Massive Telomere Loss Is an Early Event of DNA Damage-induced Apoptosis*

Received for publication, July 9, 2002, and in revised form, October 10, 2002
Published, JBC Papers in Press, October 29, 2002, DOI 10.1074/jbc.M206818200

Rafael Ramírez‡§, Julia Carracedo, Rosario Jiménez, Andrés Canela¶¶, Eloísa Herrera**,**, Pedro Aljama‡, and María A. Blasco¶¶

From the Unidad de Investigación, Hospital Universitario Reina Sofía, 14004 Córdoba, Spain and Department of Immunology and Oncology, National Centre of Biotechnology, 28049 Madrid, Spain

Chromosomal stability and cell viability require a proficient telomeric end-capping function. In particular, telomere dysfunction because of either critical telomere shortening or because of mutation of telomere-binding proteins results in increased apoptosis and/or cell arrest. Here, we show that, in turn, DNA damage-induced apoptosis results in a dramatic telomere loss. In particular, using flow cytometry for simultaneous detection of telomere length and apoptosis, we show that cells undergoing apoptosis upon DNA damage also exhibit a rapid and dramatic loss of telomeric sequences. This telomere loss occurs at early stages of apoptosis, because it does not require caspase-3 activation, and it is induced by loss of the mitochondrial membrane potential (ΔΨm) and production of reactive oxygen species. These observations suggest a direct effect of mitochondrial dysfunction on telomeres.

Somatic cells are exposed to a great variety of DNA damaging agents, which may result in genomic instability. All organisms are equipped with mechanisms to recognize and respond to DNA damage, either by triggering cell cycle arrest or by undergoing cell death by apoptosis (1). Apoptosis is a form of cell death that has been described as distinct from necrotic cell death. The biochemical characteristics of apoptosis include (i) loss of plasma membrane phospholipid asymmetry, (ii) activation of a cascade of caspases, and (iii) loss of the mitochondrial barrier function (2).

Since the seminal work of Muller in the 1930s, we know that the ends of chromosomes have a special structure that distinguishes them from DNA breaks and protects them from fusion (3). Telomeres are composed of tandem repeats of non-coding DNA sequences (TTAGGG in all vertebrates) and of associated proteins (4, 5). Both a minimal length of TTAGGG repeats at the telomeres and telomere-binding proteins are essential to preserve functional telomeres (6). It is likely that telomeres are protected from cellular activities by their ability to form a higher order structure known as the T-loop (7). Loss of TTAGGG repeats during cell division or with increasing age may result in telomere dysfunction and, therefore, delimit life span. Telomere shortening is prevented in those cell types that activate the enzyme telomerase, a reverse transcriptase that synthesizes telomeric repeats de novo at chromosome ends. Telomerase has two essential components, a catalytic subunit (hTERT) and a small RNA molecule that contains the template use to synthesize new telomeric repeats (hTER) (8). Telomerase activity has been found in germ cells and in most tumors, but only weak or no activity is detected in normal somatic cells (9). Ectopic expression of telomerase in somatic mortal cells results in telomere elongation and indefinite extension of the life span, indicating that telomerase activity is sufficient for immortal growth (10). In addition, it has been shown recently (11, 12) that re-introduction of telomerase is able to specifically elongate critically short telomeres and to prevent chromosomal instability in the telomerase-deficient mouse model.

Genes involved in signaling DNA damage are important for apoptosis, such as p53 (13–15), poly(ADP-ribose) polymerase (PARP)1 (16) or the DNA-dependent protein kinase (DNA-PK) complex (17). In addition, proteins important for double strand break repair, such as the components of the DNA-PK and MRE11 complexes, which are involved in non-homologous end joining of double strand DNA breaks and in homologous recombination, have been shown to be also located at the telomeres and to influence telomere function (18–21). A connection between telomerase activity and resistance to apoptosis has also been established (22). In particular, inhibition of telomerase and telomere shortening below a critical length results in apoptosis in various cell types, whereas induction of telomerase activity is associated with resistance to apoptosis (23–25). Here, we show that the opposite is also true and that DNA damage-induced apoptosis results in a dramatic telomere shortening in those cells undergoing apoptosis. In addition, we determine that this massive telomere shortening is one of the early events of DNA damage-induced apoptosis. All together, these results support a role for telomeres as key sensors of DNA damage.

