Expression of Human Telomerase (hTERT) Does Not Prevent Stress-induced Senescence in Normal Human Fibroblasts but Protects the Cells from Stress-induced Apoptosis and Necrosis*

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Cells subjected to sub-lethal doses of stress such as irradiation or oxidative damage enter a state that closely resembles replicative senescence. What triggers stress-induced premature senescence (SIPS) and how similar this mechanism is to replicative senescence are not well understood. It has been suggested that stress-induced senescence is caused by rapid telomere shortening resulting from DNA damage. In order to test this hypothesis directly, we examined whether overexpression of the catalytic subunit of human telomerase (hTERT) can protect cells from SIPS. We therefore analyzed the response of four different lines of normal human fibroblasts with and without hTERT to stress induced by UV, γ-irradiation, and H₂O₂. SIPS was induced with the same efficiency in normal and hTERT-immortalized cells. This suggests that SIPS is not triggered by telomere shortening and that nonspecific DNA damage serves as a signal for induction of SIPS. Although telomerase did not protect cells from SIPS, fibroblasts expressing hTERT were more resistant to stress-induced apoptosis and necrosis. We hypothesize that healing of DNA breaks by telomerase inhibits the induction of cell death, but because healing does not provide legitimate DNA repair, it does not protect cells from SIPS.

Normal human cells in culture do not divide indefinitely and after ~60 population doublings enter irreversible growth arrest termed replicative senescence. Replicatively senescent cells are characterized by increased cell volume and a distinct flat morphology, the presence of SA-β-gal activity (1), elevated expression of p16Ink4a (p16) and p21Waf1 (p21), and hypophosphorylated Rb.

Replicative senescence is induced by progressive telomere shortening, which occurs at every cell division. When telomeres reach the critical length of less than 5 kb, the Rb and p53 pathways become activated and trigger the irreversible growth arrest (2–5). How the signal from short telomeres leads to activation of p53 and Rb pathways is not well understood. Replicative senescence in human fibroblasts can be overcome by overexpression of the catalytic subunit of telomerase (6). Telomerase elongates short telomeres, and the cells become immortalized.

Cells subjected to sub-lethal stress may enter, within a short time, a state that closely resembles replicative senescence (reviewed in Ref. 7). Cells enter stress-induced senescence (SIPS) following DNA damage (UV and γ-irradiation) (7–10), oxidative stress (H₂O₂, hyperoxia) (11–13), and treatment with histone deacetylase inhibitors (14). Cells undergoing SIPS display all the major characteristics of replicatively senescent cells; they are large and flat, stain positive for SA-β-gal, accumulate p21, and contain hypophosphorylated Rb. Furthermore, it has been shown that the expression pattern in cells undergoing SIPS is similar to that of replicatively senescent cells (15). The molecular events that trigger SIPS are far from clear. It has been observed that telomeres shorten 5–10 times faster in cells grown under chronic hyperoxia (13, 16). Accelerated telomere shortening was also observed after stress with SIPS-inducing concentrations of H₂O₂ (17). It has been suggested that induction of the p53-dependent cell cycle arrest via generation of nonspecific as well as telomere-specific DNA damage is the trigger of SIPS (7). Chen et al. (18), however, did not observe telomere shortening in the cells undergoing SIPS after H₂O₂ treatment. It has also been shown that Ras-induced senescence, which represents another type of premature senescence, is telomere-independent and cannot be rescued by overexpression of hTERT (19, 20). However, expression of oncogenic Ras triggers senescence via the mitogen-activated protein kinase pathway (21), which is likely to be a pathway distinct from DNA damage-induced senescence.

