Overexpression of human poly(ADP-ribose) polymerase in transfected hamster cells leads to increased poly(ADP-ribose)ylation and cellular sensitization to γ irradiation

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Poly(ADP-ribosylation) is a posttranslational modification of nuclear proteins catalyzed by poly(ADP-ribose) polymerase (PARP), an enzyme which uses NAD⁺ as substrate. Binding of PARP to DNA single-strand or double-strand breaks leads to enzyme activation. Inhibition of poly(ADP-ribose) formation impairs the cellular recovery from DNA damage. Here we describe stable transfectants of the Chinese hamster cell line CO60 that constitutively overexpress human PARP (COCF clones). Immunofluorescence analysis of γ-irradiation-stimulated poly(ADP-ribose) synthesis revealed consistently larger fractions of cells positive for this polymer in the COCF clones than in control clones, which failed to express human PARP. HPLC-based quantitative determination of in vivo levels of poly(ADP-ribose) confirmed this result and revealed that the basal polymer levels of undamaged cells were significantly higher in the COCF clones. The COCF clones were sensitized to the cytotoxic effects of γ irradiation compared with control transfectants and parental cells. This effect could not be explained by depletion of cellular NAD⁺ or ATP pools. Together with the well-known cellular sensitization by inhibition of poly(ADP-ribose)ylation, our data lead us to hypothesize that an optimal level of cellular poly(ADP-ribose) accumulation exists for the cellular recovery from DNA damage.

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MATERIALS AND METHODS

Antibodies and plasmids. Rabbit anti-FII serum raised against the second zinc finger of PARP (Simonin et al., 1991) and rabbit antiserum Lima IV, recognizing the well-conserved PARP NAD⁺-binding domain, were kindly provided by G. de Murcia, Strasbourg, France. Monoclonal antibody F23 recognizes the same epitope on the second zinc finger of human PARP as C9 described previously (Lamarre et al., 1988). Mouse monoclonal antibody 10H raised against poly(ADP-ribose) (Kawamitsu et al., 1984) was kindly provided by M. Miwa and T.
Sugimura, Tokyo, Japan. Phosphatase-conjugated goat anti-rabbit Ig and anti-mouse Ig were from Sigma. Cy3-conjugated donkey anti-rabbit Ig were from Dianova. Fluorescein-isothiocyanate-conjugated goat anti-mouse Ig were from Renner. Plasmid Lavi, Tel Aviv, Israel, and was maintained as described (Burkle et al., 1987). Stable transfectants of C060 cells were grown in medium supplemented with 800 μg/ml geneticin (GIBCO) and 10% fetal calf serum. All cell lines were grown in the absence of selection antibiotics for at least 2 days before any experiments were carried out.

**Stable transfection of cells.** For the generation of COCF and COCFN cell lines, exponentially growing C060 cells were transfected with 18 μg pPARP31 and 2 μg pTKneo, by means of the calcium phosphate method. Clones resistant to 800 μg/ml geneticin were analyzed by immunofluorescence and western blotting.

**Immunofluorescence.** To analyze poly(ADP-ribose) formation, cells grown on coverslips were subjected to γ irradiation (100 Gy; Cell 1000; dose rate, 1.4 Gy/min; Atomic Energy of Canada Limited) in NaCl/P, (137 mM sodium chloride, 2.7 mM potassium chloride, 1.5 mM potassium dihydrogenphosphate, 8.1 mM disodium hydrogenphosphate; 1% calcium chloride at room temperature. 10 min after the start of irradiation, cells were fixed with 10% ice-cold trichloroacetic acid and processed for immunofluorescence as described previously (Kupper et al., 1995). By means of immunofluorescence analysis, we had found that cellular poly(ADP-ribose) levels are peaking at this time point under our conditions. Monoclonal antibody 10H, which recognizes poly(ADP-ribose), was used as the first antibody. Secondary antibodies were conjugated to phosphatase isothiocyanate.