EXPERIMENTAL PROCEDURES

Cell Culture—Peripheral blood lymphocytes (PBL) were obtained from 20 ml of heparinized whole blood donated by 15 healthy volun-

* This work was supported in part by Grants 72/01 and 68/01 from the Junta de Andalucía, Fundación Nefrológica and Grants FIS 000788 (to J. C.) and 000701 (to R. R.). The Blasco laboratory was funded by SWISS BRIDGE AWARD 2000, by the Ministry of Science and Technology, Spain (PM97–0133), by European Union (EU) Grants EURATOM/9910201, FIGH-CT-1999-00062, and FIS99-1999-00055, and by the Department of Immunology and Oncology (DIO). The DIO was funded by the Spanish Council for Scientific Research and by Pharmacia Corporation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

§ To whom correspondence should be addressed: Unidad de Investigación, Hospital Universitario Reina Sofía, Avda. Menéndez Pidal S/N, Córdoba 14004, Spain. Tel.: 34-957-010452; Fax: 34-957-010452; E-mail: rramirez@hrs.sas.junta-andalucia.es.

¶ Pre-doctoral fellow of the EU.

** Supported by the DIO.

The abbreviations used are: PARP, poly(ADP-ribose) polymerase; PBL, peripheral blood lymphocytes; FISH, fluorescence in situ hybridization; CPT, camptothecin; ROS, reactive oxygen species; TRF, terminal restriction fragment; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

This is an Open Access article under the CC BY license.
teers. PBL were isolated by differential gradient centrifugation (Ficoll/ Hypaque; Amersham Biosciences). Pro-myelocytic leukemia HL60 cells (ATCC CCL 240) were obtained from the American Type Culture Collection (Manassas, VA). Cultured cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (Invitrogen), 2 mM glutamine (Invitrogen), and 1% penicillin/streptomycin (10,000 units penicillin/ml and 10 mg/ml streptomycin). Cells were grown at 37 °C in a humidified, 5% CO₂ atmosphere.

**Mice**—Wild-type and late generation telomerase-deficient mice, Terc−/−, were described elsewhere (11).

**Drugs and Apoptosis Induction**—The inhibitor of caspase-3 protease (acyetyl-Asp-Glu-Val-Asp-aldehyde; Ac-DEVD-CHO) was purchased from BD Biosciences and was used at 100 nM, and camptothecin (CPT; 10 μg/ml, 25% formamide). Hydrocortisone’s solution (0.1% sodium phosphate/EDTA, 0.1% SDS, and 100 mg/ml denaturated salmon sperm DNA. After a 15 min-stringency wash at 42 °C in 0.23 SSC, 0.1% SDS the autoradiography signal was digitalized in a phosphorimager scanner (Fuji) using ImageGauge software. All lanes were subdivided into intervals of ~1 to 2 mm. The mean size of the TRF was estimated using the formula (ODi × Li/ODi), where ODi is the density reading from interval I, and Li is the size in kbp of the interval relative to the markers. Mean TRF length was determined over the range of 2.3 to 23.1 kbp markers (broad range) and also on the basis of the intensity of the signal (narrow range), where the intervals averaged were those intervals that were higher than 1% of the total signal in that lane. The median and mode values were also derived on the basis of the narrow range determination.

**Flow Cytometric Detection of Telomere Fluorescence in Situ Hybridization (FISH)(Flow-FISH)**—For Flow-FISH, 1 × 10⁶ cells were resuspended in hybridization buffer containing 70% deionized formamide, 10 mM Tris, pH 7.0, 10% fetal calf serum, and 0.3 μg/ml of the telomere-specific fluorescein isothiocyanate-conjugated probe (fluorescein isothiocyanate-O-CCCATGCATGAAAC-G-NH₂). DNA from samples was heat-denatured for 10 min at 80 °C in a Thermomixer 5436 (Eppendorf, Netheler, Germany) followed by hybridization for 2 h at room temperature. Cells were washed in washing buffer containing 70% deionized formamide, 10 mM Tris, pH 7.0, 10% fetal calf serum, 0.1% Tween 20. After incubation for 1 h at room temperature, cells were washed and resuspended in phosphate-buffered saline, 10% fetal calf serum, RNase A at 10 μg/ml (Roche Molecular Biochemicals), and propidium iodide, incubated for 1 h at room temperature, washed, and analyzed in a FACScan flow cytometer. The telomere fluorescence signal was defined as the mean fluorescence signal in G₀/G₁ cells after subtraction of the background fluorescence signal (FISH procedure without probe). The flow cytometer was calibrated every day using fluorescein-labeled fluorescence Sphero microparticles (Pharmingen). The resulting calibration curve was used for correction of experimental fluorescence values in each experiment. Green fluorescence was measured on a linear scale, and results were expressed in molecular equivalents of soluble fluorochrome units (kMESF) (26). The relative telomere length was calculated by comparing the kMESF obtained from cells using different cell lines as telomere length controls.

