Transcriptional Down-regulation of Poly(ADP-ribose) Polymerase Gene Expression by E1A Binding to pRb Proteins Protects Murine Keratinocytes from Radiation-induced Apoptosis*

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Adenovirus E1A confers enhanced cell sensitivity to radiation and drug-induced DNA damage by a mechanism involving the binding to cellular proteins. Mutant analysis in E1A-transfected murine keratinocytes demonstrates that increased sensitivity to DNA damage requires at least E1A binding to the p300/CREB-binding protein (CBP) transcriptional coactivators and to pRb family members, indicating that this biological activity of E1A is the result of the concomitant perturbation of different cell pathways. Here we show that in the same cells E1A binding to members of the retinoblastoma protein family induces transcriptional down-regulation of the poly(ADP-ribose) polymerase (PARP) gene, coding for a NAD-dependent enzyme stimulated by DNA breaks. Inhibition of PARP expression is accompanied by a decrement of γ-irradiation-induced apoptosis, which is overridden by reconstitution of wild type levels of PARP. Hence, E1A effects on PARP transcription are a central determinant of the apoptotic sensitivity of E1A-expressing keratinocytes. Conversely, E1A binding to only p300/CBP results in an increase in PARP enzyme activity and consequently in cell death susceptibility to irradiation, which is effectively counteracted by the PARP chemical inhibitor 3-aminobenzamide. Therefore, our results identify in the E1A-mediated effects on PARP expression and activity a key molecular event involved in E1A-induced cell sensitization to genotoxic stress.

Adenovirus E1A proteins stimulate entry into S-phase of the infected cells and, in combination with E1B proteins, induce transformation. This effect is probably the consequence of perturbation of a variety of cellular basal programs, like proliferation, differentiation, and programmed cell death (1). A well characterized effect of transfected E1A alone is a marked increase in cell sensitivity to DNA damaging agents (ionizing radiation and DNA-targeted chemotherapeutic compounds such as doxorubicin and 5-fluorouracil) in epithelial tumor (2, 3) and immortalized cells (4, 5), as well as in primary embryonic fibroblasts (6, 7). At the doses employed in the cited studies, the morphology of cell death induced by these agents is that of apoptosis, which under conditions of serum deprivation and high cell density could be elicited per se by E1A transfection, without any additional stimulus (8). Therefore, it is likely that the basis of E1A-induced enhanced sensitivity to DNA damage relies at least partially in the lowering of the apoptotic threshold, by acting at a distal step in the programmed cell death pathway. E1A encodes two mRNA species, named 13S and 12S, which give respectively a 289- and a 243-amino acid protein, identical apart from an internal stretch of 46 amino acids. E1A proteins present two conserved regions (CR1 and CR2), which are known from mutational studies to be responsible for the induction of transformation and to be the site of interaction with several identified cellular proteins (reviewed in Ref. 9). These proteins are p107, p130, and p105Rb, belonging to the pRb tumor suppressor gene family and which interact primarily with CR2 with a weak cooperativity of CR1, and p300 and CREB-binding protein (CBP) of the p300/CBP family of transcriptional coactivators, which require a portion of CR1 and the N terminus for binding (10). Since these proteins have key regulatory roles in different cell pathways, the perturbations induced by their association with E1A are thought to be responsible for the complex biological activity of E1A (11). Nevertheless, the mechanism leading to E1A-induced cell sensitization and the exact role in this process of the bound cellular proteins are almost totally unclear.

This study was designed with the aim of finding downstream substrates for the action of E1A that could be involved in its sensitization to ionizing radiation-induced apoptosis. We used an established in vitro model of spontaneously immortalized murine keratinocytes, stably transfected with full-length E1A 13S or with E1A 13S deletion mutants unable to bind either p300 (mutant NdI598) or p105Rb (mutant NdI222/224) family members (5). In these cells, we demonstrate that E1A down-regulates the expression of PARP (EC 2.4.2.30), an abundant nuclear enzyme endowed with DNA damage-triggered ADP-ribosylating activity and involved in the late apoptotic cascade as a substrate/effector protein (12). By testing the behavior of the two mutant E1A-expressing cell lines, we also show that this effect is mediated by E1A binding to pRb proteins and is accompanied by apoptosis resistance to γ-rays, while E1A binding to the transcriptional integrators p300/CBP enhances PARP activity and increases irradiation-induced cell death.

