Reduced Stability of Regularly Spliced but not Alternatively Spliced p53 Protein in PARP-deficient Mouse Fibroblasts

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Abstract

The interaction between poly(ADP-ribose) polymerase (PARP) and the product of the tumor suppressor gene p53 has been described previously. PARP deficiency may affect the expression and regulation of wild-type (wt) p53. For this purpose, we have used immortalized cells derived from wt and PARP knockout mice. We have found a clearly reduced basal level of PARP expression and that the p53 protein present in PARP-deficient cells possesses characteristic features of AS p53. Our results clearly show that PARP-deficient mouse cells possess a distinct form of p53. The expression of p53 in PARP knockout cells, no significant difference of the p53 transcription rate was observed between wt and PARP-deficient cells. Interestingly, in both cell types, an additional p53 transcript representing the alternatively spliced (AS) p53 form was detected. Because of its reactivity with different specific anti-p53 antibodies, we have determined that the p53 protein present in PARP knockout mouse cells possesses characteristics of AS p53. Our results clearly show that PARP-deficient cells constitutively express the AS form of wt p53 and indicate that the regularly spliced p53 is extremely unstable in the absence of PARP. Moreover, PARP−/− cells fail to transactivate p53-responsive genes. Treatment of PARP−/− cells with genotoxic agents primarily leads to the activation of AS p53 protein.

Introduction

PARP (EC 2.4.2.30), a nuclear enzyme of M, 116,000, is an unique detector of single-stranded DNA breaks (for review see Refs. 1 and 2). This property to recognize even negligible DNA lesions is important for protection of cells from DNA damage and for the maintenance of genome integrity. PARP is an ubiquitous enzyme that is composed of three main structural domains, to which its distinct functions can be attributed. An NH₂-terminal part encompasses the DNA-binding domain with two zinc-finger motifs involved in the recognition of and binding to nicks and a bipartite nuclear localization signal. Between the second zinc-finger and the nuclear localization signal lies a DEVD tetrapeptide sequence that has been shown to be specifically cleaved during the early events of apoptosis by CPP-32/caspase-3 (3). A central automodification domain regulates the interaction between PARP and DNA. A COOH-terminal catalytic fragment encompasses the NAD-binding pocket. The physiological role of poly(ADP-ribosyl)ation reactions in numerous cellular processes, including replication, DNA repair and recombination, stress response, and apoptosis, remains to be determined. Recently, the establishment of new molecular and genetic approaches, including a transdominant inhibition of PARP (4), an antisense model (5), and generation of PARP-deficient mice by homogenous recombination (6, 7), gave evidence for the involvement of PARP in the maintenance of genome integrity. Although results obtained from studies with PARP-deficient mice are partially contradictory, this novel genetic approach offers a very promising experimental model for the elucidation of PARP function.

In response to DNA damage and environmental stress, another nuclear protein, p53 tumor suppressor, is strongly activated, resulting in its increase to high levels due to the protein stabilization and/or enhanced transcription (for review see Refs. 8 and 9). The accumulation of p53 protein leads to inhibition of cell cycle progression or induction of apoptosis, thereby preventing the proliferation of damaged cells and allowing repair of damaged DNA. The antiproliferative regulation of wt p53 has been shown to be mediated by transactivation of a variety of target genes. Most importantly, p53 regulates the transcription of several cell cycle-related genes, including gadd45 (growth arrest and DNA damage), mdm-2, and p21(WAF1/CIP1) (10). The product of the latter has been implicated in mediating the cell cycle arrest at G₁ checkpoint because of its ability to associate with and to inhibit cyclin-dependent protein kinase 2, which is necessary for cell cycle progression (11). In addition, p21 has been shown to bind directly to proliferating cell nuclear antigen and to inhibit proliferating cell nuclear antigen-dependent DNA replication in vitro. Furthermore, it was found that p53 interacts with the transcription factor IHH complex, which participates in nucleotide excision repair. wt p53 has also been implicated in maintaining of centrosome integrity and to control a spindle checkpoint. Mutations of the p53 gene result in conformational changes in the protein and loss of its suppressor function. In >50% of human tumors, the p53 gene is lost or mutated.

