Chromosomal aberrations in PARP<sup>−/−</sup> mice: Genome stabilization in immortalized cells by reintroduction of poly(ADP-ribose) polymerase cDNA


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Depletion of poly(ADP-ribose) polymerase (PARP) increases the frequency of recombination, gene amplification, sister chromatid exchanges, and micronuclei formation in cells exposed to genotoxic agents, implicating PARP in the maintenance of genomic stability. Flow cytometric analysis now has revealed an unstable tetraploid population in immortalized fibroblasts derived from PARP<sup>−/−</sup> mice. Comparative genomic hybridization detected partial chromosomal gains in 4C5-ter, 5F-ter, and 14A1-C1 in PARP<sup>−/−</sup> mice and immortalized PARP<sup>+/−</sup>-fibroblasts. Neither the chromosomal gains nor the tetraploid population were apparent in PARP<sup>−/−</sup>-cells stably transfected with PARP cDNA [PARP<sup>+/−</sup> (+PARP)], indicating negative selection of cells with these genetic aberrations after reintroduction of PARP cDNA. Although the tumor suppressor p53 was not detectable in PARP<sup>−/−</sup>-cells, p53 expression was partially restored in PARP<sup>−/−</sup> (+PARP) cells. Loss of 14D3-ter that encompasses the tumor suppressor gene Rb-1 in PARP<sup>−/−</sup>-mice was associated with a reduction in retinoblastoma(Rb) expression; increased expression of the oncogene Jun was correlated with a gain in 4C5-ter that harbors this oncogene. These results further implicate PARP in the maintenance of genomic stability and suggest that altered expression of p53, Rb, and Jun, as well as undoubtedly many other proteins may be a result of genomic instability associated with PARP deficiency.

Poly(ADP-ribose) polymerase (PARP) is involved in nuclear processes involving cleavage and rejoining of DNA, such as DNA replication, differentiation, DNA repair and recombination, apoptosis, as well as maintenance of genomic stability (1, 2). Inhibition of PARP by either chemical inhibitors (3–5) or by dominant negative mutants (6, 7), or PARP depletion by antisense inhibition of PARP by either chemical inhibitors (3–5) or by dominant negative mutants (6, 7), or PARP depletion by antisense gene expression, sister chromatin exchanges (SCE), all of which are markers of genomic instability, in cells exposed to DNA-damaging agents. PARP-deficient cell lines are hypersensitive to carcinogenic agents, implicating PARP in the maintenance of genomic stability. PARP<sup>−/−</sup>-mice deficient in PARP by either chemical inhibitors (3–5) or by dominant negative mutants (6, 7), or PARP depletion by antisense gene expression, sister chromatin exchanges (SCE), all of which are markers of genomic instability, in cells exposed to DNA-damaging agents. PARP-deficient cell lines are hypersensitive to carcinogenic agents, implicating PARP in the maintenance of genomic stability. PARP<sup>−/−</sup>-mice deficient in PARP are resistant to murine models of a number of human diseases, including focal cerebral ischemia (20), toxin-induced diabetes (21), 1-methyl-4-phenyl-1,2,3,6-tetrahydro- pyridine (MPTP)-induced Parkinsonism (22), and peroxynitrite-induced arthritis (23), suggesting that PARP activation, triggered by oxidative or nitrosative stress, plays a role in the pathophysiology of these diseases. Primary fibroblasts derived from PARP<sup>−/−</sup>-mice show an elevated frequency of SCE and micronuclei in response to treatment with genotoxic agents (16, 24), further implicating PARP in the maintenance of genomic integrity. PARP<sup>−/−</sup>-mice developed by another group exhibit extreme sensitivity to γ-irradiation and methylnitrosourea and increased genomic instability as revealed by a high level of SCE (17). Immortalized cells derived from these mice show retarded cell growth, G2/M block, and chromosomal instability on exposure to DNA-alkylating agents, presumably because of a defect in DNA repair (25).