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1 The abbreviations used are: CREB, cAMP-responsive element-binding protein; CBP, CREB-binding protein; 3-AB, 3-aminobenzamide; FL E1A, full-length E1A; PARP, poly(ADP-ribose) polymerase; DMEM, Dulbecco’s modified Eagle’s medium; Gy, gray; PBS, phosphate-buffered saline; BSA, bovine serum albumin; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pair(s); CAT, chloramphenicol acetyltransferase.
Experimental Procedures

Cells, Cell Cultures, and Treatments—Mice Pam212 cells are a spontaneously immortalized keratinocyte line (13), while Pam212-deleted transgenic cell lines carrying full-length E1A, and the NTdl598 and NTdl929/947 E1A mutants were obtained by lipofection with retroviral constructs (MD-E1a 13S, MD-E1a NTdl598, MD-E1a NTdl929/947, respectively) carrying the G418 resistance gene (14). The E1A mutants used in this study have been previously described (15, 16). Mutant NTdl598 (amino acids 2–13 deleted) binds only to members of the pRb family, p105 and p107 at least, while mutant NTdl929/947 (amino acids 122–129 deleted) binds only to p300. All transgenic cell lines expressed similar levels of E1A, as previously assessed by Western blot and immunoprecipitation (14). All cells were grown in DMEM supplemented with 10% FCS (Life Technologies), 50 units/ml penicillin, 100 units/ml streptomycin (Sigma, Milan, Italy), 1 mg/ml G418 (Life Technologies, Inc.) for the transgenic cells, and incubated at 37 °C in a 5% CO2 atmosphere. Cells were γ-irradiated with a 60Co source at 57-cm distance and at a dose rate of 1.55 Gy/min. In all experiments cells were plated in 90-mm Petri dishes 24 h before irradiation, and the time of irradiation was taken as the zero time. For irradiation in the presence of the PARP inhibitor 3-AB, cells were incubated with 5 mM 3-AB for 2 h before irradiation and subjected to time-lapse videomicroscopy scoring of cell death.

Cell Viability and Long Term Survival—Cellular viability was assessed by the crystal violet method. Briefly, cells were plated in 24-well plates (2 × 104/well), fixed at the indicated time with 1% glutaraldehyde for 10 min, washed twice with PBS, and stained by 1.5 ml of 0.1% crystal violet solution for 30 min. Wells were rinsed with distilled water units and air-dried. The mixture was then dissolved in the solubilization buffer. The absorbance was read at 590 nm after taking the dye up in 10% acetic acid. The long term survival was determined by a clonogenic survival assay as described previously (17).

Cell Extracts and Western Blotting—Cells were plated 24 h before treatment, and the determination of the cell number was done by classical trypanoscopy procedure; for protein extraction, cells were scraped in cold PBS (pH 7.4), spun, and resuspended in lysis buffer (62.5 mM Tris (pH 6.8), 6 mM urea, 10% glycerol, 2% SDS, 0.003% bromphenol blue, 5% 2-mercaptoethanol). Cells were then lysed by sonication on ice for 20 s (micritops at limit, 40% duty cycle, Sonicator Vibracell, Sonics and Materials, Dunbury, CT) and incubated 15 min at 65 °C before loading.

Activity Gel—The PARP automodification activity was detected on the gel using the incorporation of [32P]NAD into [32P]poly(ADP-ribose) as described (19). The extracts prepared from control and treated cells were electrophoresed in a 7.5% polyacrylamide gel. Thereafter the gel was soaked at room temperature with shaking for 30 min in renaturation buffer (50 mM Tris-HCl (pH 8.0), 3 mM 2-mercaptoethanol), for 30 min in renaturation buffer containing 6 mM guanidine hydrochloride, and finally for 30 min in renaturation buffer. The enzymatic reaction was carried out for 1 h at a 37 °C in 3 ml of 100 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 1 mM NAD, 1 mM dithiothreitol, and 5 μCi/ml [32P]NAD (800 Ci/mM, NEN Life Science Products). The incorporated [32P]poly(ADP-ribose) was moved by washes with cold 10% trichloroacetic acid followed by washes with cold 1 M HCl. The gel was dried and subjected to autoradiography using Kodak X-Omat AR5 films. Alternatively radioactive bands were detected and quantified with a PhosphorImager 445 SI (Molecular Dynamics, Sunnyvale, CA).