The fact that both proteins, p53 and PARP, contribute to the capability of cells to recover from DNA damage or to commit to apoptosis leads to the assumption that the two proteins may be functionally related. Recently, we have found that p53 protein and PARP form tight complexes (12) in primary rat cells constitutively overexpressing temperature-sensitive p53(135val) mutant. The complex formation led to unusual cytoplasmic sequestration of the nuclear enzyme PARP by mutant p53(135val) (12). Moreover, we have observed ADP-ribosylation of mutant but not wt form of p53 in these cells. However, the purified wt p53 proteins were readily modified by poly(ADP-ribose) chains in vitro (13). The binding of p53 protein to specific p53 consensus sequence prevented its modification, indicating that the lack of ADP-ribosylation of wt p53 in vivo was due to the DNA binding (13).

The link between poly(ADP-ribosyl)ation reactions and p53 regulation was also reported by another group that has established PARP-deficient human cell lines. These cell lines showing reduced baseline levels of p53 failed to accumulate p53 in response to etoposide...
(VP-16) treatment and subsequently showed a decrease in VP-16-induced apoptosis (14). Decrease of constitutive expression of wt p53 was observed in PARP-deficient mouse cells (15). Interestingly, PARP deficiency did not affect the ability of cells to undergo apoptosis (16).

The aim of this study was to investigate whether PARP status affects the regulation of wt p53. We have found a clearly reduced basal level of regularly spliced (RS) wt p53 protein in PARP-deficient cells. Because of its reactivity with different specific anti-p53 antibodies, we have determined that the p53 present in PARP-deficient mouse cells possesses characteristic features of AS p53.

Our results clearly show that PARP-deficient cells constitutively express the AS form of wt p53 and that RS p53 is extremely unstable in the absence of PARP. Moreover, PARP−/− cells fail to transactivate p53-responsive genes. Treatment of PARP−/− cells with genotoxic agents primarily leads to activation of AS p53 protein.

Materials and Methods

Cells. Immortalized MEFs were obtained from PARP+/+ (A-19) and PARP−/− (A-11 and A-12) mice (6). Cells were grown in DMEM supplemented with 10% FCS in an atmosphere of 7.5% CO2.

Plasmids. Plasmids encoding full-length human wt p53 and murine wt p53 were kindly supplied by B. Vogelstein (Johns Hopkins University, School of Medicine, Baltimore, MD) and M. Oren (The Weizmann Institute, Rehovot, Israel), respectively. Plasmids encoding transcription reporters that contain the luciferase gene (expressed from a minimal thymidine kinase promoter, which is regulated by a p53 binding site, and derived from the cyclin G promoter) pWT30tkluc or control ptkluc were a kind gift of K. Okamoto (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Antibodies. Different anti-p53 antibodies recognizing distinct epitopes were used. Anti-p53 antibodies PAb421 (Ab-1), PAb240 (Ab-3), and PAb246 (Ab-4) were obtained from Oncogene Research Products (Cambridge, MA.). CM-1 was obtained from Novocastra Laboratories Ltd. (Newcastle upon Tyne, England). Specific antibodies to the COOH-terminal 17 aa unique to AS p53 form (Ab-10) were from Oncogene Research Products. Monoclonal anti-p53 antibodies PAb122 were from Boehringer (Mannheim, Germany).

Anti-waf-1 antibodies (Ab-5) were from Oncogene Research Products, anti-mdm-2 (SGM-14) antibodies were from DAKO A/S (Glostrup, Denmark), and anti-PARP antibodies were from Boehringer. Appropriate secondary antibodies linked to horseradish peroxidase, Cy-2, or Cy-3 were from Amersham (Aylesbury, UK).

Cell Treatment. Cells were treated with 0.35 μM doxorubicin hydrochloride (Farmalittra Carlo Erba AG, Zug, Switzerland) for 6 and 24 h and with 0.5 mM actinomycin D (Boehringer) for 24 and 48 h.

Indirect IF Microscopy. Cells were grown on coverslips, rinsed with PBS, fixed in ice-cold methanol-acetone (3:2) for 20 min, and washed with PBS. The cells were incubated with anti-p53 antibodies at appropriate concentrations, and the immune complexes were detected by incubation with secondary antibodies covalently coupled to Cy-2 or Cy-3 (Amersham International; Ref. 12). For visualization of nuclei, cells were additionally stained for 10 min with 4.6-diamidino-2-phenylindole (1 μg/ml in PBS).

