Expression of the novel tumour suppressor p33ING1 is independent of p53

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Summary A recently cloned tumour suppressor candidate, p33ING1, has been shown in vitro to collaborate with p53 to execute growth arrest and apoptosis. However, it is unclear as to how the expression of ING1 is regulated in normal and stress conditions. Using a p53-knockout mouse model, we investigated if the expression of ING1 was dependent on p53 status. Based on our findings, we therefore conclude that the expression of ING1 is independent of p53 status. UV induction of ING1 in keratinocytes suggests that ING1 may play a role in cellular stress response and skin carcinogenesis. © 2000 Cancer Research Campaign

Keywords: p33ING1; p53; in vivo expression; tumour suppressor gene; UV irradiation

p33ING1, which encodes a 33-kD nuclear protein, was cloned by subtractive hybridization and subsequent selection of transforming genetic suppressor elements (Garkavtsev et al, 1996). Overexpression of the sense ING1 inhibits cell growth (Garkavtsev et al, 1996). Specifically, flow cytometry analysis shows that normal fibroblasts transfected with the ING1 gene are arrested in the G0/G1 phase of the cell cycle (Garkavtsev et al, 1996). It is observed that this ING1-induced arrest is achieved by up-regulating p53-dependent activation of the p21WAF1 promoter (Garkavtsev et al, 1998). It is further noted that neither ING1 nor p53 alone can exert arrest and that both proteins can physically associate with one another, as demonstrated by immunoprecipitation (Garkavtsev et al, 1998). In addition to the function of cell growth arrest, ING1 has been found to play a role in senescence (Garkavtsev et al, 1997). Expression of ING1 is 8- to 10-fold higher in senescent cells than in young proliferation-competent human diploid fibroblasts. Cells expressing antisense ING1 can increase their replicative life by 7-fold. In apoptosis, Helbing and colleagues (1997) reported that elevated expression of ING1 enhances serum starvation-induced cell death in p19 mouse teratocarcinoma and NIH 3T3 cells. Recently, another report demonstrates that apoptosis can be enhanced by adenovirus-mediated transfer of both ING1 and p53, further underscoring the interdependent relationship between the two genes (Shinoura et al, 1999). Evidence also suggests that ING1 is capable of sensitizing cells to chemotherapeutic agents, such as etoposide and γ-irradiation (Garkavtsev et al, 1998). Although the mechanism of such sensitizing ability is unclear, its apoptotic function may be involved.

p53, which encodes a nuclear protein, regulates a number of downstream targets such as p21WAF1, GADD45, bax, and bel-2 and carries out tumour suppressive functions, much like ING1. Studies over more than a decade indicate that p53 is a key mediator of cell cycle regulation, apoptosis, DNA repair, senescence, and sensitization to chemotherapeutic agents (Bond et al, 1994; Miyashita et al, 1994; Smith et al, 1994; Li et al, 1997; 2000; Bunz et al, 1998). Regarded as a ‘guardian of the genome’, p53 holds the title of being the most frequently mutated gene known to date (Hollstein et al, 1991; 1995; 1998). More than 50% of human malignancies of epithelial, mesenchymal, haematopoietic, lymphoid, and central nervous system origin have been shown to contain a mutated form of p53. Evidence suggests that loss of normal p53 function is associated with cell transformation in vitro and the development of neoplasms in vivo (Finlay et al, 1989). Under stress conditions, such as those genotoxic in nature, levels of p53 protein rapidly increase in the cell. The upregulation induces the expression of p21WAF1, a potent inhibitor of cyclin-dependent kinase activity, which inhibits cell cycle progression from G1 to S phase (Shaulsky et al, 1991; El-Deiry et al, 1993). It has also been demonstrated that p53 maintains genomic stability by enhancing DNA repair and apoptosis. We recently demonstrated that loss of wild-type p53 function results in reduced DNA repair and apoptosis in mouse keratinocytes and fibroblasts after UV irradiation (Li et al, 1996; 1997; 1998a; Tron et al, 1998a; 1998b). As a result of reduced DNA repair and apoptosis, mice with abnormal p53 function, either by gene knockout or overexpression of mutant p53, are predisposed to UV-induced skin cancer development (Li et al, 1995; 1998b).

