Cell Death and Differentiation (2003) 10, 397–399

www.nature.com/cdd

Editorial

p53: 25 years of research and more questions to answer

JC Bourdon[1], V De Laurenzi[2,3], G Melino[2,3] and D Lane*,[1]

[1] Department of Surgery and Molecular Oncology, Ninewells Hospital and Medical School, Cancer Research UK, Cell Transformation Research Group, University of Dundee, Dundee DD1 9SY, UK
[2] Biochemistry Laboratory, IDI-IRCCS, c/o Department of Experimental Medicine and Biochemical Sciences, University of Rome 'Tor Vergata', 00133 Rome, Italy
[3] MRC Toxicology Unit, Hodgkin Building, University of Leicester, PO Box 138, Lancaster Road, Leicester LE1 9HN, UK
* Corresponding author: D. Lane. E-mail: j.bourdon@dundee.ac.uk

Cell Death and Differentiation (2003) **10,** 397–399. doi:10.1038/sj.cdd.4401243

The p53 tumor suppressor protein, first discovered in 1979, acts as a major node in a complex signalling pathway evolved to sense a broad range of cellular stresses such as DNA damage, oncogene activation, viral infection and ribonucleotide depletion. The p53 network, normally switched 'off', is activated by such cellular stresses that can alter normal cell cycle progression or induce mutations of the genome leading to oncogenic transformation. Activated p53 protein stops the cell cycle or, in many cases, switches 'on' the programmed cell death (apoptosis) pathways forcing damaged cells to commit suicide.[1] The p53 protein therefore prevents the multiplication of stressed cells that are more likely than undamaged cells both to contain mutations and exhibit abnormal cellular growth. Hence, the p53 protein, the guardian of the genome, is a critical inhibitor of tumour development explaining why it is the most frequently mutated gene in human cancers.[2–5]

This issue of *Cell Death Differentiation* contains, together with some new work on p53, six reviews and commentary articles partly summarising current knowledge in the field and outlining the main questions that still have to be answered by researchers in this area.

The mechanisms by which p53 accomplishes all its biological functions are still not completely understood. However, in the last decade, it has been shown that p53 is a transcription factor that specifically binds to sequences of DNA.[6,7] Based on the alignments of the p53 responsive elements (p53RE) which have already been identified,[8,9] 96% of the p53RE are composed of at least three repeats of the DNA sequence RRRCWWGYYY (where R=G or A, W=A or T and Y=C or T) separated by 0–13 bp. p53 activates expression of the genes containing such p53RE in their intron or promoter sequences. In the last few years, it has been established that the ability of p53 to modulate gene expression is necessary for its tumour suppressor activity. Hence, mice that are genetically engineered to express a transactivation-defective mutant p53 protein from the endogenous locus as a knockin allele are defective in both cell cycle arrest and apoptosis in response to cellular stress.[10] These animals, as in the p53−/− mouse model, are cancer prone, indicating that p53 must regulate the expression of genes involved in cell cycle arrest and cell death to prevent carcinogenesis. Therefore, identification of transcriptional targets of p53 is critical in discerning pathways by which p53 affects global cellular outcomes such as growth arrest and cell death. Identification of the cyclin-dependent kinase inhibitor Waf as a p53-responsive gene helps to explain how p53 can induce cell cycle arrest.[6,11,12] Recently, several p53-inducible genes that encode for proteins with apoptotic potential have been identified. The p53-inducible proapoptotic genes are involved in several death pathways (Figure 1): the death-receptor pathway (CD95/Fas, TNF, TRAIL, PIDD), the mitochondrial pathway (Bax, Noxa, PUMA, P53AIP, Bid), and the recently described endoplasmic reticulum (ER) stress pathway (Scotin).[9,13–21] As a protein-folding compartment, the ER is exquisitely sensitive to alterations in homeostasis that disrupt the ER function (ER-stress).[22,23] ER stresses include ER calcium store depletion, inhibition of glycosylation, reduction of disulphide bonds, expression of mutant protein or protein subunits, overexpression of wild-type (wt) protein, expression of viral proteins and hypoxia. The mechanisms of ER-stress mediated apoptosis are still unclear. One possible mechanism is the release of calcium from the ER upon ER-stress, which triggers the opening of the calcium-sensitive mitochondrial permeability transition pore (PTP), allowing the release of cytochrome *c* from the mitochondria to the cytosol and thus activating the caspase cascade.[24–26] Scotin is a protein located in the ER and the nuclear membrane. Loss of Scotin expression upon antisense expression inhibits strongly ER-stress-mediated apoptosis. The apoptotic pathways are not completely independent and a complex interplay occurs between them. In addition, very recently, a new apoptotic pathway, similar to that activated by Drosophila p53, has been described in human cells, adding additional complexity to the system. p53 increases the expression of HTRA2/Omi, an IAP-binding serine protease, which will then bind and cleave CIAP1 removing the block on caspase activation.[27] p53 also induces the expression of proteins involved in the more downstream phases of apoptosis such as APAF-1[28,29] and Caspase-6.[30]