RNA Isolation and Northern Blotting. Total cellular RNA was prepared by the guanidium isothiocyanate, phenol, and chloroform extraction procedure. mRNA was isolated on poly(dT) Sepharose beads (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturer’s protocol. mRNA samples (1 μg) were separated through 1% agarose gel and transferred onto nylon membranes (Gene Screen; DuPont). Membranes were hybridized with a fluorescein-labeled p53 cDNA probe (Gene Image; Amersham International) and stringently washed. After a blocking step, the hybrids were detected by incubation with anti-fluorescein-alkaline phosphatase conjugate. After the excess conjugate was washed off, probe-bound alkaline phosphatase was used to catalyze light production in the presence of substrate. Chemiluminescence was detected by autoradiography.

Immunoblotting. Proteins dissolved in reduced SDS sample buffer were loaded on SDS-polyacrylamide gels, electrophoretically separated, and transferred onto polyvinylidene difluoride membrane (Amersham International). Equal loading of total proteins was confirmed by Ponceau S staining. Immunodetection of antigens was performed with specific antibodies. The immune complexes were detected autoradiographically using appropriate peroxidase-conjugated second antibodies and enhanced chemiluminescent detection reagent ECL + (Amersham International). In some cases, blots were stripped and used for further incubation.

Transactivation of the p53-responsive Genes. To determine the intrinsic ability of cells to transactivate p53-responsive genes, MEFs were transfected in triplicate with transcription reporters containing the luciferase gene expressed from a minimal thymidine kinase promoter regulated by a p53-binding site derived from the cyclin G promoter. In some additional assays, plasmid encoding murine wt p53 was cotransfected. Forty-eight h after transfection, cells were lysed and luciferase activity was measured using chemiluminescent substrate (Promega Corporation, Madison, WI). Activity (mean of triplicate determination ± SD) was expressed as fold induction relative to cells transfected with long terminal repeat control vector.

Results

Expression of p53 Protein in PARP-deficient Mouse Cells. To determine whether the inactivation of the PARP gene has any effect on the basal level of p53 protein, we performed immunoblotting experiments. In the first tests, we used the highly sensitive anti-p53 antibody PAb421, which recognizes an epitope located in the COOH terminus of p53 protein (aa 363–372) and is known to react with either wt or mutant p53 form. As shown in Fig. 1, p53-positive signals were detected in nuclei isolated from wt MEFs (Lanes 3 and 4) but not in nuclei prepared from two different PARP−/− cell lines (Lanes 1 and 2). Sequential incubation of the blot with anti-PARP antibodies revealed the presence of a M116,000 protein band representing full-length PARP in wt but not in knockout mouse fibroblasts. Nuclei isolated from mouse liver were used as positive control for PARP expression (Fig. 1, Lane 6). A PARP-positive signal of comparable intensity with that for wt MEFs was observed. To prove whether the lack of the PAb421 immunoreactivity was due to the detection limit of

![Fig. 1. Expression of wt p53 protein in mouse cells. Nuclear proteins isolated from wt (+/+) and from PARP−/− (−/−) A-11 and A-12) mouse fibroblasts and from mouse liver were separated on 10% SDS gels and blotted onto polyvinylidene difluoride membranes. Immunoblotting was performed using the anti-p53 antibody PAb421 and, subsequently, the anti-PARP antibody. Twenty μg of protein were loaded in each lane, with exception of Lane 4, in which 10 μg of protein were loaded. Lane 1, A-11; Lane 2, A-12; Lanes 3 and 4, wt cells; Lane 5, marker; Lane 6, mouse liver.](image-url)
the immunoblotting assay, we have monitored p53 protein expression at single cell level by IF microscopy. The nuclei of wt MEFs were strongly stained using PAb421, but cells from PARP knockout mice didn’t give any characteristic nuclear signal with these antibodies (Fig. 2A, top). Weak cytoplasmic staining obtained with PAb421 seems to be nonspecific because it was also observed in the control assay, in which the first antibody was omitted (data not shown). These experiments clearly showed that PAb421 monoclonal antibodies react with wt p53 protein in normal mouse fibroblasts but fail to detect it in PARP-deficient cells, either by immunoblotting or IF. We have, therefore, repeated experiments using other anti-p53 antibodies exhibiting distinct immunological specificity. Using PAb122, which spans the common epitope with PAb421, we have obtained a p53 signal in normal but not in PARP-deficient fibroblasts (data not shown). Upon incubation with the monoclonal PAb240 antibody, which binds to an epitope between aa 207–212 and rabbit polyclonal anti-p53 antibody CM-1, recognizing a broader epitope(s) range, we have detected p53-positive signals not only in wt MEFs but also in both PARP-deficient cell lines (Fig. 2A, middle). However, the staining pattern of PARP-deficient cells differed from that observed for wt counterparts. Polyclonal CM-1 antibodies stained strongly and uniformly the nuclei of wt mouse fibroblasts, whereas in PARP−/− cells, they gave markedly weaker signals. Nuclear staining was not homogenous but was restricted to a few dots. In these cells, CM-1 antibodies reacted additionally with perinuclear and cytoplasmic structures.