In the present study, flow cytometry revealed that immortalized fibroblasts derived from PARP<sup>−/−</sup>-mice exhibit mixed ploidy, including a tetraploid cell population, which is also indicative of genomic instability. We characterized the genetic alterations associated with PARP depletion by comparative genomic hybridization (CGH) analysis (26, 27) of genomic DNA from both wild-type and PARP<sup>−/−</sup>-mice as well as from immortalized fibroblasts derived from these animals. With a limit of detection of 5–10 Mb (28), this cytogenetic technique detects unbalanced chromosomal gains and losses in test DNA as a measure of genetic instability. Although CGH now is widely used as a powerful tool for generating maps of DNA copy number changes in human tumor genomes, only two studies to date have demonstrated its potential for evaluating genetic instability in transgenic mouse models (29, 30). CGH analysis revealed partial gains in chromosomes 4, 5, and 14, and partial loss of chromosome 14 in PARP<sup>−/−</sup>-mice as well as from immortalized fibroblasts derived from these animals. With a limit of detection of 5–10 Mb (28), this cytogenetic technique detects unbalanced chromosomal gains and losses in test DNA as a measure of genetic instability. Although CGH now is widely used as a powerful tool for generating maps of DNA copy number changes in human tumor genomes, only two studies to date have demonstrated its potential for evaluating genetic instability in transgenic mouse models (29, 30). CGH analysis revealed partial gains in chromosomes 4, 5, and 14, and partial loss of chromosome 14 in PARP<sup>−/−</sup>-mice as well as from immortalized fibroblasts derived from these animals. With a limit of detection of 5–10 Mb (28), this cytogenetic technique detects unbalanced chromosomal gains and losses in test DNA as a measure of genetic instability. 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Abbreviations: PARP, poly(ADP-ribose); PARP, poly(ADP-ribose) polymerase; SCE, sister chromatid exchanges; CGH, comparative genomic hybridization; PCNA, proliferating cell nuclear antigen; topo I, topoisomerase I; RT-PCR, reverse transcription-PCR; Rb, retinoblastoma.

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Materials and Methods

Cell Lines, Vectors, and Transfection. Homozygous PARP−/− mice that were generated by disrupting exon 2 of the PARP gene by homologous recombination (16) and wild-type (PARP+/+) littermates (strain 129/Sv × C57BL/6; female) were used in the present study. Wild-type (PARP+/+ clone A19) and PARP−/− (clone A1) fibroblasts were immortalized spontaneously by a standard 3T3 protocol (16) and cultured in DMEM supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml). Immortalized PARP−/− fibroblasts were cotransfected by Lipofectamine (Life Technologies, Grand Island, NY) with human PARP (pCD12) cDNA (31) and the plasmid pTracer-CMV (a zeocin-based vector; Invitrogen). This vector was used because the PARP−/− fibroblasts express a neomycin resistance gene that was introduced during establishment of the PARP knockout mice. Stable transfectants were selected in growth medium containing zeocin (500 μg/ml).

Immunoblot Analysis. SDS/PAGE and transfer of proteins to nitrocellulose membranes were performed according to standard procedures. Membranes were stained with Ponceau S (0.5%) to confirm equal loading and transfer of proteins. Membranes were incubated with antibodies to PARP (1:2,000 dilution; BioMol, Plymouth Meeting, PA), PAR (1:250; gift from M. Miwa, Japan), p53 (1:20 dilution; PAB421, Calbiochem), retinoblastoma (Rb) (1:200 dilution; clone IF8, Santa Cruz Biotechnology), glutamate dehydrogenase (1:1,000; Biodesign International, Kennebunkport, ME), Jun (1:1,000 dilution, Calbiochem), proliferating cell nuclear antigen (PCNA) (1:800; Calbiochem), or topoisomerase I (topo I) (1:2,500; TopoGen, Columbus, OH). After subsequent incubation with appropriate horseradish peroxidase-conjugated antibodies to mouse or rabbit IgG (1:3,000 dilution), immune complexes were detected by enhanced chemiluminescence (Pierce).

Flow Cytometry. Nuclei were prepared for flow cytometric analysis as described (33). Cells were exposed to trypsin and resuspended in 100 μl of a solution containing 250 mM sucrose, 40 mM sodium citrate (pH 7.6), and 5% (vol/vol) DMSO. The cells were lysed for 10 min in a solution containing 3.4 mM sodium citrate, 0.1% (vol/vol) NP-40, 1.5 mM spermine tetrahydrochloride, and 0.5 mM Tris-HCl (pH 7.6). After incubation of lysates for 10 min with ribonuclease A (0.1 mg/ml), nuclei were stained for 15 min with propidium iodide (0.42 mg/ml), filtered through a 37-μm nylon mesh, and analyzed with a dual-laser flow cytometer (FACSScan, Becton Dickinson).