The increasing numbers of new p53-responsive genes identified in the last years[31] and the identification of two p53 homologues, p63 and p73,[32–35] suggest that there is still a lot of work to be done to define clearly the p53-dependent pathways. Identification and characterisation of novel p53-target genes, using modern genomics and proteomics techniques, is a particularly efficient way to identify novel cellular pathways controlled by this gene. In this issue of CDD,

Sax and El-Deiry[31] summarise the p53-inducible genes and describe their functions according to their ability to control cell cycle and/or apoptosis.

Although it is generally believed that p53 effects are exerted through the activation of transcription, it is becoming evident that p53 is also capable of repressing transcription. Ho and Benchimol[36] illustrate the mechanism through which p53 can repress transcription and describe some of the cases in which the repressive effects play a role. Once again large-scale screening will help to define these repressive pathways.

Activated p53 can induce both cell cycle arrest and apoptosis, but here again scientists face an open question, what determines whether cells stop proliferating or die? The choice between life and death could be because of the ability of p53 to bind and transactivate preferential subsets of genes. For example, some tumour-derived p53 mutants can transactivate as efficiently as wt p53, the promoter of the CDK inhibitor p21, but cannot transactivate the promoter of the proapoptotic gene Bax.[37,38] Reciprocally, the p53 mutant 121F can transactivate Bax as efficiently as wt p53 but not the p21 promoter.[39]

Regulators of p53 transcriptional activity can also play a role in the choice between life and death. For example, ASPP has been shown to enhance the DNA binding and transcriptional activity of p53 on the promoters of proapoptotic genes *in vivo*. Interestingly, the expression of ASPP is frequently downregulated in human breast cancer expressing wt but not mutant p53.[40] Recently, an inhibitor of ASPP has been identified, iASPP. Both human iASPP and its homologue in *Caenorhabditic elegans*, Ce-iASPP, can inhibit p53-mediated apoptosis. Therefore, iASPP is the most phylogenetically conserved inhibitor of p53 identified so far, suggesting that it may play a critical role in controlling p53 and therefore be involved in carcinogenesis.[41]

The N&C by Weber and Zambetti[42] and the review by Oren,[43] analyse the current hypotheses that are believed to explain the different behaviour of a cell in response to p53 activation. Different levels of p53 could trigger different responses depending on high or low affinity of the responsive promoters; cell context: different cell types, expressing different cofactors, would have different responses; block of the apoptotic pathway downstream of p53 by upregulation of antiapoptotic genes can also determine cell fate; different post-translational modifications of p53 would determine the promoter specificity of the protein and therefore the pathway that is activated.[42] Additionally, proapoptotic proteins often require specific stimuli in order to trigger apoptotic pathways (i.e. binding of CD95L, or Fas ligand, to CD95, binding of TRAIL to DR5, phosphorylation of Bax for its translocation from the cytoplasm to the mitochondria, etc.), suggesting therefore that additional cellular signalling (both intracellular and extracellular) represents another level of control over cell fate. According to this model, p53 would be required to provide the proapoptotic protein network, while cell signalling would be necessary to provide adequate stimuli to fire the apoptotic cascade network. Thus, the interdependence between the cell signalling (cell context) and p53 may protect the cell from inappropriate induction of cell death in response to cellular damage and only trigger apoptosis when death is the last resort (Figure 1).