**Western blot.** Crude protein extracts were prepared, separated electrophoretically and blotted as described previously (Kiippel et al., 1995). Blots were incubated overnight at 4°C with anti-FII serum diluted 1:2000 in NaCl/P, 0.3% Tween-20, 5% dry milk, or with hybridoma supernatant of F23 diluted 1:200 in NaCl/P, 0.3% Tween-20, 5% dry milk. Blots were developed with alkaline-phosphatase-conjugated goat anti-rabbit immunoglobulins.

**Quantitation of poly(ADP-ribose) level.** This procedure was carried out as described previously (Jacobson et al., 1984). Poly(ADP-ribose) was purified from trichloroacetic-acid-insoluble cell extracts by means of boronate chromatography and was enzymatically digested to yield the nucleosides adenosine, ribosyladenosine and diribosyladenosine. Nucleosides were derivatized into fluorescent etheno compounds by chloroacetaldehyde incubation, followed by PBA-Heptate chromatography and reversed-phase HPLC (Varian) coupled with fluorometric detection (Varian). Quantitation was carried out with etheno nucleoside standards.

**Quantitation of the cellular content of NAD⁺ and ATP.** Cells grown on petri dishes were γ irradiated (14 Gy) in NaCl/P, plus 1 mM calcium chloride at room temperature and thereafter precipitated at various times with 0.5 M perchloric acid. NAD⁺ content was measured with a NAD⁺ cycling assay (Jacobson and Jacobson, 1976). For determination of ATP contents, cells were lysed at different times with 8 M guanidine hydrochloride, and ATP bioluminescence assays were performed (Lundin et al., 1976).

**Cell survival.** Irradiation of cell monolayers and determination of cell survival was performed as described previously (Kupper et al., 1995). Exponentially growing cell monolayers were subjected to γ irradiation at a dose rate of 14Gy/min in NaCl/P, at room temperature. After irradiation, NaCl/P, was replaced with medium, and cells were incubated for 16 h under routine cell-culture conditions. Cells were trypsinized, and appropriate dilutions of cells were plated onto 10-cm petri dishes. Eight days later, colonies were fixed with 10% formaldehyde in NaCl/P, and stained with 0.1% crystal violet. The number of colonies consisting of more than 50 cells was determined.

**RESULTS**

**Generation of stably transfected cell lines.** To obtain cell lines stably overexpressing human PARP, plasmid pPARP31, which carries the human PARP cDNA under the transcriptional control of the human cytomegalovirus immediate early promotor/enhancer, was transfected with a plasmid conferring resistance to geneticin into C060 hamster cells. Geneticin-resistant clones were screened by immunofluorescence and western blotting. The western blot (Fig. 1A) was developed with a polyclonal antiserum raised against the second zinc finger of human PARP (anti-FII; Simonin et al., 1991), which cross-reacts with PARP molecules from various mammalian species including hamster. In three of the clones obtained, which were designated COCF1, 2 and 4, overexpression of the 113-kDa human PARP is evident. Densitometric analysis of this western blot revealed that the level of PARP was about sevenfold that of the parental cell line.
CO60, while fivefold overexpression of human PARP in COCF clones was detected in an equivalent western blot that was probed with polyclonal rabbit antiserum Lim4 raised against the highly conserved NAD\(^+-\)binding domain of human PARP (data not shown). The latter antiserum was shown to recognize PARP of all mammalian species tested (Grube and Burkle, 1992). By means of monoclonal antibody F23, which recognizes human but not hamster PARP, several geneticin-resistant clones were identified that failed to express human PARP (Fig. 1B). Three of these clones, called COCFN1, 2 and 4, served with parental CO60 cells as negative controls for further experiments.