To further our understanding of the relationship between p53 and ING1, we first investigated how the expression of ING1 behaves in the context of p53 using a knockout mouse model. We also looked at whether ING1 expression relies on the status of p53 in UV-irradiated conditions. Our results indicate that ING1 expression is independent of wild-type p53 function and that ING1 may play a role in cellular stress response and skin carcinogenesis.
MATERIALS AND METHODS

Animals

p53+/– and p53–/– mice were purchased from Taconic (New York, NY, USA). p53+/– mice were carried a disrupted, nonfunctional p53 gene, created by homologous recombination in an embryonic stem cell line and by microinjection of the stem cells into 3.5-day-old C57BL/6 blastocysts (Donehower et al., 1992).

Cell culture

Normal human epithelial keratinocytes (NHEK) were obtained from the Tissue Bank of Vancouver General Hospital. They were maintained in Keratinocyte-SFM medium (Canadian Life Technologies, Burlington, Ontario, Canada). Human HaCaT keratinocytes (kindly provided by Dr NE Fusenig, DKFZ, Heidelberg, Germany) were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 5% fetal calf serum (Canadian Life Technologies), 100 units ml–1 penicillin and 100 µg ml–1 streptomycin at 37°C in a 5% CO2 atmosphere.

Dermal fibroblasts of p53+/– and p53–/– mice were isolated from 4-week-old mice. The mice were sacrificed by cervical dislocation and a 2 × 2 cm skin biopsy was dissected from the dorsal area. The hair was removed and the skin biopsy was disinfected with 2.5% sodium hypochlorite. The skin tissue was then minced and incubated in DMEM containing 200 units ml–1 collagenase (Sigma, St Louis, MO, USA) at 37°C for 6 h. The digested skin tissue was centrifuged at 1000 rpm for 10 min and the pellet washed with pre-warmed DMEM twice. The cells were resuspended in DMEM containing 10% FBS and incubated at 37°C in a 5% CO2 atmosphere.

UVB irradiation

Medium was removed and the cells were exposed to UVB (290–320 nm) using a bank of four unfiltered FS40 sunlamps (Westinghouse, Bloomfield, NJ, USA). The intensity of the UV light was measured by the IL 700 radiometer fitted with a WN 320 filter and an A127 quartz diffuser (International Light, Newburyport, MA, USA). Medium was replaced and cells were incubated in a 5% CO2 incubator at 37°C after UVB irradiation.

RT-PCR

Total RNA was extracted by TriZol reagent (Canadian Life Technologies) and the concentrations were determined by UV spectrophotometry. 5 µg of total RNA was reverse-transcribed into cDNA in the presence of 10 units µl–1 of SUPERSCRIPT II RNase H– Reverse Transcriptase (Canadian Life Technologies), 5X first-strand buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl2), 100 mM DTT, 10 mM dNTP mix (10 mM each of dATP, dGTP, dCTP and dTTP at pH 7.0), and 2 pmole of the forward primer (5′-AGAAGTTGGAACACTCGATG-3′) and 10 mM of the reverse primer (5′-AGAAGTTGGAACACTCGATG-3′), and 5 units µl–1 of the Taq DNA polymerase. Amplification was carried out as follows: 1. initial denaturation at 94°C for 3 min; 2. denaturation at 95°C for 45 s; 3. annealing at 50°C for 1 min; 4. polymerization at 72°C for 2 min; 5. repeat of steps 2–4 for 40 cycles; and 6. final polymerization at 72°C for 5 min. Samples were then electrophoresed on 1% agarose gels containing 0.5 µg ml–1 of ethidium bromide and visualized under UV light. Reaction mix with pCI-ING1 plasmid DNA (kind gift from Dr K Riabowol, University of Calgary, USA) was used as a positive control. For semi-quantitative PCR, 2 µl of the cDNA samples from reverse transcription were diluted at 10–1 and 100–1. They were then amplified by PCR as described above.

Northern blot analysis

Total RNA was extracted by TriZol reagent and the concentrations were determined by UV spectrophotometry. Samples were heated to 65°C and run on 1% agarose gels containing formaldehyde and 0.5 µg ml–1 ethidium bromide. After separation, capillary transfer to nitrocellulose was performed overnight at room temperature and its efficiency assessed by UV light. The blot was then baked for 2 h in a vacuum oven at 80°C. Pre-hybridization was carried out by incubating the blot with a mixture containing 6X SSPE, 5X Denhardt’s reagent, 0.5% SDS, and 100 µg ml–1 yeast tRNA for 1 h at 65°C. The ING1 probe was first made by amplifying a 477-bp fragment by PCR using primer 1 (5′-GATCCCTGAAGGAGCTAGACG-3′) and primer 2 (5′-AGAAGTTGGAACACTCGATG-3′) and then labelling it with α32P dCTP (10 µCi ml–1) according to the manufacturer protocol in the Random Primers DNA Labeling System (Canadian Life Technologies). Hybridization was done by incubating the blot with the labelled probe at 65°C for 16–24 h. Filters were washed with 2X SSC/0.1% SDS once for 15 min at room temperature and three washes 20 min each at 65°C. Blots were visualized on X-ray films after an overnight exposure.