**Normal Level of p53 Transcription in PARP−/− Cells.** To prove whether the obvious decrease of wt p53 protein amounts in PARP-deficient cells is due to reduced protein stability or, rather, decreased transcription rate, we checked the level of p53 mRNA by

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Fig. 2. Different p53 staining pattern in PARP-deficient cells. A, untreated wt (wt) and PARP-deficient cells (KO) were stained with three different anti-p53 antibodies B, p53 staining of actinomycin D-treated cells. Nuclei were visualized with 4,6-diamidino-2-phenylindole.
Northern blotting in subsequent experiments. As shown in Fig. 3, the positive p53 signals were detected in all analyzed mouse samples. Surprisingly, an even stronger p53 signal was detected in one PARP-deficient cell line, as compared with normal mouse fibroblasts, despite lower mRNA loading, as evidenced by control probing with GAPDH. A weaker p53 signal observed for mouse liver samples correlated with lower amounts of loaded mRNA. The careful inspection of the Northern blot revealed an additional band hybridized with labeled p53 probe. A higher band, which was approximately 100 bp larger than the lower band, was present in samples from mouse fibroblasts but not from the mouse liver. The position of the additional p53 signal and ratio between both bands coincided with that reported for the AS p53 transcript.

**Induction of p53 Protein in Response to Doxorubicin.** Because in normal cells wt p53 protein is constitutively expressed in very low amounts due to its short half-life but becomes stabilized in response to treatment with DNA-damaging agents, we have addressed the question of whether treatment with doxorubicin would transiently induce p53 protein in PARP-deficient cells. As depicted in Fig. 4 (top), a significant increase of wt p53 level in normal MEFs occurred already 6 h after doxorubicin application, and further p53 activation was observed at 24 h. Concomitantly with the increase of wt p53 protein, mdm-2-positive signals appeared, reaching the highest level 24 h posttreatment. However, in both PARP-deficient cell lines, no induction of PAb421 immunoreactive p53 form could be detected in response to doxorubicin treatment. Northern blot analysis revealed presence of both p53 splice variants in tested mouse cells. Therefore, it was advisable to probe the blots with antibodies allowing detection of both wt p53 forms. Indeed, incubation of the blots with anti-p53 PAb240 also revealed p53-positive signals in PARP-deficient cells. Interestingly, accurate inspection of the blots shows that the kinetics of p53 induction after doxorubicin treatment in PARP-deficient cells differed from that observed in wt cells. Six h after drug application, the intensity of p53 PAb240 reactive band markedly decreased to a barely detectable level. At 24 h, p53 positive signals were again observed. No detectable activation of mdm-2 protein could be found in PARP-deficient cells.

**Increased Level of Alternative Spliced p53 in PARP-deficient Cells.** Considering the above results showing the lack of p53 reactivity in PARP-deficient cells, either control or doxorubicin treated, with PAb421 and positive signals with PAb240, it appeared possible that these cells solely express the AS form of wt p53 at the protein level. To prove this presumption, we have performed immunoblotting experiments using the specific polyclonal antibody Ab-10 raised against the 17-aa sequence unique to AS p53. To ensure that Ab-10 antibody does not cross-react with the RS form, we have probed it with p53 proteins of different origin that possess a COOH terminus characteristic of the RS p53 form. Results shown in Fig. 5 illustrate that RS p53 proteins from human and mouse were reactive with PAb421 but not with Ab-10, thereby demonstrating that Ab-10 is highly specific for AS p53. Interestingly, this antibody stained p53 either in wt or in PARP-deficient mouse cells (Fig. 6, top). However, the latter gave a stronger p53 signal, indicating that PARP-deficient cells produce higher amounts of AS p53 protein than the wt counterparts.