Southern blot analysis revealed different integration patterns of transfected plasmid pPARP31 in the three COCFN clones on the one hand, and in COCF1 and COCF2 on the other hand, while COCF2 and COCF4 had the same integration pattern (data not shown). The latter finding was confirmed by fluorescence in-situ hybridization on chromosomes (data not shown). However, these two, clones which were isolated from equivalent petri dishes derived from the same transfection, behaved differently with respect to cell morphology and proliferation rate. The cell-doubling time of COCF2 was 23 h, while it is only 12 h for COCF4. COCFN4 and COCF1 cannot be distinguished by Southern blot analysis, but differ with respect to human PARP expression (COCFN4 is negative, COCF1 positive), cell-doubling time (11.5 h for COCFN4 and 16 h for COCF1), and with respect to their sensitivity to \( \gamma \) irradiation.

**Analysis of poly(ADP-ribose) formation.** By means of immunofluorescence analysis, overexpression of human PARP was detected in almost 100\% of the COCF4 cell population, while resident hamster PARP in COCFN4 cells was not detected under these conditions (data not shown). The effect of this PARP overexpression on cellular poly(ADP-ribosylation) was investigated by immunofluorescence (Burkle et al., 1993) and by HPLC analysis (Jacobson et al., 1984). For immunofluorescence analysis, cells growing on coverslips were subjected to increasing doses of \( \gamma \) irradiation to stimulate PARP activity through the formation of DNA strand breaks. In the range of doses studied (11.5–42 Gy), poly(ADP-ribose)-specific immunostaining of cell nuclei was consistently stronger in the human-PARP-positive clones than in the negative ones. Fig. 2 shows representative experiments carried out with COCF4 cells and COCFN4 control cells, which were chosen because of their nearly identical proliferation rates (see above). In unirradiated cells of any of the clones, no nuclear poly(ADP-ribose) staining was detectable, confirming our earlier results obtained with different C060 transfectants (Kupper et al., 1995).

To quantitate the poly(ADP-ribose) levels, cells growing on petri dishes were treated with 14 Gy \( \gamma \) irradiation. Poly(ADP-ribose) was purified from cells by means of boronate chromatography and subjected to HPLC analysis as described in Materials and Methods. In Fig. 3 the results obtained with the clones COCF4 and COCFN4 are depicted. In clone COCF4 the basal levels of poly(ADP-ribose) are 2.6-fold higher than in the control clone COCFN4 (4 pmol/10\(^7\) cells in COCF4 versus 1.5 pmol/10\(^7\) cells in COCFN4). Likewise, treatment of the cells
Cells are given. The human-PARP-overexpressing clone shows enhanced radiosensitivity in COCF clones, survival assays were performed after 16 h in growth medium. Cells were plated in quadruplicate for the determination of cell survival. Mean values of the surviving fractions from at least three independent experiments with each clone and standard deviations are depicted. (B) Combined survival curves for COCF1, -2 and -4 (filled circles) or COCFN1, -2 and -4 cells (open circles). Surviving fractions were determined as described in (A). Mean values of the surviving fractions from at least three independent experiments done with each clone were used to calculate the combined survival curves for COCF and COCFN clones. Standard deviations are indicated.

![Graph A](image1)

**Fig. 4.** Survival of COCF and COCFN clones after irradiation. (A) Exponentially growing COCF4 (filled circles) and COCFN4 cells (open circles) were γ irradiated in NaCl/P₃ as indicated. After incubation for 16 h in growth medium, cells were plated in quadruplicate for the determination of cell survival. Mean values of the surviving fractions from at least three independent experiments with each clone and standard deviations are depicted. (B) Combined survival curves for COCF1, -2 and -4 (filled circles) or COCFN1, -2 and -4 cells (open circles). Surviving fractions were determined as described in (A). Mean values of the surviving fractions from at least three independent experiments done with each clone were used to calculate the combined survival curves for COCF and COCFN clones. Standard deviations are indicated.

with 14 Gy irradiation resulted in 2.3-fold higher peak levels of poly(ADP-ribose) in COCF4 cells (37.6 pmol/10⁷ cells) than in COCFN4 cells (15.9 pmol/10⁷ cells).