CGH. Normal DNA was extracted from spleen tissue of normal mice (FVB) and test DNA was prepared from liver tissue of wild-type and PARP−/− mice, as well as from immortalized PARP−/− and PARP−/− (+PARP) fibroblasts according to standard protocols. Differences in the source of the DNA (spleen, liver, or cell lines) does not affect CGH results (26, 27). Normal metaphase chromosomes for CGH were prepared from a spleen culture of C57BL/6 mice as described (30). Labeling, hybridization, and detection of DNA were performed as described (30, 34). Normal DNA and test DNA were labeled in a nick-translation reaction in which dTTP was replaced by digoxigenin-11-dUTP (Boehringer Mannheim) (normal DNA) or biotin-16-dUTP (Boehringer Mannheim) (test DNA). A total of 500 ng each of labeled normal and test DNA was precipitated with ethanol in the presence of salmon sperm DNA (3 μg) and excess mouse Cot-1 DNA (50 μg) (GIBCO/BRL), and the precipitates were dried and resuspended in 15 μl of hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate). The DNA was denatured at 80°C for 10 min and allowed to preanneal for 3 h at 37°C. Normal metaphase chromosomes were denatured at 80°C for 2 min in 2× SSC containing 70% formamide and then were dehydrated through an ethanol series (70%, 90%, and 100%). The probe mixture was applied to the denatured metaphase chromosomes under a coverslip and sealed with rubber cement, and hybridization was performed for 4 days at 37°C. The biotin-labeled test DNA was visualized with FITC-conjugated avidin (Vector Laboratories), and the digoxigenin-labeled control DNA was detected with mouse anti-digoxigenin (Sigma) and tetramethylrhodamine isothiocyanate-conjugated goat antibodies to mouse IgG (Sigma). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and embedded in antifading agent.

Microscopy and Digital Image Analysis. Gray scale images of FITC-labeled test DNA, the tetramethylrhodamine isothiocyanate-labeled control DNA, and the 4′,6-diamidino-2-phenylindole (DAPI) counterstain from at least eight metaphase spreads for each hybridization were acquired with a cooled charge-coupled device camera (CH250; Photometrics, Tucson, AZ) that was connected to a Leica DMRB-E microscope equipped with fluorescence-specific optical filters TR1, TR2, and TR3 (Chroma Technology, Brattleboro, VT). Quantitative evaluation of hybridization was performed with a custom computer program developed for analysis of mouse chromosomes that was based on a human CGH program (30, 35). Average ratio profiles were computed as the mean value of at least eight ratio images. Fluorescence ratio was defined as the ratio of the total test (green) to the total control (red) fluorescence at each position along the length of each chromosome; chromosomal regions with a fluorescence ratio of ≥1.25 were interpreted as a gain, whereas regions with a ratio of ≤0.75 were interpreted as a loss.

PCR and Reverse Transcription-PCR (RT-PCR). Unique oligonucleotide primer pairs for human and mouse PARP, p53, and Rb-1 genes and mRNA were designed and prepared. Total RNA, purified from cell pellets or liver tissue with an RNA extraction kit (Amersham Pharmacia Biotech), was subjected to RT-PCR with a Perkin-Elmer Gene Amp EZ RTTh RNA PCR kit. The reaction mix (50 μl) contained 300 μM each of dGTP, dATP, dTTP, and dCTP, 0.45 μM of each primer, 1 μg of total RNA, and rTTh DNA polymerase (5 units). With an Ampliton II PCR machine (Thermolyne, Dubuque, IA), RNA was transcribed at 65°C for 40 min, and DNA was amplified by an initial incubation at 95°C for 2 min, followed by 40 cycles of 95°C for 1 min, 60°C for 1.5 min, and 65°C for 0.5 min, and a final extension at 70°C for 22 min. For PCR, genomic DNA was prepared according to standard protocols and amplified as above. The PCR products then were separated by electrophoresis in a 1.5% agarose gel and visualized by ethidium bromide staining.