Western blot analysis

Cells were harvested by scraping and solubilized by the Triple detergent lysis buffer containing 50 mM Tris-Cl pH 8.0, 150 mM NaCl, 0.02% sodium azide, 1% sodium dodecylsulphate, 1% Nonidet P-40, 100 µg ml–1 phenylmethylsulphonyl fluoride, 1 µg ml–1 aprotinin, 1 µg ml–1 leupeptins and 1 µg ml–1 pepstatin. Concentrations of proteins were determined by the DC Protein Assay (BioRad, Mississauga, Ontario, Canada). 50 µg lane–1 of proteins were separated on 10% polyacrylamide/SDS gels and electroblotted onto a nitrocellulose filter. The filter was then blocked with 5% skimmed milk for 1 h and incubated with 1:2000 polyclonal rabbit p33ING1 antibody (PharMingen, Mississauga, Ontario, Canada) for 1 h at room temperature, followed by three washes with PBS 0.02% Tween-20 (PBS-T). The filter was then incubated with 1:10 000 goat anti-rabbit IgG (Calbiochem, San Diego, CA, USA) at room temperature for 1 h, followed by three washes with PBS-T. The signals were detected with the ECL Western blotting detection system (New England Biolab, Guelph, Ontario, Canada). A 1:2000 dilution of β-actin antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) was used as an internal control for each blot.
Immunohistochemistry

All samples were frozen-sectioned at six-micron and mounted onto saline-coated slides. They were then fixed in cold acetone for 2 min. Using the ImmunnoCruz Staining Systems (Santa Cruz Biotechnology Inc) serial sections were first blocked with horse serum for 20 min, then incubated with 1:500 p33ING1 polyclonal antibody for 2 h at room temperature, followed by two washes in PBS each for 2 min. Next, the sections were incubated with biotin-labelled anti-rabbit secondary antibody with avidin–biotin–peroxidase complex for 30 min, followed by two washes in PBS each for 2 min and staining with the HRP substrate containing DAB chromogen and peroxidase substrate for 30 s to 5 min. Sections were immediately dehydrated two times in 95% ethanol each for 10 min, two times in 100% ethanol each for 10 s, and three times in xylenes each for 10 s. Slides were added with permanent mounting medium, covered with glass coverslip, and observed under a light microscope.

RESULTS

In vivo expression of ING1 is independent of p53

Studies that have examined the relationship between ING1 and p53 have mostly been done in vitro (Garkavtsev et al, 1998; Shinoura et al, 1999). Since p53 is a transcription factor that is known to initiate a whole host of molecular events by transactivating genes, we investigated if p53 could be an upstream regulator of ING1 by first examining whether ING1 was expressed in organs from p53+/+ and p53−/− mice. Results from RT-PCR showed that ING1 was expressed in the brain, liver, lung, heart and skin of both p53+/+ and p53−/− mice (Figure 1A). Quantitative RT-PCR indicated that ING1 mRNA levels were virtually equal in the heart of p53+/+ and p53−/− mice (Figure 1B). The data suggest that ING1 expression is independent of p53 status. To further confirm the p53-independent expression of ING1, we used Northern blot analysis to compare mRNA levels in the brain, liver, lung, heart, skin, kidney, testis, and thymus of p53+/+ and p53−/− mice. Our results showed that there was no significant difference in ING1 mRNA expression in all eight organs examined (Figure 1C).

Next, we investigated whether p53 status affects ING1 expression at the post-transcriptional level. We compared ING1 protein levels in the brain, liver, lung, heart, and skin between p53+/+ and p53−/− mice. Figure 2 shows that there is no substantial difference in the levels of 33-kD ING1 protein between p53+/+ and p53−/− mice in all five organs examined. Recently, two isoforms of the p33ING1 gene, which encode 47-kD and 24-kD proteins, have been found (Saito et al, 2000). The anti-ING1 antibody we used predominantly detected the 33-kD isoform. To confirm the p53-independent expression of ING1 from the Western analysis, we used immunohistochemical staining to look at ING1 protein expression in the brain between the p53+/+ and p53−/− groups. Our results show that p53+/+ and p53−/− mice have similar levels of ING1 protein (Figure 3).