**p53 Induction by Actinomycin D Treatment.** Treatment of mouse cells with low concentrations of actinomycin D is known to enhance the generation of AS p53 form. On the other hand, one cannot exclude the possibility that, in response to this drug, the RS form would also be affected. To distinguish between overlapping bands representing two p53 forms, it was necessary to modify the conditions of their electrophoretic separation. Unfortunately, at a constant acrylamide concentration, the two p53 forms were not resolved satisfactorily. We have found their optimal resolution on gradient gels (4–10%). As shown in Fig. 6, Ab-10-immunodetectable AS p53 protein was induced by actinomycin. Moreover, the recorded AS p53 enhancement at 24 h was stronger in PARP-deficient cells than in normal mouse fibroblasts. AS p53-responsive signals were sustained for an additional 24 h. It was interesting to prove whether actinomycin D also affected the expression of the RS p53 form. Immunoblotting...
with PAb421 antibody, allowing its selective detection, revealed the increase of p53 signal following actinomycin D solely in normal MEFs. However, in PARP-deficient cells, actinomycin D treatment did not affect the expression of PAb421-immunoreactive p53 form. Subsequent incubation of the blot with PAb240 or CM-1 antibodies allowed concomitant monitoring of both naturally occurring p53 forms. Surprisingly, sequential immunoblotting revealed appearance of an additional, slightly higher protein band. On the basis of immunoreactivity with different anti-p53 antibodies, we can assume that this additionally visualized protein band represent AS p53 form. An increase in p53 concentration in response to actinomycin D was evident. Interestingly, despite stimulation of the p53 protein, especially its AS form, no induction of mdm-2 could be detected, even at 48 h. On the other hand, significant activation of waf-1 protein in PARP-deficient cell lines occurred 24 h after actinomycin D application. The staining pattern of actinomycin D-treated cells with CM-1 (Fig. 2B) antibody was comparable with that of controls. However, in response to actinomycin D, the reactivity of wt as well as PARP-deficient cells with Ab-10 recognizing exclusively AS p53 was significantly affected. Weak cytoplasmic staining observed in untreated controls was strongly enhanced after treatment. Extremely intense signals were found in postmitotic cells.

**Lack of Transactivation of p53-responsive Genes in PARP-deficient Cells.** To determine the functional consequence the reduction of the basal level of RS p53 protein, we measured the intrinsic activity to transactivate p53-responsive genes in normal and PARP-deficient cells. wt fibroblasts stimulated the p53-mediated luciferase activity ~8-fold (Fig. 7). Simultaneous overexpression of murine wt p53 enhanced additionally the luciferase activity. However, in PARP−/− cells, no considerable induction of p53-mediated luciferase activity could be detected. Concomitant overexpression of wt p53 did not increase the p53-mediated induction of luciferase activity.

This lack of the transactivating ability in the presence of coexpressed wt p53 was surprising, considering the fact that the transfection efficiency in both cell types was comparable. This was evidenced by cotransfection with β-galactosidase or green fluorescent protein. However, differing concentrations of the overexpressed wt p53 between wt and PARP knockout cells could explain this effect. To prove this possibility, we additionally analyzed the extracts by immunoblotting for the expression of wt p53. In samples of normal mouse cells, markedly enhanced concentration of PAb421-reactive wt p53 protein was detected. In PARP−/− cells, despite the cotransfection of wt p53, no PAb421 immunoreactivity could be detected, indicating that the RS wt p53 has reduced stability in the absence of functional PARP.

Taken together, these results clearly show that the significant decrease of the basal level of RS p53 form in PARP-deficient cells reduces their endogenous potential to specifically regulate p53-responsive genes. The obvious decrease of the amounts of the RS wt p53 in PARP−/− cells is due to the reduced protein stability. Moreover, depending on PARP status, different p53 forms are activated in response to DNA damage. In wt mouse cells RS p53 protein was primarily induced, whereas in PARP-deficient cells, the AS p53 was activated.

**Discussion**

The wt p53 protein acts as a superior control factor in cellular proliferation and maintenance of genome integrity. Its regulatory
activities are mediated not only by the sequence-specific DNA binding domain but also by the COOH-terminal part harboring target sites for a variety of cellular kinases and binding region for a number of transcription factor IIIH-associated proteins, including XPD, XPB, and CSB strand-specific DNA repair enzymes (8). The most important feature of the COOH-terminal part is the ability to recognize the DNA structures resulting from damage-induced lesions. Therefore, one could expect that shortening or mutation of p53 protein at its COOH terminus would alter the p53 activity.