We therefore designed an experiment to test the hypothesis that accumulation of DNA breaks in telomeres and rapid telomere shortening was the primary cause of SIPS. We also aimed to test whether nonspecific DNA damage could cause senescence or whether the senescent phenotype was strictly telomere-dependent. We compared the induction of SIPS by UV and γ-irradiation and H₂O₂ in four different lines of normal human fibroblasts with and without hTERT. Our predictions were that if SIPS was triggered by telomere shortening, the presence of telomerase activity should render the cells more resistant to SIPS. On the other hand, if SIPS could be caused by nonspecific DNA damage, then overexpression of hTERT would have no effect. We observed no difference in the induction of SIPS between fibroblasts that did or did not express hTERT, as monitored by morphological changes, growth arrest, and SA-β-gal activity. This result suggests that SIPS is trig-
gered by nonspecific DNA damage and most likely is telomere-independent. We also tested whether SIPS required p21 by inducing SIPS in p21−/− human fibroblasts. p21 accumulates in cells entering replicative senescence, and it has been demonstrated that p21−/− fibroblasts do not undergo replicative senescence (22). We observed that SIPS was attenuated in p21−/− cells, suggesting that SIPS and replicative senescence share some common pathways. However, the inhibition of SIPS in p21−/− cells was ~50%, in contrast to replicative senescence, which is completely blocked in these cells.

We have also found that hTERT-expressing cells were more resistant to apoptosis and necrosis induced by UV and γ-irradiation. A protective effect of telomerase on induction of apoptosis was recently reported (23–27). It has been proposed that telomerase may have additional functions (24) and may attenuate apoptosis by some interaction with the apoptotic machinery (26). We observed the strongest protective effect of hTERT in cells treated with UV and γ-irradiation, known inducers of DNA double-strand breaks, and almost no protection against H2O2 damage, which affects many other cell compartments in addition to DNA. This suggests that the protective mechanism works at the DNA level. We hypothesize that telomerase protects cells from apoptosis and necrosis by a healing process, such as adding telomeric repeats to broken DNA ends.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**

WI-38, IMR-90, and LF1 are normal human lung fibroblasts. WI-38 fibroblasts were provided by J. Smith; IMR-90 cells were from the Coriell Institute for Medical Research, and LF1 fibroblasts were a kind gift from J. Sedivy (see Ref. 22). HCA2 human foreskin fibroblasts were isolated in our laboratory. The normal cells were used at population doubling of 34–37, 35–38, 20–23, and 26–30 for WI-38, IMR-90, LF1, and HCA2, respectively. WI-38-hTERT, IMR-90-hTERT, and HCA2-hTERT were kind gifts from J. Campisi. LF1-hTERT, LF1p21−/−, and LFp21−/−–hTERT were kindly provided by J. Sedivy (20, 28). Cells were grown in minimum essential media with Earle’s salts or minimum essential media with Earle’s salts supplemented with 10% fetal calf serum, nonessential amino acids, and sodium pyruvate.

**Treatment of Cells with Various Agents**

Cells were seeded at 5 × 10^5–10^6 cells per 100-mm tissue culture dish, grown for 2–3 days (until the first contacts between the cells became visible, but before confluence), and subjected to the following treatments.

**γ-Irradiation**—Cells were irradiated using Gammacell 1000 (Atomic Energy of Canada, Ltd.) at the dose of 5.5 kilorads (for most treatments), at 1 kilorad per 1.08 min. The culture medium was changed immediately after irradiation.

**H2O2 Treatment**—Culture medium containing H2O2 was added to the cells and incubated for 2 h at 37°C. Then cells were washed once with PBS and a fresh culture medium was added.

**SA-β-gal**

Ten days after treatment cells were fixed and stained for SA-β-gal as described (1). Cells were counted under the microscope, and a minimum of 500 cells was counted for each coverslip. The percent β-galactosidase-positive cells from the total number of cells was calculated.

**Thymidine Incorporation**

Thymidine incorporation assay was performed as described (29). Briefly, cells were grown on coverslips, 3–4 days after treatment, and [3H]thymidine was added to the cells. Cells were then incubated for an additional 36 h and fixed and subjected to autoradiography, and the percentage of cells incorporating tritiated thymidine was determined.

**Southern Analysis**

Ten days after treatment genomic DNA was extracted from 2 × 10^6 cells, digested with the mixture of restriction enzymes (AluI, HaeIII, HpalI, and HpaII), separated by electrophoresis on a 0.8% agarose gel, and transferred to nylon filters (Hybond-N+) under alkali conditions, according to the manufacturer’s instructions. Membranes were hybridized for 12 h at 55°C with a radiolabeled (TTAGGG)_4 probe, washed, and subjected to autoradiography.

