Gain of function mutations in p53

Dirk Dittmer, Sibani Pati, Gerard Zambetti, Shelley Chu, Angelika K. Teresky, Mary Moore, Cathy Finlay & Arnold J. Levine

We report that the expression of murine or human mutant p53 proteins in cells with no endogenous p53 proteins confers new or additional phenotypes upon these cells. Mutant p53 proteins expressed in cell lines lacking p53 resulted in either enhanced tumorigenic potential in nude mice ((10)3 cells) or enhanced plating efficiency in agar cell culture (human SAOS–2 cells). Also, mutant human p53 alleles, unlike the wild-type p53 protein, could also enhance the expression of a test gene regulated by the multi-drug resistance enhancer-promoter element. These data demonstrate a gain of function associated with p53 mutations in addition to the loss of function shown previously to be associated with mutations in this tumour suppressor gene.

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544–1014, USA

Correspondence should be adressed to A.J.L.

The p53 gene behaves like a tumour suppressor gene in that mutations at both alleles are selected for in murine[1] as well as human[2,3] cancers. Wild-type p53 protein suppresses the transformation of cells in culture[4] and blocks the tumorigenicity of cancerous cells in nude mice[5]. Wild-type p53 is a transcription factor[6–8] which binds to specific DNA sequences[9–12] adjacent to p53 responsive genes. The mutations in the p53 gene that occur in murine or human cancers[13–15] produce p53 proteins which fail to bind to DNA[9,10,12,13,16] and fail to promote the transcription of a normally responsive promoter-enhancer element and gene[6–8]. This then is the expected loss of function mutation of a p53 tumour suppressor gene product. Consistent with this interpretation is the fact that the oncogene products of the DNA tumour viruses bind to the p53 protein and inactivate its ability to act as a transcription factor[6,7,17,18]. The cellular oncogene *mdm-2* also binds to p53 and abolishes its ability to act as a transcription factor[19].

In some cases, mutant p53 proteins appear to act in a dominant negative fashion entering into oligomeric protein complexes with wild-type p53 and blocking its ability to function[4]. The transformation of cells in culture by mutant p53 proteins, with an endogenous wild-type p53 protein in such cells[20,21], is thought to be an example of this phenotype. In this way, p53 may act as an oncogene[22–24].

Human carcinomas clearly select for p53 missense mutations resulting in faulty proteins persisting in the cancerous cells[14,15]. This observation suggests that these altered p53 proteins may contribute some function (that is, a gain of function) and are not just the result of loss of function mutations. This idea, however, has been difficult to test because of the dominant negative phenotype of mutant p53 proteins. The way to avoid this complication is to introduce a mutant p53 allele into a cell that is devoid of all endogenous p53 protein and test for an acquired or new phenotype. This has been done previously in only one series of experiments[25,26] where mutant p53 protein was expressed in a pre-B-cell line, not containing any p53 protein. Expression of the mutant p53 protein in these cells then prevented the regression of tumours in syngeneic animals. This was, however, a complicated phenotype to interpret.

p53 transfection and expression

In order to test more systematically this gain of function hypothesis, a series of experiments were undertaken using mutant human and murine p53 alleles. The effects of the expression of mutant p53 proteins were tested in two different cell lines, which are devoid of endogenous p53 protein expression. The murine fibroblast cell line (10)3 (ref. 27)and the human osteosarcoma cell lines SAOS–2 (ref. 28) are both deficient for p53 expression. Partial cDNA-genomic clones[29] for human p53 linked to the gene conferring resistance to G418 were transfected into SAOS–2 and (10)3 cells. Transfection of wild-type p53 reduced plating efficiency $\leq$ 90% in (10)3 cells and SAOS–2 cells, respectively. No permanent (10)3 (0/5) or SAOS–2 (0/12) cell lines expressing wild-type p53 could be established. These results are in accord with previous observations that reintroduction of wild-type p53 genes into transformed cells inhibits growth[5,21,30–33].