**TUNEL Assay—**Apoptosis was measured using a kit based on the TUNEL (Roche Molecular Biochemicals). In accordance with the manufacturer’s instructions, 10⁶ cells fixed with 4% paraformaldehyde for 30 min at room temperature, washed, and permeabilized for 2 min in ice with 0.1% Triton X-100. After washing, cells were decanted and resuspended in 50 μl of TUNEL reaction mixture (5 μl TUNEL enzyme containing terminal deoxynucleotidyltransferase, mixed with 45 μl of TUNEL label containing phycoerythrin-dUTP and dNTP nucleotides) or in 50 μl of TUNEL Label as negative control. After 60 min at 37 °C in a humidified atmosphere, cells were washed three times in wash buffer (tris-buffered saline, 0.1% NaN₃, + 10 autologous serum) and analyzed by flow cytometry.

**Statistical Analysis—**Differences between means were analyzed by analysis of variance followed by the Duncan test. Results are presented as the mean ± S.D. of experiments performed in triplicate.

**RESULTS**

**Cells Undergoing Apoptosis Show Dramatically Shortened Telomeres**—To determine whether DNA damage directly affects telomere length, we simultaneously measured telomere length and apoptosis in normal human PBL, as well as in a human tumor cell line, HL60 (see “Experimental Procedures”), or after treatment with various DNA damaging agents including CPT, etoposide (Topo 1), and UV radiation (Rad UV). For
telomere length determinations, we performed quantitative FISH hybridization using a telomere-specific peptide nucleic acid probe, and measured telomere fluorescence by flow cytometry (Flow-FISH; see "Experimental Procedures"). The intensity of telomeric fluorescence is proportional to telomere length (see "Experimental Procedures"). Fig. 1A shows a representative example of simultaneous analysis by flow cytometry of telomere fluorescence and apoptosis in HL60 cells that were either untreated or treated with CPT (Fig. 1A). In the absence of CPT treatment, telomeres showed the characteristic distribution of fluorescence and no apoptosis was detected in these cells (R1 peak in Fig. 1A, upper panel). Following CPT treatment, telomeric Flow-FISH showed a two-peak bimodal distribution of cells, depending on their telomere length/fluorescence, one peak showing normal-length telomeres (R1) and another with dramatically shortened telomeres (R2), as indicated by their lower telomeric fluorescence intensity. On cell sorting of the two peaks and determination of apoptosis, the R2 peak was shown to contain apoptotic cells, whereas the R1 peak contained normal viable cells, indicating that those cells that are undergoing apoptosis also show a dramatic telomere shortening (Fig. 1B, bottom panel). Indeed, all forms of DNA damage tested resulted in increased apoptosis, concomitant with a significant telomere shortening in both the normal PBL and the HL60 cell line (Table I). Table I also shows that those cells with higher rates of apoptosis also had the shortest telomeres (see apoptosis/telomere ratios). In some cases, Flow-FISH results were confirmed by Southern blot analysis of TRF, which contain the TTAGGG telomeric repeats, as well as various lengths of subtelomeric sequences. Fig. 1B shows that treatment with two other DNA damaging agents, UV and CPT, resulted in the appearance of shorter TRFs, indicating telomere shortening in the treated cells.

A time course study performed in HL60 cells treated with CPT showed that shortening in telomere length is already evident after 2 h in culture and that it peaks after 6 h (Fig. 2). Associated with the decrease in telomere length, an increase was also found in the percentage of apoptotic cells (16 ± 3% through 71 ± 10%) following 12 h of cell culture (Fig. 2). In all experiments more than 90% of the apoptotic cells were found in the low telomere subset; this is in contrast to that observed in the cell subset with normal telomere length in which the apoptosis was less than 5% (data not shown).