UV-induced ING1 expression is independent of p53

p53 plays a significant role in responding to DNA damage. Stress events, such as ionizing irradiation, ultraviolet light (UV) irradiation and exposure to genotoxic drugs, induce the accumulation of p53, which then results in cell cycle arrest, DNA repair and/or apoptosis (El-Diery et al, 1993; Li et al, 1996; 1997; 1998a; Tron et al, 1998b). Various downstream p53-mediated targets such as p21WAF1, GADD45, bax, and bcl-2 have been shown to be involved in these events. To test the hypothesis that ING1 may also be a downstream target of p53 in stress conditions, we exposed normal human epithelial keratinocytes (NHEK) and a keratinocyte cell line (HaCaT), which carries mutated p53 alleles (Lehman et al, 1993), to UVB irradiation and examined the levels of ING1 protein. Our results demonstrate that 33-kD ING1 protein is induced in both NHEK and HaCaT cells after UVB irradiation (Figure 4A and 4B). At 40 mJ cm\(^{-2}\), there is a two-fold increase in the level of ING1 protein in both types of cells. It is also noted that the expression of ING1 protein in HaCaT cells is dose-dependent, with a maximum induction at 80 mJ cm\(^{-2}\). The observation of similar levels of ING1 induction in NHEK and HaCaT cells by UV irradiation further confirms the p53-independent expression of ING1.

To confirm that UV induces ING1 expression at the transcriptional level, ING1 mRNA levels were examined in HaCaT cells over a time-course. Figure 4C shows that ING1 mRNA levels increase with time, starting at 4 h and peaking at 24 h.

Using human fibroblasts, Garkavtsev and colleagues (1998) found that DNA damaging agent, adriamycin, did not induce ING1.
expression. To determine if UV-induced ING1 in keratinocytes was tissue-specific, we exposed dermal fibroblasts isolated from p53<sup>+/+</sup> and p53<sup>−/−</sup> mice to 40 mJ cm<sup>−2</sup> of UVB. Our data show that neither p53<sup>+/+</sup> nor p53<sup>−/−</sup> fibroblasts exhibit any change in ING1 expression (Figure 5). Taken together, we demonstrate that UV-induction of ING1 is cell type-specific and p53-independent.

**DISCUSSION**

Our understanding of the biological function of ING1 has improved over the last 4 years. One of the reasons that this gene has gained increasing attention from the biological community is that, though it has no structural similarity with p53, both genes share many of the tumour suppressive functions, including growth arrest, apoptosis, senescence and sensitization to drug treatment. As well, ING1 has been shown to physically associate with p53, further pointing to the importance of its role in carcinogenesis (Garkavtsev et al, 1998). Current studies show that overexpression of ING1 inhibits cell growth, while chronic expression of ING1 antisense constructs promotes cell transformation (Garkavtsev et al, 1996; 1998). In addition, it was found that the function of cell growth control is dependent on the activity of both ING1 and p53, and p21<sup>INP</sup> has been shown to be their downstream effector (Garkavtsev et al, 1996; 1998).

Although ING1 shares functional similarities with p53, it is not known how the expression of ING1 is regulated. Since p53 is a well-known transcriptional factor for many downstream targets (El-Deiry et al, 1993; Miyashita and Reed, 1995; Owen-Schaub et al, 1995), we examined if p53 had an effect on ING1 expression in both normal and stress environments. Most studies on ING1 came primarily from in vitro analysis using long-term cultured cell lines. Since many genetic changes may occur in this type of system, we chose to use the p53-knockout mouse model for this study. We compared the ING1 mRNA and protein levels in a number of organs from p53<sup>−/−</sup> and p53<sup>+/+</sup> mice and no significant difference was observed in these two groups (Figures 1–3).

We also investigated if ING1 could be induced under stress conditions where p53 is frequently upregulated (Hall et al, 1993; Li et al, 1998a). We exposed fibroblasts from p53<sup>−/−</sup> and p53<sup>+/+</sup> mice, NHEK and a keratinocyte cell line, HaCaT, to UVB and compared the ING1 protein levels in these cells. HaCaT is a spontaneously immortalized, non-tumorigenic human keratinocyte cell line that behaves phenotypically like its normal counterpart in terms of patterns of growth and differentiation (Boukamp et al, 1988). Besides the advantage of being similar in many respects with its normal counterpart, this cell line lacks the functional p53 gene, allowing the study of the relationship between p53 and its associates. Our results indicate that ING1 is induced in both NHEK and HaCaT cells, regardless of p53 status, further confirming our previous observation of ING1 p53-independent expression. The fact that ING1 is not induced in fibroblasts after UV irradiation suggests that UV-induction of ING1 expression is cell type-specific, consistent with the data reported by Garkavtsev and colleagues (1998) where they found no induction of ING1 expression in fibroblasts treated with adriamycin, a chemotherapy drug. Similarly, Zeremski et al (1999) reported that there was a lack of effect of p53 status on ING1 levels in murine mammary gland cell lines under normal and stress conditions.