On the contrary, plating efficiency following transfection of mutant p53 constructs was as expected for transfection with G418-linked expression constructs. About 20–50%

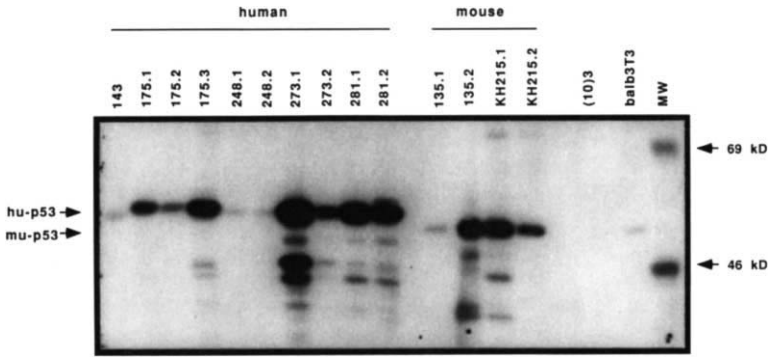

Fig. 1 Immunoprecipitation of p53 protein synthesized in (10)3 cell lines. Cell lines described in Table 1 were labelled with ^{35}S–methionine and the soluble p53 protein in these extracts was immunoprecipitated. The autoradiograph of the SDS–polyacrylamide gel of the immunoprecipitates is shown. (10)3 cells alone have no detectable p53 protein and Balb 3T3 cells indicate the level of wild-type p53 in these cells. hu–p53, position of human p53 protein; mu–p53, position of mouse p53 protein. In some lanes the bands of lower molecular weight (50–36,000 daltons) are thought to be the proteolytic products of p53 that react with the antibody used.

of the clones tested expressed mutant p53 protein. This proportion is similar to the one observed for transfection of mutant p53 expression vectors into cells expressing endogenous wild-type p53 (refs 29,34). Several independent cell lines were derived for each p53 mutant and p53 expression levels were confirmed by metabolic labelling and immunoprecipitation using p53 specific antibodies (Fig. 1). Typically mutant p53 protein was expressed at high levels. Some of the cell lines expressing mutant p53 proteins had slightly altered morphologies but this was not a consistent observation. p53 expression was detectable in SAOS–2 derived cell lines after prolonged passage even in the absence of G418 selection *in vitro* (data not shown) and in nude mouse tumours formed by (10)3 derived cell lines *in vivo*. This demonstrates that high level expression of exogenous mutant p53 is maintained in these cells even in the absence of drug selection. The (10)3 and SAOS–2 cell lines, as well as the derivatives of these cell lines expressing p53 protein, have low to non-detectable levels of the mdm–2 protein[19].

Table 1 Tumorigenic potential of mutant p53 alleles in (10)3 cells

	Cell line	Tumorigenicity in nude mice
Parental	(10)3	0/10
Vector alone	(10)3/V1	0/3
	(10)3/V2	0/3
	(10)3/V3	0/3
Human mutant		
143 V to A	(10)3/143	3/3
175 R to H	(10)3/175.1	3/3
	(10)3/175.2	3/3
	(10)3/175.3	1/3
248 R to W	(10)3/248.1	3/3
	(10)3/248.2	1/3
273 R to H	(10)3/273.1	3/3
281 D to G	(10)3/281.1	3/3
	(10)3/281.2	1/3
Mouse mutant		
KH215	(10)3/KH215.1	1/3
	(10)3/KH215.2	5/6
135 A to V	(10)3/135.1	0/3
	(10)3/135.2	0/3

The parental cell lines (10)3, these same cells containing a plasmid vector without a p53 gene (vector alone) or these cells with a variety of human or murine mutant p53 genes (human mutant, mouse mutant) were inoculated (5×10^6 cells) into nude mice. Tumorigenicity is given by the number of mice with tumours over the total number of mice inoculated. Mutant p53 genes are indicated by the codon mutated (143, 175 etc.) and the amino acid change (V to A, R to H) using the one letter code for amino acids. Cell lines are named for the mutant used, that is (10)3/175, and independent lines given additional numbers (10)3/175.1, (10)3/175.2, etc.