The Telomere Shortening Associated with DNA Damage-induced Apoptosis Is Independent of Caspase-3 Activation—One of the molecular events associated with DNA damage-induced apoptosis is the cleavage of caspase-3 pro-enzyme into a caspase-3 active form. To determine whether the cells showing short telomeres upon DNA damage also express the active form of caspase-3, flow cytometry was used to simultaneously detect telomere fluorescence and caspase-3 activity (see "Experimental Procedures"). Five different experiments were carried out using CPT as the DNA damage agent, and a representative set of results is shown in Fig. 3A. The fraction of CPT-treated cells showing the shortest telomeres by Flow-FISH (R2 peak in Fig. 3A) also exhibited the higher levels of caspase-3 activity (Fig. 3A).

To determine whether the telomere shortening associated with DNA damage-induced apoptosis was dependent on caspase-3 activation, we treated the cells with Ac-DEVD-CHO, a caspase-3 inhibitor. As expected, inhibition of caspase-3 activation by Ac-DEVD-CHO prevented both caspase-3 activity and apoptosis in CPT-treated cells (Fig. 3B). However, Ac-DEVD-CHO did not prevent telomere shortening in CPT-treated cells (Fig. 3B). Mitochondria Are Key Regulators of Apoptosis-induced Telomere Degradation—The relationship between apoptosis-in-

---

**Fig. 1.** Telomere length determinations by flow cytometry (Flow-FISH) or by terminal restriction fragment analysis (TRF). A, representative histograms of telomere fluorescence in untreated (−CPT) or camptothecin-treated (+CPT) cells. After CPT treatment two different subsets of cells were observed according to their telomere length/fluorescence, the R1 peak showing normal length telomeres and the R2 peak showing short telomeres. TUNEL assay indicates that those cells with short telomeres (R2) are undergoing apoptosis compared with the subpopulation of cells with normal telomeres (R1). B, representative examples of TRF analysis of untreated and UV-, and CPT-treated cells. Notice that upon treatment with DNA damaging agents, lower molecular weight TRFs appear (short TRFs) in the treated cells, in agreement with telomere shortening in these cells.
duced telomere shortening and mitochondrial function was also addressed using flow cytometry. In particular, we focused on two key mitochondrial events that occur during apoptosis: (i) the generation of ROS, and (ii) the loss of mitochondrial membrane potential ($\Delta\psi_m$). Fig. 4A shows that the subset of CPT-treated cells showing short telomeres (peak R2) also exhibited a decrease in $\Delta\psi_m$, again indicating that cells undergoing apoptosis have shorter telomeres (Fig. 4A, upper panel). Treatment with CPT also increased ROS production, coincidental with increased apoptosis and concomitant with telomere shortening (Fig. 4B). Furthermore, complete uncoupling of the mitochondrial membrane potential by addition of protonophore mC1CCP further increased ROS production, accelerated apoptosis and concomitant with telomere shortening (Fig. 4). Indeed, all CPT-treated cells were in the R2 peak of short telomeres when mC1CCP was used (Fig. 4A).

Relationship between Apoptosis-induced Telomere Shortening and PARP1 Degradation—We found here that the subset of CPT-treated cells that exhibited shorter telomere length (peak R2) also showed PARP1 cleavage (Fig. 5, lane 2). In contrast, PARP1 cleavage was not observed in the subset of cells with normal telomere length (Fig. 5, lane 1). Telomere Shortening Associated with DNA Damage-induced Apoptosis Is Independent of p53 Status—The human tumor cell line HL60 used for this study lacks the p53 gene because of a deletion (27). To determine the effect of p53 in apoptosis-induced telomere shortening, we transiently transfected wild-type p53 into HL60 cells, and these cells were named HL60SN3. As expected, greater susceptibility to apoptosis was observed in p53-transfected HL60 cells (HL60SN3) upon DNA damage than in HL60 control cells, apoptosis being observed in 87.6 and 69.1% of cells in each case (Fig. 6). Average telomere length was also shorter in the p53-transfected HL60 (HL60SN3) cells than in the HL60 p53-null cells, whether they were untreated or had been treated with CPT (see Table II and Fig. 6), which agrees with the higher rates of apoptosis in the p53-positive cells (Fig. 6). However, the ratio of apoptosis to telomere length (Table II) was similar in both cases, suggesting that p53 status does not influence the telomere shortening