Keratinocyte-specific UVB induction of ING1 may have biological significance, as keratinocytes are the primary target of
UVB in the skin. The induced ING1 protein may contribute to the various cellular responses, such as apoptosis and DNA repair, after UVB irradiation.

In this study, we found that wild-type p53 is not required for the expression of ING1 in both normal and stress conditions, although their partnership has been shown to be required for exerting biological effects such as growth inhibition and apoptosis (Garkavtsev et al., 1998; Shinoura et al., 1999). The UV induction of ING1 seen in our keratinocytes therefore suggests two possibilities: 1. ING1 is capable of responding to stress in terms of expression independent of p53 but cannot perform its cellular activities without the functional p53; and 2. ING1 is capable of responding to stress and carries out its functions independent of p53. Further functional studies are needed to decipher the role of ING1 in cellular stress response. Because we have previously shown that p53 gene knockout predisposes animals to UV-induced skin cancer development, reduces UV-damaged DNA repair, and lowers the apoptotic rate (Li et al., 1995; 1996; 1997; 1998), investigations on how ING1 cooperates with p53 in keratinocytes to modulate cell cycle arrest, DNA repair, and apoptosis will further determine the functional significance of ING1 in UV-induced stress response in particular and skin carcinogenesis in general. To support the notion that ING1 plays a role in skin carcinogenesis, we have recently found that UV-induced murine squamous carcinomas express significantly higher levels of ING1 regardless of p53 status in comparison to normal skin (data not shown). Although current mutational studies indicate that very few alterations are found in ING1 gene in breast, ovarian, and gastric cancers (Ohmori et al., 1999; Oki et al., 1999; Toyama et al., 1999), ING1 mutation may only be specific to UV-induced skin cancers. Future studies on ING1 mutational status in skin tumours will help reveal the nature of its ING1 induction.

Taken together, our results demonstrate that ING1 expression is independent of p53 status and that UV-induction of ING1 in human keratinocytes suggests a role of ING1 in cellular stress response and skin carcinogenesis.

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REFERENCES

Bond JA, Wylie FS and Wynford-Thomas D (1994) Escape from senescence in human diploid fibroblasts induced directly by mutant p53. Oncogene 9: 1885–1889

Boukamp P, Petrussevksa RT, Breitkreutz D, Hornung J, Markham A and Fusieng NE (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol 106: 761–771

Burz F, Dutta A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW and Vogelstein B (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282: 1497–1501

Denechower LA, Harvey M, Slagle BL, McCarthy MJ, Montgomery CA Jr, Butel JS and Bradley A (1992) Mice deficient for p53 are developmentally normal but have defects in multiple p53 target tissues. Cell 75: 805–816

Finlay CA, Hinds PW and Levine AJ (1989) The p53 proto-oncogene can act as a suppressor of transformation. Cell 57: 1083–1093

Garkavtsev I, Kazarov A, Gudkov A and Riabowol K (1996) Suppression of the novel growth inhibitor p33ING1 promotes neoplastic transformation. Nat Genet 14: 415–420

Garkavtsev I and Riabowol K (1997) Extension of the replicative life span of human diploid fibroblasts by inhibition of the p33ING1 candidate tumour suppressor. Mol Cell Biol 17: 2014–2019

Garkavtsev I, Grigorian IA, Osovskaya VS, Chernov MV, Shamakot PM and Gudkov AV (1996) The candidate tumour suppressor p33ING1 cooperates with p53 in cell growth control. Nature 391: 295–298

Hall PA, McKeever PH, Menage HD, Dover R and Lane DP (1993) High levels of p53 protein in UV-irradiated normal human skin. Oncogene 8: 203–207

Helbing CC, Veilette C, Riabowol K, Johnston RN and Garkavtsev I (1997) A novel candidate tumour suppressor, ING1, is involved in the regulation of apoptosis. Cancer Res 57: 1255–1258