Tumorigenicity of p53-expressing cell lines

Each of the parental cell lines, the cloned cells transfected with the vector alone (no mutant p53) and cloned cell lines expressing mutant p53 were assayed for colony formation in soft agar and tumorigenicity in nude mice (Tables 1 and 2). None of the parental cell lines nor clones transfected with the vector alone (no p53 coding regions) formed colonies in soft agar (0/4, SAOS–2) or tumours in nude mice (0/3 for each of three independent clones, (10)3). So far (greater than 356 days post injection), no tumours have been observed in animals injected with the (10)3 parental cells. Also, animals injected with (10)3 cells expressing the temperature sensitive mouse mutant p53 alleles (Ala135Val)[21], which exhibits a partial wild-type character, did not develop tumours. In contrast, all (10)3 derived cell lines expressing "hot spot" human mutants of p53 (mutations in codons 143, 175, 248, 273, 281) formed tumours in nude mice when 5×10^6 cells per mouse were injected (Table 1). The tumours continued to express human p53 protein as determined by immunoblot analysis (Fig. 2). Cell lines derived from representative tumours were equally viable if initially seeded in the presence or absence of 800 μg ml^{-1} G418 and expressed mutant p53 protein as judged by metabolic labelling and immunoprecipitation (data not shown). In contrast, SAOS–2 cell lines expressing human mutant p53 did not form tumours in nude mice when 1×10^7 cells were injected per mouse. Unlike the parent line, however, SAOS–2 cells expressing either the 175 or 273 mutant allele had a low but readily measurable plating efficiency in soft agar (Table 2). The level of p53 protein expression in a SAOS–2 cell line did not correlate with the ability of these cells to form colonies in agar.

p53 gain of function

These results demonstrate that mutant p53 expression confers a growth advantage in the absence of endogenous wild-type p53 protein. Thus, these mutants may not only act as dominant negative mutants to eliminate wild-type p53 function but may also have gained an additional function to support cellular growth in the absence of any

Table 2 Growth in agar of mutant p53 alleles in SAOS–2 cells

	Cell line	Growth in soft agar (%)	Tumorigenicity
Parental	SAOS–2	0	0/2
Vector alone	SAOS–2/V1	0	nd
	SAOS–2/V2	0	nd
	SAOS–2/V3	0	0/2
	SAOS–2/V4	0	nd
Human mutant			
143 V to A	SAOS–2/143.1	0	0/2
175 R to H	SAOS–2/175.1	0.28	0/2
	SAOS–2/175.2	0.29	nd
248 R to W	SAOS–2/248.1	≤0.05	0/2
273 R to H	SAOS–2/273.1	0.174	0/2
281 D to G	SAOS–2/281.1	≤0.05	0/2
	SAOS–2/281.2	≤0.05	nd

The parental line (SAOS–2), vector alone or mutant p53 genes are as described in Table 1 as is the nomenclature of the cell lines. For growth in soft agar 1×10^4 cells were plated and the percentage of these cells that formed colonies of greater than 30 cells is presented. Tumorigenicity is as measured in Table 1 except 1×10^7 cells were injected per animal.

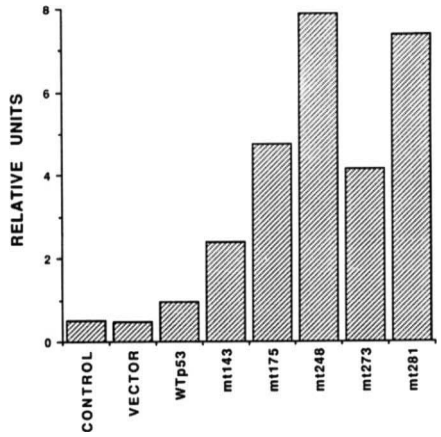

Fig. 3 Relative levels of CAT activity in cell lines transfected with mdr-CAT reporter gene and p53 wild-type or mutant clones. (10)1 cells were transfected with the mdr-CAT reporter gene alone (control), the reporter and the vector with no p53 gene (vector), the wild-type p53 gene (WT p53) and the series of mutant p53 clones (mt 143, 175, 248, 273, 281). The relative levels of CAT activity were assayed as described[8] and quantitated using a Phosphorimager and Image Quant software (Molecular Dynamics).

wild-type p53 protein. Alternatively, the wild-type p53 protein may possess both a growth suppressive and growth promoting function. Mutations that only eliminate the growth-suppressive function of p53 could then result in the constitutive expression of a growth-promoting function, resulting in deregulated cell growth.