Time-lapse Videomicroscopy—Cells were plated in 25-cm2 flasks and incubated in 5% CO2 atmosphere under a time-lapse videomicroscopy apparatus (20). Briefly, randomly chosen fields of 100 cells were monitored at a rate of one frame every 3 min for 72 h. Cells were considered to undergo apoptotic cell death when exhibiting membrane blebbing, shrinkage, and fragmentation in apoptotic bodies, followed in some cases by membrane disruption and a morphology of secondary necrosis. The scoring was carried out manually marking the dying cells on a transparency attached to the time-lapse monitor. Results are expressed as either the cumulative number of apoptotic events at the final time point or plotted against time with scoring every 5 h. For experiments involving transient expression of the pCNDNA3-mPARP vector cells were plated in 24-well plates, transfected as reported, and incubated in a 5% CO2 chamber under the time-lapse videomicroscopy apparatus (20).

Involvement of PARP in E1A Effects on Apoptosis

Plasmid Constructs—Murine genomic DNA extracted from Pam212 cells by the DNeasy Tissue Kit (Qiagen) was PCR-amplified following standard procedures with the primers 5'-GGGAATTTCTTTCCGAGCGCA-3' (upstream, from position 328 of GenBank accession number M14553) and 5'-AAATCTCCTGTCCTTGCATTCT-3' (downstream, from 741 to 760), which give a 451-bp product spanning a portion of the murine PARP 5' regulatory region highly homologous to the previously characterized rat PARP promoter from −237 to +13, see Ref. 39. The resulting amplification product was cloned in the pCRII vector, sequenced, digested with SstI and XhoI, and subcloned into the pCAT-Basic vector (Promega, Madison, WI) in a NotI site and subsequently used for the normalization of the pCAT-Basic vectors. The pCAT3-Basic vector (Promega, Madison, WI) was used as a loading control. Cells were plated in 24-well plates, transfected as reported, and incubated in a 5% CO2 chamber under the time-lapse videomicroscopy apparatus.

Transparent Transfection and CAT Assay—For CAT assay in the wild type and E1A Pam212 keratinocytes the plasmids pCAT3-B-mPARP and pCAT3-Control were used, the latter containing SV40 promoter and enhancer sequences and being a standard for comparison of CAT activities in different cell lines. Transparent transfection of these CAT reporter plasmids and of the pCNDNA3-mPARP expression vector was carried out using a lipofection procedure. Cells were plated in 24-well plates at 8 × 104 cells/well, being 70% confluent 24 h after. For each well of cells 0.4 μl of plasmid DNA and 2 μl of LipofectAMINE 2000 Reagent (Life Technologies, Inc.) were diluted separately in 50 μl of cell culture medium and incubated at room temperature for 10 min. The diluted DNA and cationic lipid were combined and incubated further for 20 min. The growth medium was removed from...
cells and 0.5 ml of DMEM added, then the plasmid-lipid complexes (100 μl) were directly added to each well. Cells were incubated for 5 h, the DMEM replaced with complete medium and further incubated for additional 24 h. CAT activity was then monitored by liquid scintillation counting of CAT reaction products. 48 h post-transfection cell extracts obtained with 100 μl of xylene were incubated in a reaction mix containing 3H-labeled chloramphenicol and n-butylryl coenzyme A. The n-butylryl chloramphenicol partitions mainly into the xylene phase, while unmodified chloramphenicol remains predominantly in the aqueous phase (22). The xylene phase was mixed with scintillant and counted in a scintillation counter. The ratio of the counts obtained for each cell line with pCAT3-B-mPARP and pCAT3-Control, normalized to the protein content of the sample determined by the BCA Protein assay (Pierce), gave the level of activity of the PARP murine promoter. For normalization of the transfection efficiency of the pCDNA3-mPARP vector, an aliquot of the transfected cells was subjected to activity gel determination of PARP as described previously, and only those samples giving a PARP activity comparable with the wild type Pam212 keratinocytes (80%) were subjected to the time-lapse analysis.