**Consequences of human PARP overexpression on cell survival.** Poly(ADP-ribosylation) has been postulated to play a role in several cellular responses to DNA damage (reviewed by Alt-Carin and Richter, 1987; de Murcia and Menissier de Murcia, 1994; Lindahl et al., 1995). To study possible biological effects of human PARP overexpression and increased poly(ADP-ribose) formation in COCF clones, survival assays were performed after γ irradiation. In Fig. 4A, survival data for COCF4 and COCFN4 cells are given. The human-PARP-overexpressing clone shows reduced survival after γ irradiation compared with the negative clone. Fig. 4B shows combined survival data for all the COCF and COCFN clones. The survival curve of the parental cell line CO60 (not depicted in Fig. 4) coincides with the COCFN4 curve. The figure shows that radiosensitization is a general feature of the PARP-overexpressing (COCF) clones. The dose modification factor at 10% survival (LD₁₀₀) was 1.3.

**NAD⁺ and ATP contents after γ irradiation.** According to the cell-suicide hypothesis, the poly(ADP-ribosylation) of cells subjected to excessive DNA damage can cause NAD⁺, and consequently, ATP depletion and energy starvation (Berger and Berger, 1986). To analyse whether the impaired cell survival observed in the COCF clones could be due to such a mechanism, we studied cellular NAD⁺ and ATP contents at different times after γ irradiation. For these experiments, we chose the highest irradiation dose used in the survival assays (14 Gy). Severe decreases of the cellular NAD⁺ content were not observed in COCF4 (−35%) or in COCFN4 (−20%) (Fig. 5). Since there were no detectable decreases of ATP levels (Fig. 5), the impaired cell survival in the COCF transfectants exhibiting increased poly(ADP-ribosylation) cannot be explained by energy starvation.

**DISCUSSION**

We stably transfected a human-PARP-cDNA expression construct into the Chinese hamster cell line CO60 to see whether this could be a strategy to increase poly(ADP-ribosylation) in living cells above normal levels. After two subcloning procedures, we established transfectants, called COCF1, 2 and 4, that displayed constitutive overexpression of human PARP in almost 100% of the cells. Geneticin-resistant clones without detectable expression of human PARP were chosen as negative controls (COCFN1, 2 and 4). Immunofluorescence analysis of poly(ADP-ribose) formation in γ-irradiated cells revealed that the fraction of cells positive for this polymer was consistently higher in the COCF clones (Fig. 2). This was confirmed by quantitation of in vivo levels of poly(ADP-ribose) with an HILPC-based detection method, yielding higher poly(ADP-ribose) levels both with and without γ irradiation in COCF4 cells compared with COCFN4 control cells (Fig. 3). The result of increased poly(ADP-ribose) levels in irradiated cells is remarkable in that PARP is an abundantly expressed housekeeping protein, and the level of poly(ADP-ribosylation) has been thought to depend on the number of DNA breaks formed after carcinogen treatment rather than on the number of enzyme molecules available. We are currently investigating whether this increased polymer accumulation is caused by the mere overexpression of PARP or is due to qualitative differences between endogenous (hamster) and exogenous (human) PARP.
The catalytic function of PARP was attributed to a dimerized form (Mendoza Alvarez and Alvarez Gonzalez, 1993; Panizeter and Althaus, 1994). That we observed increased poly(ADP-ribose)ylation in the COCF clones did not necessarily prove that plasmid pPARP31, which was used in the transfection, codes for a catalytically functional enzyme, since a catalytically inactive human PARP molecule heterodimerized to a hirnster PARP molecule could serve as an acceptor for the polymer. However, sequence analysis of the PARP cDNA insert of pPARP31 revealed only two amino acid substitutions in comparison with one of the three sequences published (Uchida et al., 1987). First, at codon 70 we found Glu instead of Gln, the same exchange being present in PARP cDNA sequences published by two other groups (Cherneny et al., 1987; Kurosaki et al., 1987). Second, at codon 762 we found Ala instead of Val, which is present in all the published sequences (Cherneny et al., 1987; Kurosaki et al., 1987; Uchida et al., 1987). However, we detected Ala at this position in several independent NM1149 phages of the primary cDNA library of human embryonic fibroblasts that we screened (Küpper and Bürkle, 1992), and in another human PARP cDNA clone (kindly provided by G. de Murcia), which had been isolated independently and proved to be functional (Giner et al., 1992). Thus, this difference probably represents a sequence polymorphism. To definitely prove that plasmid pPARP31 encodes a catalytically active human PARP, we subcloned the cDNA insert into a yeast expression vector and transformed strain W303-1B of S. cerevisiae, which is considered to be devoid of poly(ADP-ribose)ylation. In agreement with an earlier report (Kaiser et al., 1992), transformation of yeast resulted in poly(ADP-ribose) formation, which could be detected by immunofluorescence (data not shown). Moreover, we confirmed the results of Kaiser et al. showing that human PARP expression caused growth retardation in yeast, which could be reversed by adding 2.5 mM of the ADP-ribosylation inhibitor 3-methoxybenzamide to the culture medium (data not shown).