AS p53 form represents a naturally occurring COOH-terminal truncated p53 variant (17) in which 96 nucleotides of the 3’ end of intron 10 were inserted between original nucleotides 1091 and 1092 of the mouse gene. This p53 variant first cloned and sequenced from transformed fibroblasts is not a feature of tumor cells but was also shown to be expressed in normal cells and tissues (17). Alternative splicing of the primary p53 transcript generates two distinct p53 mRNAs that code for two distinct wt p53 proteins: the full-length RS p53 and an AS p53, in which 17 new aa substitute for the last 26 COOH-terminal residues of RS p53. Alternative splicing of the p53 transcript seems to be not a general phenomenon of mammalian tissues but, rather, a species-specific event. It was detected in mouse but not in human and rat tissues. This AS form of wt p53, which represents 30% of the total p53 of murine cells and tissues, was suggested to be expressed mostly at the G2 phase of the cell cycle. What is the biological role of AS p53? Functionally, the AS p53 protein differs from the RS p53 in that it is constitutively active in sequence-specific DNA binding (18) and is devoid of activities ascribed to the COOH domain. Furthermore, by heterocomplex formation with RS p53, AS p53 could alter the normal function of RS p53 (19).

In this study, we have shown that, in PARP-deficient cells, RS wt p53 is reduced to an undetectable level, as evidenced by immunoblotting and IF microscopy using anti-p53 antibody PAb421. Furthermore, upon application of antibodies recognizing epitopes common for both p53 splice forms, we have detected considerable amounts of p53 protein in PARP-knockout cells. Subsequent immunoblotting with the antibody directed against the 17-aa motif unique for AS form of wt p53 demonstrated that p53 protein expressed in PARP-deficient cells represents AS p53. Despite its slightly lower molar mass, the AS p53 protein runs more slowly in the SDS gel than the RS counterpart. It is obvious that not only the size but also the primary structure of a protein are critical for its electrophoretic mobility. It became most evident in the case of human and murine wt p53. Although molecular weights of both p53 proteins barely differ, human wt p53 runs in the SDS gel to a markedly higher position than the murine p53.

Interestingly, the two p53 splice forms possess quite distinct characteristics with respect to their induction, localization, and ability to transactivate responsive genes and are possibly differentially regulated, which was reflected in the different responses of the cells to genotoxic agents. The kinetics of p53 activation in PARP-deficient cells following doxorubicin or actinomycin D treatment differed from that observed in the wt counterparts. Induction of RS p53 protein accompanied by increase of mdm-2 level occurred solely in wt but not in PARP-deficient cells. Surprisingly, in response to actinomycin D, the level of waf-1 protein increased at 24 and 48 h in PARP−/−cells despite the lack of RS p53. Therefore, it seems that the observed waf-1 up-regulation is mediated by an additional p53-independent pathway, as reported previously (20).

These data complete and extend those obtained by Agarwal et al. (15) and provide new information about the status and regulation of p53 protein in PARP-deficient cells. In their study, the authors used Pab240 antibody reacting with an epitope common for both p53 forms, and for this reason, they could not distinguish between two splice forms of wt p53. We used five different anti-p53 antibodies recognizing distinct epitopes and additionally used gels that enabled separation of two p53 forms. This combined approach facilitated discrimination between two p53 splice forms.

Our results demonstrating the reduction of RS p53 protein to an undetectable level after inactivation of PARP gene is in concordance with published data obtained with human cell lines and indicate that PARP is necessary for stabilization of RS p53. The data are also consistent with findings of Vaziri et al. (21) showing that inhibition of PARP activity in human fibroblasts leads to abrogation of p53-mediated expression of p21 and mdm-2 in response to DNA damage. On the other hand, the PARP deficiency does not seem to affect the stability of AS form of p53. It is open to speculation how PARP affects the stability of RS p53. Is the protein-protein interaction and complex formation sufficient to protect p53 from untimely degradation, or is PARP enzymatic activity necessary? The latter suggestion was also postulated by Vaziri et al. (21). To elucidate this problem in the mouse, which, in some aspects of p53 regulation, essentially differs from other species, we are considering further experiments.

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References


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