How could a mutant p53 protein accomplish this? The mutant p53 protein fails to bind to DNA and activate transcription. In spite of this, mutant p53 proteins have been shown to stimulate the expression of some genes, such as the multi-drug resistant gene[35]. In this case, the mutant p53 protein could bind to a transcription factor and the complex could then activate (or inactivate a repressor) to promote transcription of such a test gene. To determine whether the mutant p53 alleles that confer the gain of tumorigenic potential can also activate the multi-drug resistance gene in these cells with no p53 protein, the following experiment was performed: (10)3 and (10)1 cells were transfected with either (i) human wild-type p53, (ii) mutant p53 cDNA clones (mutations at codons 143, 175, 248, 273 and 281), or (iii) the vector with no p53 gene, and the enhancer-promoter region of the multi-drug resistant gene regulating a test gene, chloramphemicol transacetylase[35]. Extracts from these cells were then tested for enzyme activity (Fig. 3). In the absence of p53 (mutant or wild-type), there was a low level of CAT activity (Fig. 3, vector and control) which was unaffected by the presence of wild-type p53 human protein (WT p53, Fig. 3). In contrast, the p53 mutant proteins stimulated the expression of the CAT gene in these cells (Fig. 3). This is a clear example of a gain of function not shared by the wild-type protein.

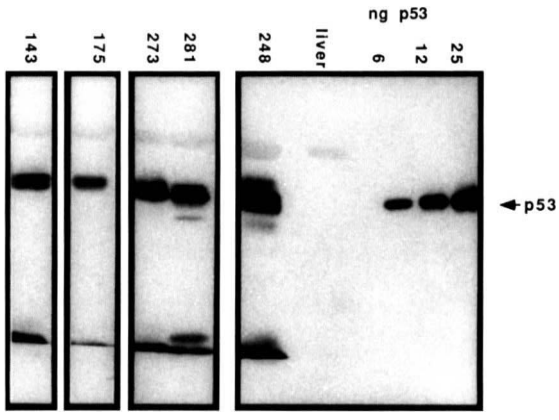

Fig. 2 Immunoblot of p53 protein expressed in tumours from nude mice. (10)3 cell lines with human p53 mutant clones (143, 175, 273, 281, 248) were inoculated into nude mice. The resulting tumours (0.5–1.0 cm) were used to analyse p53 protein expression in these tumour cells. Liver from the nude mouse has no detectable p53 protein (wild-type is too low to detect) and 6, 12, 25 ng of purified p53 are included as a standard in this assay. 250 µg of protein were analysed in each experimental lane.

Discussion

Our results demonstrate that missense mutant p53 alleles produce an altered protein that can confer a gain of a new function upon a cell. This takes the form of an enhanced growth advantage. These results both confirm and extend the previous observations of Rotter and colleagues[22,26]. It should be pointed out, however, that different cell lines may well respond differently to the expression of mutant p53 protein. The (10)3 cells could produce tumours in nude mice when they expressed mutant p53 proteins, but the SAOS–2 cells did not have that phenotype. Even different mutant alleles conferred different phenotypes upon these cell lines. Codon 175 and 273 mutations permitted SAOS–2 cells to grow in agar while codon 281 or 248 mutations did not. A codon 135 mutation in the murine gene p53 failed to convert (10)3 cells to a tumorigenic cell line while an insertion mutation at codon

215 (KH215, Table 1) did impart a tumorigenic potential and a gain of function. The ability of some mutant p53 proteins to enhance the expression of the multi-drug resistance gene[35] (Fig. 3) demonstrates that mutant p53 proteins can alter cellular gene expression in a manner distinct from the wild-type protein. This provides a mechanism for the way in which mutant p53 alleles can add new growth potential to cells.

