53. For example, the PI3-K/AKT pathway mediates the phosphorylation and regulation of key apoptotic effector proteins including BAD, caspase 9, XIAP (and possibly others) in a manner that inhibits the onset of apoptosis. Pro-apoptotic stimuli or the withdrawal of survival signals, in addition to lowering the threshold for p53 induction, may prime the apoptotic pathway for engagement in a manner that would synergise with the action of p53.

Relevant to the link between p53 and PI3-K signalling is the finding that the WISP-1 (Wnt-1-induced secreted protein) growth factor can block DNA damage-induced, p53-dependent apoptosis (but not death receptor-mediated apoptosis) by a mechanism which involves activation of the AKT pathway [43]. WISP-1 action leads to up-regulation of the anti-apoptotic protein Bcl-X_L and a corresponding inhibition of cytochrome c release from the mitochondrion. This provides another example of how a p53-independent pathway can profoundly and specifically influence the outcome of p53 action.

The ability of the Myc protein to repress expression of p21, which contributes to the sensitisation of cells to apoptosis by Myc, provides yet another example of a p53-independent route that can cooperate with p53 in the induction of apoptosis. The p21 protein is not only a mediator of cell cycle arrest, but can also protect cells against apoptosis. In addition to stimulation by p53, p21 expression also requires the action of the stress-inducible Miz-1 (Myc-interacting zinc finger-1) protein. However, in the presence of Myc, Miz-1 recruits Myc to the p21 promoter resulting in the repression of p21 transcription [44,45]. This effect is specific to the p21 promoter and loss of p21 expression therefore removes a p53-dependent inhibitor of cell death in a manner that does not affect p53-dependent expression of pro-apoptotic factors such as PUMA, PIG3 and BAX, thereby favouring the initiation of apoptosis.

5. Concluding remarks

Induction of p53 can occur in response to a range of genotoxic or non-genotoxic stresses leading to the biological outcomes of growth arrest or apoptosis. It is now clear that there is a large and complex range of factors that contribute to the choice between these two general outcomes including the cell type, the type and intensity of initiating stress, p53 levels, the presence of p53 co-activators or regulators and the p53-MDM2 regulatory feedback loop which is itself the major target of inducers of the p53 pathway. Considerable insight has been gained over the past few years into the molecular mechanisms by which many of these factors selectively favour arrest or apoptosis but these are only partly understood and a comprehensive understanding of the complex inter-relationship between these different factors and how they contribute to an integrated decision concerning the biological outcome of inducing p53 has not yet been achieved. Understanding these mechanisms is currently a major challenge in p53 research which may ultimately provide novel

targets and approaches to therapeutic manipulation of the p53 pathway in the treatment of cancer.

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