To study influences of PARP overexpression on the cellular response(s) after DNA damage, we performed survival assays on our transfectants after treatment with γ irradiation. Surprisingly, the COCF clones displayed increased radioresistance compared with the COCFN clones (Fig. 4). The same result was obtained when the clones were treated with the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (data not shown). Since excessive PARP activation can lead to NAD+ depletion and consequently to cell death (Berger and Berger, 1986; Heller et al., 1995; Zhang et al., 1994), we analyzed NAD+ and ATP consumption at the highest γ dose (14 Gy) applied for the survival assays. Our results show that neither NAD+ nor ATP depletion was the reason for the increased sensitivity in the COCF clones. Recently, data on a similar cell system were reported. CHO-9 Chinese hamster cells and a mutagen-hypersensitive derivative thereof were stably transfected to express human PARP (Fritz et al., 1994). These transfecteds displayed reduced sensitivity towards the alkylating agent methyl methanesulfonate compared with the respective parental cell line. However, since only one transfectant from each cell line was investigated, and since survival was compared with the parental cell line rather than with control clones, it is hard to exclude that the observed effects were caused by clonal variation or by the influence of selection. A strong argument that clonal variation is not the reason for the observed difference in radioresistance between our COCF (expressing) and COCFN (non-expressing) clones is that COCFN4 and COCF1 are of identical clonal origin, as judged from the integration pattern of the human PARP cDNA construct (data not shown), but markedly differ in their sensitivity to DNA damage. Furthermore, we recently characterized several stable transfecants of a rat cell line overexpressing human PARP cDNA from pPARP31. None of the clones that displayed increased poly(ADP-ribosyl)ation upon γ irradiation or treatment with the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine was protected against the inflicted DNA damage (Berges, F., Bürlke, A., Küpper, J.-H. and Zeller, W., unpublished results). A report of Schizosaccharomyces pombe transfectants expressing human PARP showed sensitization to γ irradiation compared with untransformed fission yeast, an organism which seems not to possess a poly(ADP-ribose)ylation pathway (Avila et al., 1994).

We and others have recently shown that trans-dominant inhibition of poly(ADP-ribosyl)ation is associated with sensitization to DNA damage (Küpper et al., 1995; Schreiber et al., 1995). Together with the results of this report, we hypothesize that the level of poly(ADP-ribose) accumulation upon DNA damage is strictly controlled, and any manipulation of polymer steady-state levels interferes with the cellular recovery from DNA damage. An alternative but less likely hypothesis is that there are species-specific differences in the complexity of poly(ADP-ribose). According to the protein-shuttle model proposed by Althaus, poly(ADP-ribose) has a role in the transient removal of histones from DNA to facilitate DNA repair (Althaus, 1992). It is conceivable that the polymer synthesized by human PARP is less suited to interact with hamster histones.

The study of further biochemical and biological consequences of higher-than-normal poly(ADP-ribose) levels in living cells should contribute to a better understanding of the cellular function(s) of poly(ADP-ribose)ylation.

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REFERENCES