These data make an interesting prediction. Tumours with mutant missense p53 proteins may well be more aggressive or have a poorer prognosis than tumours with no p53 proteins (deletions have only the loss of function mutation). There is already a partial test of these ideas with cervical carcinomas. About 90% of cervical carcinomas express the human papillomavirus oncogenes E6 and E7. The E6 protein binds to p53 and promotes the proteolytic degradation of the p53 protein, so these cells are like a null p53 mutant[36,37]. The remaining 10% of cervical cancers do not have human papillomavirus present and some of these instead have p53 missense mutations[38]. These tumours are more aggressive and invasive than the human papillomavirus positive tumours and the prognosis is worse in these cases. Thus, it may be that a missense p53 mutation contributing a gain of a new function is worse than no p53 gene product at all. This is consistent with the results demonstrating that p53 missense mutations in breast[41] and gastric[42] cancers have a much poorer prognosis than those cancers with wild-type p53 protein. More specific tests of these ideas should now be undertaken.

Methodology

Cell lines and plasmids. The (10)3 and (10)1 cell lines are described in Harvey and Levine[27]. (10)3 is a permanent murine cell line passaged so as to be contact inhibited. It did not produce colonies in agar and did not induce tumours in nude mice when 5×10^6 cells were injected into these animals. The p53 gene has a stop codon at position 173 (out of 290 codons). There is no detectable p53 protein or fragment in these cells[27]. The SAOS–2 cells are human osteosarcoma cells that have deletions of the p53 genes. The SAOS–2 cell line (obtained from V. Rotter, The Weizmann Institute, Rehovot, Israel) did not produce colonies in agar suspension cultures and tumours in animals. The murine p53 wild-type and mutant plasmids are described in ref. 4 while the human p53 wild-type and mutant constructions are described in ref. 29. DNA transfection procedures are as described previously[29].

Phenotypes of cell lines. To measure growth of cells in soft agar, cells were seeded in 0.3% noble agar (Difco) on top of a 0.5% bottom layer. 1×10^4 cells were plated per 5 ml prepared in DMEM with 15% fetal bovine serum containing 0.8 μg ml^{-1} of G418 (Gibco-BRL). Colonies >30 cells were counted after 3 wk. The results (Table 2) are presented as the percentage of cells that made colonies.

Tumorigenicity in nude mice was measured by injecting 5×10^6 cells ((10)3 series) or 1×10^7 cells (SAOS–2 series) into Balb c/J nu/nu mice. The results (Table 1) are presented as the number of mice with tumours over the total number of mice injected. On average palpable tumours were first detected at 23 days (s.d. = 16 days with $n = 25$). Tumours grew progressively until they were 0.5–1.0 cm in size and became necrotic. The animals were sacrificed at that time. Animals displaying no tumour after 12 wk (5 times s.d.) were considered tumour free.

Immunoprecipitation. Cells were labeled with ^{35}S–methionine, soluble protein extracts prepared and the p53 protein analyzed by immunoprecipitation using antibody PAb421 as described[39]. 5×10^6 cpm were loaded in each lane of the gel. Immunoblot analysis was carried out with antibody PAb1801 (Oncogene Science) using 250 μg of extracts from tumours. A peroxidase-conjugated anti-mouse IgG (Cappel) was employed to detect the p53–antibody complex and the band was visualized using ECL systems (Amersham), as described[40].

CAT analysis. The DNA transfections, preparation of extracts and CAT enzyme assay are as described[8]. The multi-drug resistance gene construct has been described[35]. The MDR promoter fragment was 1.8 kb. It has no TATA box but has a number of other transcription factor binding sites. These experiments were carried out 3–9 times, depending upon the mutant p53 allele tested. The results shown in Fig. 3 are from one experiment in which the panel of mutant alleles were tested at the same time in the same cells to control for transfection efficiencies. Based upon these data, it is not clear whether the relative differences between the stimulation of the MDR promoter by different p53 mutant alleles are meaningful.

Received 19 January; accepted 8 February 1992.