**RESULTS**

**Short and Long Term Survival of Wild Type and E1A-transformed Keratinocytes Exposed to γ-irradiation**—We assessed the response to 5 Gy of γ-irradiation of a panel of murine Pam212 keratinocytes stably expressing full-length (FL) and containing 3H-labeled chloramphenicol and n-butylryl coenzyme A. The n-butylryl chloramphenicol partitions mainly into the xylene phase, while unmodified chloramphenicol remains predominantly in the aqueous phase (22). The xylene phase was mixed with scintillant and counted in a scintillation counter. The ratio of the counts obtained for each cell line with pCAT3-B-mPARP and pCAT3-Control, normalized to the protein content of the sample determined by the BCA Protein assay (Pierce), gave the level of activity of the PARP murine promoter. For normalization of the transfection efficiency of the pCDNA3-mPARP vector, an aliquot of the transfected cells was subjected to activity gel determination of PARP as described previously, and only those samples giving a PARP activity comparable with the wild type Pam212 keratinocytes (80%) were subjected to the time-lapse analysis.

**Effects of E1A Binding to p300 and pRb Family Members on PARP Expression and Activity**—E1A binding to its cellular targets provokes perturbation of unknown pathways that could be implicated in the cell sensitivity to genotoxic stress. Since one of the determinants of cell radiosensitivity is the PARP nuclear enzyme, we wanted to verify if the activity of this protein could be implicated in the modulation of cell response to γ-ray by E1A, due to E1A binding to the p300/CBP transcriptional coactivator, to pRb proteins, or both. Fig. 2 reports the level of PARP activity in basal conditions and after 5 Gy of γ-irradiation (assessed by the activity gel method) and the level of PARP expression (assessed by Western blotting and semi-quantitative RT-PCR) for the wild type and the E1A-expressing Pam212 keratinocytes. The binding of E1A to only pRb proteins (NTdl598 mutant) induced a reduction of PARP enzymatic activity paralleled by a decrease in PARP protein and mRNA content to values under the basal physiologic values for these cells. On the contrary, the mutant NTdl992/947, 947, able to bind only p300/CBP, did not affect PARP expression but induced a marked increment of PARP activity, quantified as about three times the basal wild type levels (data not shown). The binding of E1A to both sets of proteins (FL E1A) resulted in a decrease of PARP expression comparable with that induced by the NTdl598 mutant, but the level of enzyme activity was the same as in untransfected keratinocytes. The pattern of PARP activity in the cell panel obtained after γ-irradiation was substantially analogous to that of the untreated cells, showing only, as expected, a generalized slight increment of signal attributable to DNA damage-induced PARP overactivation, since PARP protein levels remained unchanged (not shown). Therefore, E1A affects PARP activity differently by binding pRb proteins or p300/CBP, the first effect being a down-regulation of PARP expression at the mRNA and protein level and the second an opposite action of increment of PARP activity without any change in gene expression. The overall effect of FL E1A on PARP appears to be the combinative result of the effects induced by the two mutants, that is a down-regulation functionally compensated by an increased enzymatic activity.

**Transcriptional Activity of the Mouse PARP Promoter in Wild Type and E1A-transformed Keratinocytes**—The down-regulation of PARP mRNA steady state levels in cells expressing E1A proteins able to bind pRb (NTdl508 and FL E1A, Fig. 2C) prompted us to investigate if this effect could be due to lowering of the PARP transcriptional activity. Since the regulatory sequences in the rat PARP proximal promoter have been identi-
Involvement of PARP in E1A Effects on Apoptosis

**FIG. 2.** E1A affects PARP expression and activity differently by binding pRb proteins or p300/CBP. Equal amounts of extracts from each cell line were subjected to activity gel with (upper panel) and without (lower panel) previous exposure to 5 Gy of γ-irradiation (the γ-ray-treated extracts were prepared 4 h from irradiation) (A) and to Western blot analysis (B), to evaluate PARP activity and expression levels. Both Western blotting and activity gel analysis were carried out in cell extracts obtained as described under “Experimental Procedures.” A representative experiment is reported. (C), graphic representation of PARP mRNA levels evaluated by an internal standard-based (β-actin) semiquantitative RT-PCR technique (see “Experimental Procedures” for details). Amplification products were run on an agarose gel and the ethidium bromide-stained bands were quantitated by densitometric analysis. Each histogram represents the mean ± S.E. of three to four values of PARP amplification products normalized to the starting cDNA volumes and referred to the corresponding β-actin values.

To reconcile the increased apoptotic susceptibility with the absence of change in radiosensitivity of the NTdl922/947 keratinocytes (reported in Fig. 1 as viability and clonogenic potential), we performed a scoring of the mitotic events in this mutant, which demonstrates a marked increment of the proliferation rate. This increased mitotic activity could compensate for the enhanced cell death observed and is indicative of the complexity of effects exerted by E1A on the pathways leading to cell proliferation and death.