Owing to its deadly function, p53 activity must be tightly regulated. In the absence of cellular stress, p53 is a short-lived protein because of its rapid degradation by the proteasome. Mdm2, one of p53 target genes, encodes an E3 ubiquitin

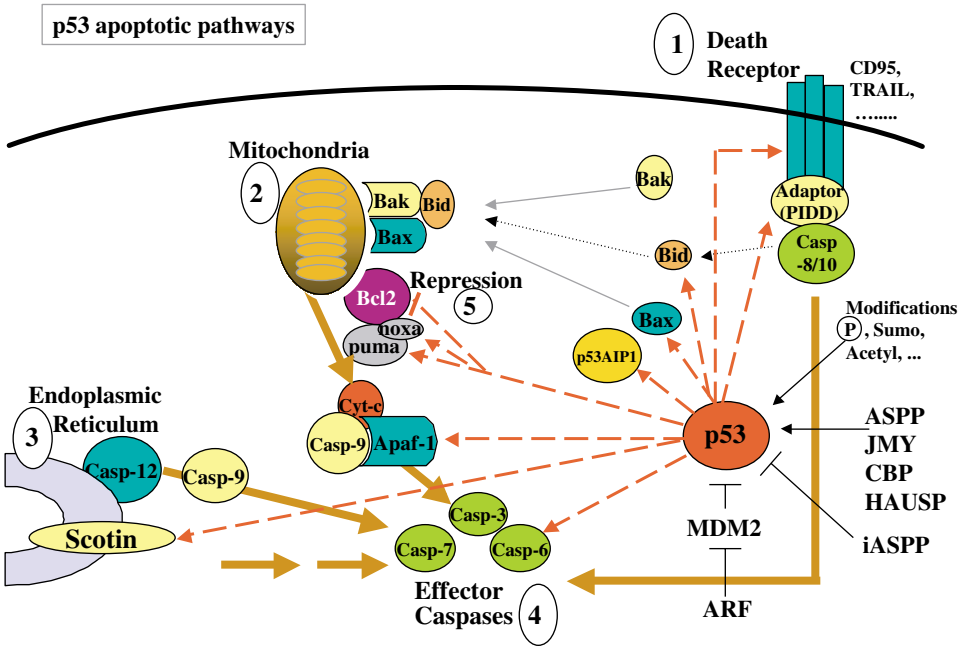

Figure 1 Schematic representation of the different apoptotic pathways controlled by p53. In response to cellular stress, p53 transactivates proapoptotic genes activating (1) the death receptor pathway (pidd, CD95, TRAIL, Bid), (2) the mitochondrial pathway (bax, noxa, puma, p53aip1, apaf-1) and (3) the ER pathway (Scotin). All the different pathways converge to a common downstream pathway (4), where caspase 6 is directly transactivated by p53, thus modulating the sensitivity of the cell to die. Finally, p53 can also repress the transcription of relevant prosurvival genes (5), as shown for Bcl2. The activity of p53 is controlled in different ways depending on the cell context: post-translational modifications (phosphorylation, sumoylation, acetylation), cofactors (ASPP, JMY, CBP) and inhibitors (MDM2, iASPP)

ligase that binds p53 and promotes its ubiquination and degradation. Other post-translational modifications of p53 including phosphorylation, acetylation and sumoylation, affect p53 stability and function. Again, although a large number of data have been generated describing p53 modifications, we do not have a clear picture of their exact role in regulating p53 function. In the N&C of this issue, Xu[44] summarises the important role of post-translational modifications in the regulation of p53 function. Xu[44] also suggests that new techniques now available (knockin and phospho-/acetylation-specific antibodies) should be used to define the role of the many modifications identified. A recent advancement in the understanding of p53 regulation is given by the elucidation of the role exerted by ATM.[45] Extremely few double-stranded breaks (DSB) are sufficient to trigger within minutes the autophosphorylation of ATM with dissociation of their homo-dimer;[45] this activates the kinase activity of ATM on other substrates, such as p53.[46,47]

Despite the huge amount of work carried out on p53 in the last 10 years, many questions on its function still remain partly unanswered: What are the stimuli leading to p53 activation? Which post-translational modifications are essential for p53 function? How does p53 induce apoptosis? How does a cell decide between life and death after p53 is induced? What is the function of the more recently identified p53 homologues, p63 and p73, and do these genes interact with each other leading to cell cycle control and differentiation?