Acknowledgements

We wish to thank K. James for assistance with the manuscript. The work was supported by an NIH grant P01 CA41086 A.J.L. and by a Public Health Service grant R01 CA55036 from the National Cancer Institute (C. Finlay). C.F. is a Special Fellow of the Leukemia Society.

1. Johnson, P. & Benchimol, S. Friend virus induced murine erythroleukaemia: The p53 locus, in *Cancer Surveys* Vol. **12**, (ed. A.J. Levine) 137–151 (Cold Spring Harbor Press, New York, 1992).
2. Baker, S.J. *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinoma. *Science* **244**, 217–221 (1989).
3. Baker, S.J., Markowitz, S., Fearon, E.R., Willson J.K.U. & Vogelstein, B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* **249**, 912–915 (1990).
4. Finlay, C.A., Hinds, P.W. & Levine, A.J. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* **57**, 1083–1093 (1989).
5. Chen, P.-L., Chen, Y., Bookstein, R. & Lee, W.-H. Genetic mechanisms of tumour suppression by the human p53 gene. *Science* **250**, 1576–1579 (1990).
6. Farmer, G.E. *et al.* Wild-type p53 activates transcription *in vitro*. *Nature* **358**, 83–86 (1992).
7. Kern, S. *et al.* Oncogenic forms of p53 inhibit p53-regulated gene expression. *Science* **256**, 827–32 (1992).
8. Zambetti, G.P., Bargonetti, J., Walker, K., Prives, C. & Levine, A.J. Wild-type p53 mediates positive regulation of gene expression through a specific DNA sequence element. *Genes Dev.* **6**, 1143–1152 (1992).
9. Bargonetti, J., Friedman, P.N., Kern, S.E., Vogelstein, B. & Prives, C. Wild-type but not mutant p53 immunopurified proteins bind to sequences adjacent to the SV40 origin of replication. *Cell* **65**, 1083–1091 (1991).
10. El-Deiry, W.S., Kern, S.E., Pietenpol, J.A., Kinzler, K.W. & Vogelstein, B. Definition of a consensus binding site for p53. *Nature Genet.* **1**, 45–49 (1992).
11. Funk, W.D. *et al.* A transcriptionally active DNA-binding site for human p53 protein complexes. *Molec. cell. Biol.* **12**, 2866–2871 (1992).
12. Kern, S.E. *et al.* Identification of p53 as a sequence-specific DNA-binding protein. *Science* **252**, 1708–1711 (1991).
13. Barnonetti, J. *et al.* Site-specific binding of wild-type p53 to cellular DNA is inhibited by SV40 T antigen and mutant p53. *Genes Dev.* **6**, 1886–1898 (1992).
14. Levine, A.J., Momand, J. & Finlay, C.A. The p53 tumour suppressor gene. *Nature* **351**, 453–456 (1991).
15. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C.C. p53 mutations in human cancers. *Science* **253**, 49–53 (1991).
16. Kern, S.E. *et al.* Mutant p53 proteins bind DNA abnormally in vitro. *Oncogene* **6**, 131–136 (1991).
17. Mietz, J.A., Unger, T., Huibregtse, J.M. & Howley, P.M. The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T-antigen and by HPV-16 E6 oncoprotein. *EMBO J.* **11**, 5013–5020 (1992).
18. Yew, P.R. & Berk, A.J. Inhibition of p53 transactivat ion required for transformation by adenovirus E1B 55 Kd protein. *Nature* **357**, 82–85 (1992).
19. Momand, J., Zambetti, G.P., Olson, D.C., George, D. & Levine, A.J. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53 mediated transactivation. *Cell* **69**, 1237–1245 (1992).
20. Zambetti, G.P., Olson, D., Labow, M. & Levine, A.J. A mutant p53 protein is required for the maintenance of the transformed cell phenotype in p53 plus ras transformed cells. *Proc. natn. Acad. Sci. U.S.A.* **89**, 3952–3956 (1992).
21. Martinez, J., Georgoff, I., Martinez, J. & A.J. Levine. Cellular localization and cell cycle regulation by a temperature sensitive p53 protein. *Genes Dev.* **5**, 151–159 (1991).