### Table I

| Cells    | Treatment   | Apoptotic cells | Telomere length | % apoptotic cells/telomere length | % apoptotic cells/telomere length |
|----------|-------------|-----------------|-----------------|----------------------------------|----------------------------------|
| PBL      | Not treated | 5 ± 2           | 17.3 ± 1.4      | ND                               | ND                               |
|          | CPT         | 34 ± 7          | 16.2 ± 1.4      | 2/17.1                           | 95/13.3                          |
|          | Topo 1      | 23 ± 5          | 15.8 ± 1.1      | 4/19.9                           | 97/13.1                          |
|          | Rad UV      | 26 ± 10         | 16.3 ± 1.2      | 3/17.2                           | 95/12.7                          |
| HL60     | Not treated | 7 ± 3           | 21.3 ± 1.4      | ND                               | ND                               |
|          | CPT         | 63 ± 12         | 18.1 ± 1.4      | 3/21.1                           | 93/15.3                          |
|          | Topo 1      | 53 ± 15         | 18.9 ± 1.1      | 2/21.2                           | 94/15.5                          |
|          | Rad UV      | 21 ± 14         | 19.4 ± 1.2      | 2/21.1                           | 92/15.1                          |

* Double staining in cells sorted from telomere length.

![Fig. 2](image) **Time course study performed in HL60 cells treated with CPT.** After CPT treatment, telomere length and apoptosis were studied in the same experiment. As this figure shows, the decrease in telomere length was associated with the increase in apoptosis.

![Fig. 3](image) **Caspase3 activity determinations using flow cytometry in untreated (-CPT) or CPT-treated cells (+CPT).** A, upon CPT treatment, caspase-3 activity is greater in the subpopulation of cells with short telomeres (R1 peak) than in those showing normal length telomeres (R2 peak). B, effect of caspase-3 inhibitor Ac-DEVD-CHO on caspase activity, apoptosis, and telomere length. Ac-DEVD-CHO treatment inhibits both caspase-3 activation and apoptosis in CPT-treated cells but does not inhibit telomere shortening. These observations indicate that telomere shortening occurs prior to caspase-3 activation.
associated with DNA damage-induced apoptosis, at least in HL60 cells (Table II).

Changes in Telomerase Activity Associated with DNA Damage-induced Apoptosis and Telomere Shortening—To study whether telomere shortening triggered by apoptosis could be due, at least in part, to telomerase inhibition in these cells as a consequence of apoptosis, we studied telomerase activity using the telomeric repeat amplification protocol assay in HL60 cells before and after treatment with CPT (see “Experimental Procedures”). Following CPT treatment, a moderate decrease in telomerase activity could be detected in HL60 cells (Fig. 7).

Cells Derived from Terc−/− Mice with Short Telomeres Exhibit Increased Apoptosis after CPT Treatment—Lymphocytes derived from wild-type, second-generation (G2) and fifth-generation (G5) Terc−/− mice were used to further investigate the relationship between susceptibility to apoptosis and telomerase length (11). Although G2 Terc−/− mice have telomeres that are similar in length to those of wild-types, G5 Terc−/− mouse telomeres are an average of 40% shorter than those of the wild-type controls (11). Furthermore, G5 Terc−/− mice display a proportion of chromosomes that lack detectable TTAGGG repeats at the telomeres, resulting in greater chromosomal instability (11). After CPT treatment (see “Experimental Procedures”), apoptosis occurs in 46 ± 9% of G5 Terc−/− lymphocytes, compared with only 6 ± 2% of wild-type lymphocytes (Fig. 8). Similarly, phytohemagglutinin A stimulation also resulted in a rise in lymphocyte apoptosis in G5 Terc−/− mice, in contrast to what we observed in lymphocytes from wild-type mice (Fig. 8).

DISCUSSION

Cells with short telomeres are more sensitive to DNA damage (28–30). In this study, we have demonstrated that cells undergoing apoptosis show dramatically shortened telomeres. Our results indicate that caspase-3 activation is not neces-
Telomeres and Apoptosis.

These results further support an association between short apoptosis-induced telomere shortening and PARP1 degradation (12). Our data support the idea of a relationship between apoptosis and chromosome end-capping mouse suggested that PARP1 activity is not directly involved in analysis of telomere length and function in a PARP1-deficient regulator of telomere length and function, although a recent caspases into two fragments of 115 and 85 kDa, respectively, being cleaved by agents. PARP1 has been also suggested as a potential caspase activation (31).