Results

An Unstable Tetraploid Population in Immortalized PARP−/− Cells. One marker of genomic instability in cells is the development of tetraploidy or aneuploidy, which is typical of many tumors and is associated with progression to malignancy or metastasis (36). Tetraploidy results when cells exit from mitosis in the absence of either chromosome segregation or cytokinesis; such cells are genetically unstable and become aneuploid at subsequent mitoses (37). Flow cytometric analysis of immortalized fibroblasts derived from PARP knockout mice (clone A1) revealed the existence of a tetraploid population of cells (Fig. 1). After cell synchronization and release from either aphidicolin block at the G1-S transition or serum deprivation, DNA histograms of wild-type cells (clone A19) (Fig. 1A) showed a typical pattern characterized by two major peaks of nuclei at G0-G1 (haploid) and G2-M (diploid) phases of the cell cycle. In contrast, in addition to these two major peaks, DNA histograms of PARP−/− cells (clone A1) (Fig. 1B) showed a third peak corresponding to the G2-M peak of an unstable tetraploid cell population in these cells. Similar to those of wild-type cells, DNA histograms of PARP−/− cells stably transfected with PARP cDNA [PARP−/− (+PARP)] (clone A3–2) and synchronized by serum deprivation exhibited only the two major peaks of nuclei at
Aphidicolin-induced G1-S block (extracts of wild-type, but not PARP antibodies to PAR. PARP expression also was confirmed in tissue in wild-type and PARP thus, RT-PCR analysis detected mouse or human PARP transcripts
cytometric analysis. In addition to the two major peaks of nuclei at G0-G1 and
that normally would have been repaired.
G2-M peak of an unstable tetraploid cell population (arrows).

G0-G1 and G2-M (Fig. 1C). Thus, stable transfection of PARP−/− cells with PARP cDNA appeared to confer genomic stability to the PARP−/−(+PARP) cells. Loss of PARP may allow the emergence and survival of cells with gross genetic abnormalities that normally would have been repaired.

Lack of p53 Protein Caused by PARP Deficiency in Immortalized PARP−/− Cells; Partial Restoration of p53 Expression by Reintroduction of PARP cDNA. Inactivation or loss of the tumor suppressor protein p53 in diploid cells results in the formation of unstable tetraploid cells predisposed to chromosome segregation abnormalities (38). We therefore investigated whether development of the unstable population of tetraploid cells in immortalized PARP−/− fibroblasts might be associated with loss of p53 expression. Immunoblot analysis with antibodies to PAR confirmed the lack of immunoreactive PARP in immortalized PARP−/− cells and its presence in wild-type and PARP−/−(+PARP) cells (Fig. 2A). PARP−/− (+PARP) cells were stably transfected with human PARP cDNA; thus, RT-PCR analysis detected mouse or human PARP transcripts in wild-type and PARP−/−(+PARP) cells, respectively, but not in PARP−/− cells. Reconstitution of PARP activity in PARP−/− (+PARP) cells was further verified by immunoblot analysis with antibodies to PAR. PARP expression also was confirmed in tissue extracts of wild-type, but not PARP−/−, mice, by immunoblot analysis with anti-PAR; probing of the blot with anti-PAR revealed negligible poly(ADP-ribose)ylation of nuclear proteins in PARP−/− tissue extracts (data not shown).

p53 was detected in lysates of wild-type cells, but not in PARP−/− cell extracts, by immunoblot analysis with antibodies to p53 (PAb421) (Fig. 2B). Stable transfection with PARP cDNA partially restored p53 expression in the PARP−/−(+PARP) cells. Consistent with other studies (39), the decrease in p53 expression in PARP−/− cells was not attributable to lower p53 transcript levels or a decrease in copy number, as revealed by RT-PCR analysis of RNA and PCR analysis of genomic DNA from these cells. This finding suggests that the lack of p53 in PARP−/− cells may be the result of reduced protein stability and that PARP may be involved in p53 stabilization and accumulation. Because the loss of p53 allows the survival of cells with severe DNA damage, thus, promoting tetraploidy (40), down-regulation of p53 expression in PARP−/− cells may contribute, at least in part, to the genomic instability and the development of tetraploidy in these cells.

CGH Analysis of Chromosomal Aberrations Associated with PARP Deficiency. CGH was used in the present study to map chromosomal gains and losses associated with PARP depletion. CGH analysis of DNA from liver tissue of PARP−/− mice revealed partial gains in chromosome 4 (4C5-ter), chromosome 5 (5F-ter), and chromosome 14 (14A1-C2), as well as a deletion that mapped to chromosome 14 (14D3-ter) (Fig. 3B). In contrast, CGH analysis detected no chromosomal abnormalities in wild-type (PARP+/+) mice (Fig. 3A). These results indicate that the specific chromosomal changes detected in the PARP−/− mice are attributable to PARP deficiency.