22. Eliyahu, D., Raz, A., Gruss, P., Givol, D. & Oren, M. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature* **312**, 646–649 (1984).
23. Parada, L.F., Land, H., Weinberg, R.A., Wolf, D. & Rotter, V. Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature* **312**, 649–651 (1984).
24. Jenkins, J.R., Rudge, K. & Currie, G.A. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature* **312**, 651–654 (1984).
25. Wolf, D., Admon, S., Oren, M. & Rotter, V. Abelson murine leukemia virus-transformed cells that lack p53 protein synthesis express aberrant p53 mRNA species. *Molec. cell. Biol.* **4**, 552–558 (1984).
26. Shaulsky, G., Goldfinger, N. & Rotter, V. Alterations in tumour development in vivo mediated by expression of wild-type or mutant p53 proteins. *Cancer Res.* **51**, 5232–5237 (1991).
27. Harvey, D. & Levine, A.J. p53 alteration is a common event in the spontaneous immortalization of primary BALB/C murine embryo fibroblasts. *Genes Dev.* **5**, 2375–2385 (1991).
28. Masuda, H., Miller, C., Koeffler, H.P., Battifora, H. & Cline, M.J. Rearrangement of the p53 gene in human osteogenic sarcomas. *Proc. natn. Acad. Sci. U.S.A.* **84**, 7716–7719 (1987).
29. Hinds, P.W. *et al.* Mutant p53 cDNAs from human colorectal carcinomas can cooperate with ras in transformation of primary rat cells: A comparison of the "hot spot" mutant phenotypes. *Cell Growth Diff.* **1**, 571–580 (1990).
30. Baker, S.J., Markowitz, S., Fearon, E.R., Willson, J.K.U. & Vogelstein, B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* **249**, 912–915 (1990).
31. Diller, L. *et al.* p53 functions as a cell cycle control protein in osteosarcomas. *Molec. cell. Biol.* **10**, 5772–5781 (1990).
32. Johnson, P., Gray, D., Mowat, M. & Benchimol, S. Expression of wild-type p53 is not compatible with continued growth of p53-negative tumour cells. *Molec. cell. Biol.* **11**, 1–11 (1991).
33. Mercer, W.E. *et al.* Negative growth regulation in a glioblastoma tumour cell line that conditionally expresses human wild-type p53. *Proc. natn. Acad. Sci. U.S.A.* **87**, 6166–6170 (1990).
34. Eliyahu, D., Michalovitz, D. & Oren, M. Overproduction of p53 antigen makes established cells highly tumorigenic. *Nature* **316**, 158–160 (1985).
35. Chin, K.V., Ueda, K., Pastan, I. & Gottesman, M.M. Modulation of activity of the promoter of the human MDR1 gene by ras and p53. *Science*, **255**, 459–462 (1992).
36. Werness, B.A., Levine, A.J. & Howley, P.M. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* **248**, 76–79 (1990).
37. Scheffner, M., Werness, B.A., Huibregtse, J.M., Levine, A.J. & Howley, P.M. The E6 oncoprotein encoded by human papillomavirus 16 or 18 promotes the degradation of p53. *Cell* **63**, 1129–1136 (1990).
38. Riou, G. *et al.* Association between poor prognosis in early stage invasive cervical carcinomas and nondetection of HPV DNA. *Lancet* **335**, 1171–1174 (1990).
39. Tan, T.-H., Wallis, J. & Levine, A.J. Identification of the p53 protein domain involved in formation of the simian virus 40 large T antigen-p53 protein complex. *J. Virol.* **59**, 574–583 (1986).
40. Reich, N.C. & Levine, A.J. Growth regulation of a cellular tumour antigen, p53, in nontransformed cells. *Nature* **308**, 199–201 (1984).
41. Thor, A.D. *et al.* Accumulation of p53 tumour suppressor gene protein: an independent marker of prognosis in breast cancer. *J. natn. Cancer Inst.* **84**, 845–855 (1992).
42. Martin, H.M., Filipe, M.I., Morris, R.W., Lane, D.P. & Silvestre, F. p53 expression and prognosis in gastric carcinoma. *Int. J. Cancer* **50**, 859–862 (1992).