An important experimental approach undertaken by many scientists in the last years to study p53 function, and try to answer some of these questions, has been the generation of mouse models with altered p53. A number of different models have been generated, including: transgenic animals over-expressing wt and mutated p53, knockout and knockin animals. Clarke and Hollstein[48] in their review summarise the phenotypes of the different mice and the questions raised by this experimental approach, and suggest that other animal models (tissue specific, inducible) could be of use in the understanding of p53 biology.

1. Benchimol S (2001) Cell Death Differ. 8: 1049–1051
2. Choisy-Rossi C and Yonish-Rouach E (1998) Cell Death Differ. 5: 129–131
3. Vogelstein B *et al.* (2000) Nature 408: 307–310
4. Vousden KH and Lu X (2002) Nat. Rev. Cancer 2: 594–604
5. El-Deiry WS (2001) Cell Death Differ. 8: 1066–1075
6. El-Deiry WS *et al.* (1993) Cell 75: 817–825
7. Funk WD *et al.* (1992) Mol. Cell. Biol. 12: 2866–2871
8. Bourdon JC *et al.* (1997) Oncogene 14: 85–94
9. Bourdon JC (2002) J. Cell. Biol. 158: 235–246
10. Jimenez GS *et al.* (2000) Nat. Genet. 26: 37–43
11. Harper JW *et al.* (1993) Cell 75(4): 805–816
12. Castedo M *et al.* (2002) Cell Death Differ. 9: 1287–1293
13. Miyashita T and Reed JC (1995) Cell 80: 293–299
14. Muller M *et al.* (1998) J. Exp. Med. 188: 2033–2045
15. Oda E *et al.* N (2000) Science 288: 1053–1058
16. Oda K *et al.* Y (2000) Cell 102: 849–862
17. Munsch D *et al.* (2000) J. Biol. Chem. 275: 3867–3872
18. Nakano K and Vousden KH (2001) Mol. Cell 7: 683–694
19. Yu J *et al.* (2001) Mol. Cell 7: 673–682
20. Klocke BJ *et al.* (2002) Cell Death Differ. 9: 1063–1068
21. Santamaria AB *et al.* (2002) Cell Death Differ. 9: 549–560
22. Kaufman R J (1999) Genes Dev. 13: 1211–1233
23. Ferri KF and Kroemer G (2001) Nat. Cell Biol. 3: E255–E263
24. Ichas F *et al.* (1997). Cell 89: 1145–1153
25. Ichas F and Mazat JP (1998) Biochim. Biophys. Acta. 1366: 33–50
26. Szalai G *et al.* (1999) EMBO J. 18: 6349–6361
27. Jin S *et al.* (2003) Genes Dev. 17: 359–367
28. Fortin A *et al.* (2001) J. Cell Biol. 155: 207–216
29. Moroni MC *et al.* (2001) Nat. Cell Biol. 3: 552–558
30. MacLachlan TK and El-Deiry WS (2002) Proc. Natl. Acad. Sci. USA 99: 9492–9497
31. Sax JK and El-Deiry WS (2003) Cell Death Differ. 10: 413–417
32. Levrero M (1999) Cell Death Differ. 6: 1146–1153
33. Levrero M *et al.* (2000) J. Cell Sci. 113: 1661–1670
34. Melino G *et al.* (2002) Nat. Rev. Cancer 2: 605–615
35. Stiewe T and Putzer BM (2002) Cell Death Differ. 9: 237–245
36. Ho J and Benchimol S (2003) Cell Death Differ. 10: 404–408
37. Friedlander P *et al.* (1996) Mol. Cell. Biol. 16: 4961–4971
38. Ludwig RL *et al.* (1996) Mol. Cell. Biol. 16: 4952–4960
39. Saller E *et al.* (1999) EMBO J. 18: 4424–4437
40. Samuels-Lev Y *et al.* (2001). Mol. Cell 8: 781–794
41. Bergamaschi D *et al.* (2003) Nat. Genet. 33: 162–167
42. Weber JD and Zambetti GP (2003) Cell Death Differ. 10: 409–412
43. Oren M (2003) Cell Death Differ. 10: 431–442
44. Xu Y (2003) Cell Death Differ. 10: 400–403
45. Bakkenist CJ and Kastan MB (2003) Nature 421: 499–506
46. Banin S *et al.* (1998) Science 281: 1674–1677
47. Canman CE *et al.* (1998) Science; 281: 1677–1679
48. Clarke AR and Hollstein M (2003) Cell Death Differ. 10: 443–450