**Detection of Apoptosis and Necrosis by Acridine Orange Staining**

Cells were collected 48 and 72 h after post-treatment, stained with acridine orange, and analyzed by FACS (Beckman-Coulter, EPICS XL-MCL, using System II version 3.0) as described (30). Apoptotic and necrotic fractions were identified as described (31), using etoposide and actinomycin-treated young and old cells as standards.

**Detection of Necrosis by Release of DNA**

The cellular DNA fragmentation enzyme-linked immunosorbent assay kit (Roche Molecular Biochemicals) was used for the detection of bromodeoxyuridine (BrdUrd)-labeled DNA released from necrotic cells into the cell medium. Briefly, 24 h before stress treatment cells were incubated with BrdUrd. At various time points after stress treatments the supernatants of the cell cultures were collected, and DNA fragments were captured with an anti-DNA antibody and detected by an anti-BrdUrd antibody-peroxidase conjugate according to the manufacturer’s instructions.

**Western Blot Analysis**

Adherent fibroblasts were harvested and lysed in protein sample buffer, boiled for 10 min, and equal numbers of cells loaded on SDS-PAGE. The proteins were transferred to nitrocellulose membrane using a semidry transfer cell (Bio-Rad). Membranes were hybridized with the following antibodies: anti-p21 (WAF1/Ab1, Oncogene), anti-Rb (Rb Ab1I(1F8), LabVision), anti-p53 (pAb1801 Calbiochem), and anti-p16 (p16 Ab1(DCS-50.1/A7) LabVision). Equivalent loading of lanes was verified by hybridization with anti-actin antibodies (Calbiochem).

**RESULTS**

**hTERT Does Not Protect Fibroblasts from SIPS—Introduction of telomerase into human fibroblasts allows them to escape replicative senescence (22). To test whether telomerase could protect the cells from SIPS, we compared the induction of SIPS in normal human fibroblasts cell lines with and without telomerase. We have used four lines of fibroblasts that have been frequently used to study senescence: fetal lung fibroblasts IMR-90, WI-38, and LF1 (22) and foreskin fibroblasts HCA2. The corresponding lines (IMR90-TERT, WI-38-TERT, LF1-TERT, and HCA2-TERT) with telomerase activity were obtained from..."
the parental lines by infection with a retroviral vector containing an hTERT expression cassette. Cells were grown for at least 70 population doublings after infection to confirm the immortalized phenotype. Telomerase activity in the immortalized lines was tested by the telomeric repeat amplification protocol assay (32). All the four immortalized lines had high telomerase activity (Fig. 1).

In order to induce premature senescence cells were treated with UV and γ-irradiation or H2O2. All these treatments have been reported to induce SIPS in human fibroblasts (7). We have found that doses of 5.5 kilorads for γ-irradiation, 2 J/m² for UV irradiation, and 500 μM H2O2 are optimal for induction of SIPS by testing a wide range of doses (data not shown). The above doses irreversibly arrest cell division in more than 90% of the surviving cells.

Three days following the treatment, all cells acquired an enlarged flat morphology typical of senescent cells.

FIG. 2. SA-β-gal activity and DNA synthesis in normal and hTERT-immortalized fibroblast cell lines after stress treatments. Cells were subjected to 2 J/m² UVB, 5.5 kilorads of γ-irradiation, or treated with 500 μM H2O2 for 2 h. A. percent of SA-β-gal-positive cells. Staining was performed on day 10 after stress. The percent of SA-β-gal-positive cells was calculated by counting at least 500 cells per sample. B. DNA synthesis determined by thymidine incorporation. [3H]Thymidine was added on day 3 after stress. The percent of cells incorporating thymidine was calculated by counting at least 500 cells per sample. Experiments were repeated three times, and error bars represent the S.E.
and without hTERT. To obtain a quantitative comparison of induction of SIPS, we used SA-β-gal staining and measured the number of cells that synthesized DNA by tritiated thymidine incorporation. All the treatments induced SA-β-gal activity in more than 70% of the cells (Fig. 2A). There was some variability between the individual cell lines but no difference between the normal and hTERT expressing cells of the same line. Thymidine incorporation was strongly inhibited in all the cell lines, independent of telomerase activity (Fig. 2B).