**PARP Expression and Activity Levels Are Determinant of Apoptotic Sensitivity in Murine Keratinocytes Stably Expressing E1A Binding Mutants**—The unexpected finding of the combined proapoptotic-antiapoptotic activity of E1A was suggested by the down-regulation of PARP expression in the NTdl598-expressing keratinocytes. To directly test if this single molecular event could be a determinant of apoptotic sensitivity for the different E1A mutants, we transiently transfected the NTdl598 keratinocytes with a pcDNA3-PARP expression vector, bearing human PARP cDNA under the control of the cytomegalovirus promoter. Activity gel determination of PARP microsopy scoring of radiation-induced cumulative cell death events in the panel of E1A-transfected cells. We counted the number of cell deaths that occurred over a 72-h observation interval after irradiation in a population of 100 cells. As reported in Fig. 3A, the administration of 5 Gy of γ-irradiation to wild type keratinocytes produced cell death figures (presenting invariably the morphology of apoptosis, not shown) that were the same number of the spontaneous cell deaths observed in a 100-cell field in 72 h, about 10 apoptotic figures. This demonstrates that the administered radiation dose, at least in the time window of observation, is unable to change the apoptotic threshold of Pam212 keratinocytes. The number of deaths was instead almost tripled in the clone stably expressing full-length E1A, indicating that E1A-induced radiosensitization is accompanied by a substantial decrease of the apoptotic threshold.

When we tested the cell lines expressing the two mutant E1A constructs, we saw that the NTdl598 mutant, able to bind to pRb proteins but not to p300/CBP, completely lost the ability of E1A to lower the apoptotic threshold to γ-rays of these cells. Moreover, the binding of E1A to only p300/CBP (mutant NTdl922/947) oversensitized cells to apoptosis with respect to the wild type E1A protein. This cell death profile of the E1A mutants is in agreement with two opposite effects exerted by E1A on the apoptotic susceptibility of Pam212 cells, one negative due to binding to pRb and one positive due to binding to p300/CBP. To better characterize the possible antiapoptotic effect of E1A binding to pRb we exposed the Pam212 wild type and NTdl598 keratinocytes to increasing doses of γ-rays, obtaining the dose-response curve reported in Fig. 3B, which clearly indicates, particularly at the higher radiation doses, the antiapoptotic activity of E1A sequestering of pRb proteins.

**TABLE I**

<table>
<thead>
<tr>
<th>Wild type Pam212</th>
<th>NTdl598</th>
<th>NTdl922/947</th>
<th>FL E1A</th>
</tr>
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<tbody>
<tr>
<td>% CAT activity</td>
<td>100±18</td>
<td>15±3</td>
<td>107±15</td>
</tr>
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* A. Pacini and P. Nassi, unpublished data.
cell lines were plated in 25 cm² flasks (3 × 10⁵ cells/flask) and after 24 h exposed or not to 5 Gy of γ-irradiation, then a field of 100 cells was monitored by time-lapse videomicroscopy for the subsequent 72 h. During the time-lapse recording, cells were maintained at 37 °C in a 5% CO₂ atmosphere. The cell death scoring was as described under "Experimental Procedures." The cumulative number of cells at the time end point is reported as mean ± S.E. of four independent experiments. Symbols: hatched column, unirradiated wild type Pam212 cells; white column, wild type Pam212 cells; dark gray column, NTdl598 cells; light gray column, NTdl922/947 cells; black column, FL E1A cells. B, time-lapse dose-response curve of cumulative cell death events obtained after exposure of wild type and NTdl598-expressing keratinocytes to increasing doses (from 5 to 20 Gy) of γ-rays. Symbols: white columns, wild type Pam212 cells; gray columns, NTdl598 cells.

showed that plasmid transfection reproducibly reconstituted PARP enzymatic activity at levels comparable with those of the wild type cells or higher (not shown). Fig. 4A reports that the apoptotic sensitivity to 15 Gy of γ-irradiation is restored after 24 h from transfection of NTdl598 Pam212 cells with the PARP expression vector, becoming even higher than in the wild type cells. Conversely, the administration of 3-AB, a specific inhibitor of PARP enzymatic activity, lowered the level of cell death present in the NTdl922/947 mutant after 5 Gy of γ-irradiation. The dose of 5 mM of 3-AB administered 48 h before the exposure to γ-irradiation was chosen because it was able to decrease PARP activity by 70% in wild type Pam212 cells (not shown). As reported in Fig. 4B, 3-AB decreased the apoptotic sensitivity of cells expressing the NTdl922/947 mutation to levels corresponding to that of cells expressing the full-length E1A, demonstrating that inhibition of PARP activity acts on the apoptotic threshold of this cell model in the same manner of E1A sequestering of pRb proteins.