To investigate the effects of reintroduction of PARP cDNA into PARP−/− cells, CGH analysis also was performed on genomic DNA from immortalized PARP−/− (clone A1) and PARP−/− (+PARP) (clone A3—2) fibroblasts that had been passaged for >10 generations. The partial chromosomal gains detected at 4C5-ter, 5F-ter, and 14A1-C2 in PARP−/− mice were also present in the immortalized PARP−/− fibroblasts (Fig. 4B). However, these gains were not detected in the average ratio profiles of genomic DNA from PARP−/− (+PARP) cells (Fig. 4C). Only the partial loss of chromosome 14 was retained in these cells. Additional chromosomal aberrations were detected by CGH in both the immortalized PARP−/− and PARP−/−(+PARP) cells, which are likely attributable to the immortalization process (data not shown).

Altered Expression of Tumor Suppressor Rb-1 and theJun Oncogene in PARP−/− Mice. Deletions or gains of chromosomal regions detected by CGH may indicate the site of genes that promote further genomic instability through loss of tumor suppressor

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genes or gains of oncogenes. It was therefore of interest to assess the expression of some key genes that map to regions of chromosomal gain or loss in the PARP knockout mice, although clearly many other genes could have been chosen. The region of chromosome 14 that is deleted in PARP knockout mice (14D3-ter) encompasses the tumor suppressor gene Rb-1 (Fig. 5A) along with numerous other genes. Interestingly, immunoblot analysis of tissue extracts with antibodies to Rb revealed a marked reduction in constitutive expression of Rb in PARP knockout mice relative to that in wild-type mice. Rb expression also was decreased in immortalized PARP knockout fibroblasts compared with wild-type fibroblasts (data not shown). Similarly, the glutamate dehydrogenase (GluD) gene, a neighboring gene that also maps to 14D3, exhibits reduced expression in the PARP knockout mice as shown by lower levels of the glutamate dehydrogenase protein in tissue extracts. In addition, the oncogene Jun is located (at 4CS-C7) in the region of chromosome 4 that exhibits a gain in PARP knockout mice and cells. Immunoblot analysis of tissue extracts with antibodies to Jun confirmed that Jun expression is increased in PARP knockout mice (Fig. 5B). In contrast, no difference in protein expression of the Pena and TopI genes was detected in wild-type and PARP knockout mice (Fig. 5C); these genes map to chromosome 2B-C and 2H, respectively, regions that show no gains or losses by CGH analysis.

A marked decrease in Rb transcript levels in PARP knockout mice, as revealed by RT-PCR analysis, correlates with decreased abundance of Rb protein in these animals (Fig. 5D). In contrast, p53 transcript levels were similar in wild-type and PARP knockout mice, in agreement with CGH results showing that the Rb gene, but not the p53 gene (located in chromosome 11B2-C), is in a deleted chromosomal region. PCR analysis of DNA from liver tissue further revealed that the Rb gene copy number also is reduced in PARP knockout mice compared with wild-type mice, whereas the p53 gene copy number is unchanged (Fig. 5D). Thus, the decreases in Rb protein and transcript levels in PARP knockout mice are consistent with the loss of the Rb gene.

Discussion

Although exhibiting varying phenotypes, two groups of PARP knockout mice developed by different laboratories both exhibit increased genomic instability as indicated by elevated frequencies of SCE and micronuclei formation after treatment with DNA-damaging agents, providing support for a role for PARP in the maintenance of genomic integrity (16, 17). We have now identified a population of tetraploid cells, another indication of genetic instability (37), among immortalized fibroblasts derived from PARP knockout mice. This tetraploid cell population was no longer apparent in PARP knockout cells, suggesting that the reintroduction of PARP into PARP knockout cells may have stabilized the genome and resulted in selection against this genomically unstable population.