Two key mitochondrial events that occur during apoptosis are (i) the generation of ROS, and (ii) the loss of mitochondrial membrane potential (Δψm). We have demonstrated that cells with short telomeres showed a drop in Δψm. We have also observed increased ROS production, coinciding with increased apoptosis and concomitant with telomere shortening. These results support a key role for the mitochondria in telomere length metabolism.

PARP1 is activated during the DNA damage response, and it is involved in the base-excision repair (16), being cleaved by caspases into two fragments of 115 and 85 kDa, respectively, during apoptosis. PARP1 has been also suggested as a potential regulator of telomere length and function, although a recent analysis of telomere length and function in a PARP1-deficient mouse suggested that PARP1 activity is not directly involved in telomere length maintenance or in chromosome end-capping (12). Our data support the idea of a relationship between apoptosis-induced telomere shortening and PARP1 degradation. These results further support an association between short telomeres and apoptosis.

p53 is a key of damaged DNA and is capable of eliciting either apoptosis or cell cycle arrest, depending on the type of cell involved (14–15). p53 is also a sensor of short telomeres, because the phenotypes associated with telomeric dysfunction in the telomerase-deficient mouse model, Terc−/−, are delayed when in a p53-deficient background (11, 32). Our results show that telomere shortening associated with DNA damage-induced apoptosis is independent of p53 status.

Our results also suggest that lower telomerase activity is associated with the process of apoptosis. However, this mild decrease in telomerase activity is unlikely to account for the massive telomere loss that can be observed in apoptotic cells, and further mechanisms will have to be invoked.

Cells derived from G5 Terc−/− mice with short telomeres exhibit increased apoptosis after CPT treatment. These results suggest that telomere shortening renders lymphocytes more susceptible to apoptosis after treatment with DNA damaging agents.

In summary, we show here that cells that suffer apoptosis after DNA damage exhibit a dramatic loss of telomere length compared with non-apoptotic cells. Furthermore, such telomere shortening does not require caspase-3 activation and can be directly induced by mitochondrial membrane depolarization. All in all, these observations suggest that telomere shortening is one of the early events in DNA damage-induced apoptosis.

We also show here that p53 does not influence the telomere shortening associated with apoptosis. This is in agreement

| Cells          | Treatment | Apoptotic cells | Telomere length | % apoptotic cells/telomere length | % apoptotic cells/telomere lengtha |
|----------------|-----------|-----------------|-----------------|----------------------------------|-----------------------------------|
| HL60 p53+ (HL60SN3) | Not treated | 4 ± 5           | 19.3 ± 1.4      | ND                               | ND                               |
|                | CPT       | 54 ± 8          | 16.2 ± 1.4      | 2/18.9                           | 92/15.8                           |
| HL60 p53−     | Not treated | 9 ± 6           | 21.1 ± 1.2      | ND                               | ND                               |
|                | CPT       | 69 ± 12         | 17.8 ± 1.3      | 3/20.8                           | 93/16.2                           |

a Ratio of % apoptotic cells/telomere length.

Fig. 8. Determination of percentage of apoptotic cells in untreated, treated with CPT, or treated with phytohemagglutinin A mouse primary lymphocytes derived from wild-type, second-generation (G2) and fifth-generation (G5) Terc−/− mice, which lack telomerase activity and show progressive telomere shortening with increasing mice generations. Cells from G5 Terc−/− mice displayed increased apoptosis after CPT or phytohemagglutinin A treatment compared with similarly treated cells from wild-type or second-generation mice.

Table II: Simultaneous study of apoptosis and telomere length in wild-type and null p53 HL60 cells

Results are mean ± S.D. n = 10.
with previous observations that telomeric dysfunction triggers apoptosis in human tumor cell lines independently of their p53 status (23). Finally, we show that PBL derived from late generation telomerase-deficient mice, which have critically shortened telomeres, are more sensitive to DNA damage-induced apoptosis than the wild-type controls. These results thus indicate that (i) dramatic telomere shortening occurs as an early event of DNA damage-induced apoptosis, and that, in turn, (ii) this telomeric dysfunction may be one of the key events that signal the cellular responses associated with the apoptotic process in a way that is independent of p53 status.