**DISCUSSION**

Despite the substantial amount of data describing the details of the interaction of E1A with cellular proteins, limited information is available on how this interaction brings to the pleiotropic effects of E1A, including cell senescence to genotoxic agents. Since these agents are known to elicit a complex pathway starting with the change in activity of DNA damage sensor proteins (26), an obvious hypothesis is that E1A could directly or indirectly affect the expression and activity of these proteins to produce its profound perturbations in the downstream signals. A first confirmation of this assumption has come with the recent demonstration that p300/CBP coactivators are essential for the transactivation function of the p53 oncopspressor protein (27) and that E1A sequestration of p300/CBP disrupts functions mediated by p53 (28, 29). Since p53 is known to be activated by its direct association with DNA strand breaks (30, 31), we wanted to study the effects of E1A on another well known DNA lesion-scanning protein, the PARP enzyme.

Similarly to p53, PARP is activated by binding to breaks in DNA, binding that triggers its enzymatic domain to consume NAD for the synthesis of chains of poly(ADP-ribose) on target proteins, including PARP itself (32). We focused on an established model of spontaneously immortalized and stably E1A-transfected cells, the murine Pam212 keratinocytes, to look for a role of PARP in modulation by E1A of the response to γ-irradiation. We found that full-length E1A induced a clear down-regulation of expression of PARP both at the protein and the mRNA level and that this effect is due to transcriptional repression of the PARP promoter. Moreover, mutant analysis clearly indicated that this E1A activity is dependent on its association with pRb-related proteins. A well described mechanism of in vivo stimulation of transcription by pRb is that mediated by binding to the transcriptional activator Sp1 (33–37). Since in the rat PARP promoter five consensus sites for Sp1 are present to whom Sp1 has been demonstrated to bind strongly stimulating transcription in reporter assays (38–40), the pRb-Sp1 activator complex could be responsible for positive regulation of the PARP promoter, destroyed by E1A-induced sequestration of pRb. This activity of E1A is also associated with a reduction of apoptosis elicited by γ-ray, and the role of PARP down-regulation in this process, among the other events induced by E1A binding to pRb proteins, is sustained by the fact that PARP expression in the same mutant cell line under the control of a viral promoter restores cell sensitivity to γ-ray. An antiapoptotic activity of E1A mediated by PARP down-regulation is in agreement with the demonstration of profound protection from experimental cerebral ischemic injury in PARP null mice (41) and with the partial protection exerted in wild type mice by PARP chemical inhibitors (42). Cerebral ischemia

**FIG. 3.** E1A binding to pRb proteins counteracts sensitization to radiation-induced apoptosis due to E1A binding to p300/CBP. A, cell lines were plated in 25 cm² flasks (3 × 10⁵ cells/flask) and after 24 h exposed or not to 5 Gy of γ-irradiation, then a field of 100 cells was monitored by time-lapse videomicroscopy for the subsequent 72 h. During the time-lapse recording, cells were maintained at 37 °C in a 5% CO₂ atmosphere. The cell death scoring was as described under "Experimental Procedures." The cumulative number of cells at the time end point is reported as mean ± S.E. of four independent experiments. Symbols: hatched column, unirradiated wild type Pam212 cells; white column, wild type Pam212 cells; dark gray column, NTdl598 cells; light gray column, NTdl922/947 cells; black column, FL E1A cells.
is supposed to elicit a pathway starting with the excessive release of glutamate by overexcited neurons and resulting in the production of DNA damaging species (43), which in turn overactivate PARP with the consequent depletion of NAD and a sudden ATP fall induced by the cell effort to resynthesize NAD, ultimately responsible for death (23).

Besides our demonstration of the role of PARP activity in E1A cell sensitization to radiation-induced apoptosis, the complex effects of E1A on the expression and activation of PARP could represent a future tool for dissecting the regulation of this gene, as happened in the past for other nuclear proteins directly or indirectly affected by E1A.

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REFERENCES