CGH analysis revealed that PARP knockout mice and immortalized fibroblasts derived from these animals exhibit similar chromosomal aberrations, including gains in regions of chromosomes 4, 5, and 14. In contrast, the CGH profile of DNA from wild-type (PARP knockout) mice showed no DNA gains or losses, indicating that the chromosomal imbalances detected in the PARP knockout genome are caused by PARP deficiency. Interestingly, the chromosomal gains in the PARP knockout genome were no longer detected in the CGH profiles of DNA from PARP (+PARP) fibroblasts.
The loss of 14D3-ter that encompasses the tumor suppressor gene Rb-1 and presumably numerous other genes from the genome of PARP<sup>−/−</sup> mice was associated with a marked reduction in Rb protein, transcript, and gene copy number in these animals. Furthermore, increased expression of the oncogene Jun in the PARP<sup>−/−</sup> mice also was correlated with a gain in 4C5-ter that harbors the Jun oncogene. In contrast, there was no difference in expression of the Pcna and Top1 genes in wild-type and PARP<sup>−/−</sup> mice; these genes are considered unaffected by location within a region of chromosomal gain or loss. These results suggest that the gain or loss of large chromosomal regions, such as that encompassing Rb-1 and numerous other genes, is caused by PARP deletion and concomitant genomic instability in the PARP<sup>−/−</sup> mice.

The loss of tetraploidy and the chromosomal gains in the PARP<sup>−/−</sup> cells after stable transfection of PARP cDNA provides further support for an apparent essential role of PARP in the maintenance of genomic stability. One mechanism by which PARP may confer genetic stability is via its putative role in p53-mediated transcriptional activation of these genes (45). The location of a PAR attachment site adjacent to a proteolytic cleavage site in p53 further suggests that PAR may protect p53 from proteolysis (46); similar protection has been noted after binding of mAbs to Rb and glucosdehydrogenase (61 kDa), Jun (39 kDa), PCNA (36 kDa), and top I (100 kDa) are indicated.}

show reduced basal levels of p53 and defective p53 induction in response to DNA damage. Interestingly, the loss of the tetraploid population in the PARP<sup>−/−</sup> (+ PARP) cells further correlates with the partial restoration of p53 expression in these cells.

We recently showed that p53 is extensively poly(ADP-ribosyl)ated by PARP during early apoptosis and that degradation of the PARP attached to p53 coincides with expression of p53-responsive genes, suggesting that poly(ADP-ribosylation) may regulate p53-mediated transcriptional activation of these genes (45). The location of a PAR attachment site adjacent to a proteolytic cleavage site in p53 further suggests that PAR may protect p53 from proteolysis (46); similar protection has been noted after binding of mAbs adjacent to this region (47). The lack of regularly spliced wild-type p53 in PARP<sup>−/−</sup> cells also has been attributed to decreased protein stability, not lower levels of p53 mRNA (39). Consistently, RT-PCR and PCR analysis of RNA and DNA from immortalized wild-type and PARP<sup>−/−</sup> cells revealed that reduced expression of p53 in the PARP<sup>−/−</sup> cells was not attributable to lower levels of p53 transcripts or a decrease in p53 gene copy number. Modification of p53 by PARP therefore is implicated in p53 accumulation and stabilization (45, 46, 48), which may explain the apparent lack of p53 in PARP<sup>−/−</sup> cells. Lack of p53 in PARP<sup>−/−</sup> cells may promote further genomic alterations via different mechanisms, including abnormal centrosome amplification, which is associated with lack of wild-type p53 and also generates numerical chromosome aberrations (49).

p53 monitors genomic integrity and reduces the occurrence of mutations either by mediating cell cycle arrest in G<sub>1</sub> or G<sub>2</sub>-M or by inducing apoptosis in cells that have accumulated substantial DNA damage (50, 51). Increased expression of the p53 homolog

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p73 may compensate for the lack of wild-type p53 in immortalized PARP−/− cells (39). Consistently, the region of chromosome 4 (4C5-ter) that shows a gain in PARP−/− mice harbors the p73 gene. However, although p73, when overexpressed, can activate p53-responsive genes and induce apoptosis, it is unable to detect DNA lesions and, thus, is not induced by DNA damage (52). Both PARP activity and p53 accumulation are induced by DNA damage, and both proteins have been implicated as sensors of such damage. A functional association of PARP and p53 has been suggested by immunoprecipitation experiments (53). PARP cycles on and off the activity and p53 accumulation are induced by DNA damage, and its automodification during DNA repair in vitro facilitates access to DNA repair enzymes (54, 55). Thus, both the increased sensitivity of PARP−/− mice and cells to DNA-damaging agents (17, 25) and their genetic instability are consistent with their deficiencies in PARP and p53. Our results suggest that, in regard to the consequences of PARP deficiency in PARP−/− mice may be attributed, at least in part, to indirect effects resulting from changes in other DNA damage checkpoint proteins, such as p53.