Holstein M, Sidransky D, Vogelstein B and Harris CC (1991) p53 mutations in human cancers. Science 253: 49–53

Lehman TA, Modali R, Boukamp O, Stanek J, Bennett WP, Welsh JA, Meckelt RA, Shapard MR, Fusieng N and Rogen EM (1993) p53 mutations in human immortalized epithelial cell lines. Carcinogenesis 14: 833–839

Li G, Ho VC, Berean K and Tron VA (1995) Ultraviolet radiation induction of squamous cell carcinomas in p53 transgenic mice. Cancer Res 55: 2070–2074

Li G, Mitchell DL, Ho VC, Reed JC and Tron VA (1996) Decreased DNA repair but normal apoptosis in ultraviolet-irradiated skin of p53 transgenic mice. Am J Pathol 148: 1113–1123

Li G, Ho VC, Mitchell DL, Trotter MJ and Tron VA (1997) Differentiation-dependent p53 regulation of nucleotide excision repair in keratinocytes. Am J Pathol 150: 1457–1464

Li G and Ho VC (1998a) p53-dependent DNA repair and apoptosis respond differently to high- and low-dose ultraviolet radiation. Br J Dermatol 139: 3–10

Li G, Tron V and Ho V (1998b) Induction of squamous cell carcinoma in p53-deficient mice after ultraviolet irradiation. J Invest Dermatol 110: 72–75

Li G, Bush JA and Ho VC (2000) p53-dependent apoptosis in melanoma cells after treatment with camptothecin. J Invest Dermatol 114: 514–519

Miyashita T and Reed JC (1995) Tumour suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80: 293–299

Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B and Reed JC (1994) Tumour suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 9: 1795–1805

Ohmori N, Nagai M, Tasaka T, Koeffler HP, Riabowol K and Takahara J (1999) Decreased expression of p33ING1 mRNA in lymphoid malignancies. Am J Hematol 62: 118–119

Oki E, Maehara Y, Tokunaga E, Kakeji Y and Sugimachi K (1999) Reduced expression of p33ING1 and the relationship with p53 expression in human gastric cancer. Cancer Lett 147: 157–162

Owen-Schaub LB, Zhang W, Cusack JC, Angelo LS, Santee SM, Fujiwara T, Roth JA, Drissler AB, Zhang WW, Kruzel E and Radinsky R (1995) Wild-type human p53 and a temperature-sensitive mutant induce fas/APO-1 expression. Mol Cell Biol 15: 3032–3040

Saito A, Furukawa T, Fujikshihe S, Koyama S, Hoshii M, Hayashi Y and Horii A (2000) p24/ING1-ALTI and p47/ING1-ALTI2, distinct alternative transcripts of p33/ING1. J Hum Genet 45: 177–181

Shaulsky G, Goldfinger N, Tosky MS, Levine AJ and Rotter V (1991) Nuclear localization is essential for the activity of p53 protein. Oncogene 6: 2055–2065

Shinoura N, Muramatsu Y, Nishimura M, Yoshida Y, Saito A, Yokoyma T, Furukawa T, Horii A, Hashimoto M, Asai A, Kizuno T and Hamada H (1999) Adenovirus-mediated transfer of p33ING1 with p53 drastically augments apoptosis in gliomas. Cancer Res 59: 5521–5528

Smith ML, Chen IT, Zhan Q, Bai E, Chen CY, Gilmer TM, Kastan MB, O’Connor PM and Fornace AJ Jr (1994) Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. Science 266: 1376–1380

Toyama Y, Iwase H, Watson P, Muzik H, Saetler E, Maglocco A, DiFrancesco L, Forsyth P, Garkavtsev I, Kobayashi S and Riabowol K (1999) Suppression of ING1 expression in sporadic breast cancer. Oncogene 18: 5187–5193

Tron VA, Trotter MJ, Ishikawa T, Ho VC and Li G (1998a) p53-dependent regulation of nucleotide excision repair in murine epidermis in vivo. J Cutan Med Surg 3: 16–20

Tron VA, Trotter MJ, Tang L, Krajewska M, Reed JC, Ho VC and Li G (1998b) p53-regulated apoptosis is differentiation dependent in ultraviolet B-irradiated mouse keratinocytes. Am J Pathol 153: 579–585

Zemrzenski M, Hill I, Kwek S, Grigorian IA, Gurova K, Garkavtsev I, Diachenko L, Koomin E and Gudkov A (1999) Structure and regulation of the mouse ing1 gene. J Biol Chem 274: 32172–32181.