REFERENCES
1. Zhou, B. B., Elledge S. J. (2000) Nature 408, 433–439
2. Roy, S., and Nicholson, D. W. (2000) J. Exp. Med. 192, 647–658
3. Muller, H. J. (1938) Collect. Nat. Woods Hole 13, 181–198
4. McEachern, M. J., Krauskopf, A., Blackburn, E. H. (2000) Annu. Rev. Genet. 34, 331–358
5. de Lange, T. (1990) J. Biol. Chem. 265, 5919–5921
6. Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H., and de Lange, T. (1999) Cell 97, 503–514
7. Collins, K. (2000) Proc. Natl. Acad. Sci. U. S. A. 96, 14276–14281
8. Samper, E., Goytisolo F., Slijepcevic, P., van Jul, P., and Blasco. M. A. (2000) Mol. Cell. Biol. 20, 800–807
9. Wang, B. (2001) Oncogene 20, 1803–1815
10. Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., and de Lange, T. (1999) Mol. Cell. Biol. 19, 25–34
11. Samper, E., Flores, J. M., Blasco, M. A. (2001) Nat. Genet. 25, 347–352
12. Andoh, T. (2000) Cell. Biochem. Biophys. 33, 181–188
13. Taylor, W., and Stark, G. R. (2001) Oncogene 20, 1803–1815
14. Herbert, B., Pitts, A. E., Baker, S. I., Hamilton, W. E., Shay, J. W., and Corey, D. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 14276–14281
15. Holt, S. E., Glinsky, V. V., Ivanova, A. B., and Glinsky, G. V. (1999) Mol. Carcinog. 23, 241–248
16. Pardee, A. B., and Policino, G. (1998) Cancer Res. 58, 3645–3650
17. Samper, E., Goytisolo F., Slijepcevic, P., van Jul, P., and Blasco, M. A. (2000) Nat. Genet. 25, 347–352
18. Samper, E., Goytisolo F., Slijepcevic, P., van Jul, P., and Blasco, M. A. (2000) Mol. Cell. Biol. 20, 800–807
19. Wang, B. (2001) Oncogene 20, 1803–1815
20. Samper, E., Goytisolo F., Slijepcevic, P., van Jul, P., and Blasco, M. A. (2000) Mol. Cell. Biol. 20, 800–807
21. Zhu, X. D., Kuster, B., Mann, M., Petrini, J. H., and de Lange, T. (2000) Nat. Genet. 25, 347–352
22. Mattson, M. P., Fu, W., and Zhang, P. (2001) Mech. Ageing. Dev. 122, 659–671
23. Hahn, W. C., Stewart, S. A., Brooks, M. W., York, S. G., Eaton, K., Kurachi, A., Bejersbergen, R. L., Knoll, J. H., Meyerson, M., and Weinberg, R. A. (1999) Nat. Med. 5, 1164–1170
24. Herbert, B., Pitts, A. E., Baker, S. I., Hamilton, W. E., Shay, J. W., and Corey, D. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 14276–14281
25. Holt, S. E., Glinsky, V. V., Ivanova, A. B., and Glinsky, G. V. (1999) Mol. Carcinog. 23, 241–248
26. Henderson, L. O., Marti, G. E., Gaigalas, A., Hannon, W. H., and Vogt, R. F., Jr. (1998) Cytometry 33, 97–105
27. Shimizu, T., Pommier, Y. (1997) Leukemia 11, 1238–1244
28. Goytisolo, F. A., Samper, E., Martin-Caballero, J., Finnos, P., Herrera, E., Flores, J. M., Boulfer, S. D., and Blasco, M. A. (2000) J. Exp. Med. 192, 1625–1636
29. Wang, B., Chang, S., Weiler, S. R., Ganesan, S., Chaudhuri, J., Zhu, C., Artandi, S. E., Rudolph, K. L., Gottlieb, G. J., Chin, L., Ali, F. W., and DePinho, R. A. (2000) Nat. Genet. 26, 85–88
30. Lee, K. H., Rudolph, K. L., Yu, Y. J., Greenberg, R. A., Cannizzaro, L., Chin, L., Weiler, S. R., and DePinho, R. A. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3381–3386
31. Ben, J. G., Xia, H. L., Ju, T., and Dai, Y. R. (2001) FASEB J. 15, 123–132
32. Chin, L., Artandi, S. E., Shen, Q., Tan, A., Lee, S. L., Gottlieb, G. J., Greider, C. W., DePinho, R. A. (1999) Cell 97, 527–538
33. Hsu, H. L., Gilley, D., Galande, S. A., Hande, M. P., Allen, B., Kim, S. H., Li, G. C., Campisi, J., Kowhi-Shigematsu, T., and Chen, D. J. (2000) Genes Dev. 14, 2807–2812