We then tested whether telomerase activity was still present after the stress treatments. To this end cells from two telomerase positive cell lines IMR-90+hTERT and LF1+hTERT were analyzed by TRAP assay (32) 24 h after stress. The results (Fig. 3) indicate that telomerase activity is slightly reduced following stress. This small change in telomerase activity cannot account for induction of senescence, because a high level of telomerase activity was still present in these cells. For example, the telomerase activity in γ-irradiated and H2O2-treated LF1+hTERT cells was as high as that in untreated IMR-90+hTERT cells. We also determined telomere length in IMR-

![Fig. 3. Telomerase activity in hTERT-immortalized fibroblast cell lines after stress treatments.](image)

![Fig. 4. Telomere length in normal and hTERT-immortalized fibroblast cell lines after stress treatments.](image)

![Fig. 5. Status of p21, Rb, p53, and p16 in human fibroblasts during stress-induced senescence.](image)
90, IMR-90-hTERT, LF1, and LF1-hTERT cell lines by Southern blot analysis 10 days after stress treatments (Fig. 4). All the cell lines had long telomeres characteristic of young cells, and importantly, no telomere shortening was observed in the cells undergoing SIPS compared with control cells, suggesting that SIPS was not caused by rapid telomere shortening.

We also compared the induction of p21 and the p53 and Rb status in the wild type and hTERT-expressing cells undergoing SIPS. Adherent cells were collected 48 h after treatment and analyzed by Western blot. p21 levels were elevated in all the cells undergoing SIPS (Fig. 5A), and there was no difference in the level of p21 induction between the parental and hTERT-expressing cell lines. Rb protein levels were decreased, and Rb was mainly in the unphosphorylated state in the cells treated with γ-irradiation and H2O2 (Fig. 5B). Similar to results with p21 induction, there was no difference in the Rb status between the cells with or without telomerase. The level of p53 was strongly increased after UV treatment (Fig. 5C), and a similar level of p53 induction was observed between the cells with or without telomerase. The level of p16 (Fig. 5D) was not up-regulated in the cells undergoing SIPS.

Our results demonstrate that there is no difference in the induction of SIPS between the cells with or without telomerase activity, as assayed by SA-β-gal staining, thymidine incorporation, and the expression pattern of the major proteins regulating cell cycle arrest.

**SIPS Is Attenuated in the Absence of p21**—p21 plays a key role in the regulation of replicative senescence, and human fibroblasts with the knock out of p21 escape replicative senescence (22). In order to investigate the role of p21 in SIPS, we examined the induction of SIPS in p21+/− human fibroblasts. We have used p21+/− cells that were derived from LF1 normal human fibroblasts by double knock out (22). LF1, LFp21−/−, and LF1p21−/−hTERT cells were subjected to UV, γ-irradiation, and H2O2 as described above. Following the various treatments, some of the p21−/− cells became enlarged and elongated but not as flattened as senescent wild type LF1 cells. Induction of SA-β-gal activity (Fig. 6A) and inhibition of DNA synthesis (Fig. 6B) in p21−/− cells were also reduced —2-fold compared with the parental LF1 strain. H2O2 and γ-irradiation stress caused a decrease in levels of Rb in p21−/− cells, similar to that seen in wild type cells (Fig. 7A). Therefore, p21−/− cells under stress displayed an intermediate phenotype with some but not all the features of senescent cells. The level of p16 protein was elevated in both treated and untreated p21−/− fibroblasts (Fig. 7B), which has been observed previously (22, 28). Our results indicate that p21 is involved in SIPS in human fibroblasts; however, it is not absolutely required, and there are alternative pathways for induction of SIPS.

**hTERT Has Anti-apoptotic and Anti-necrotic Effects**—Although we did not detect any difference in the stress-induced senescence between wild type normal cells and cells with introduced telomerase, microscopic examination revealed some difference in survival following stress. Cells expressing hTERT showed better survival after treatment with UV and γ-irradiation. In order to determine what type of cell death is rescued by telomerase, we analyzed the induction of apoptosis and necrosis. Apoptosis and necrosis were assayed by acridine orange staining followed by FACS analysis (Figs. 8 and 9). This method is very sensitive and allows one to differentiate between apoptosis and necrosis in the same sample (31) (Fig. 8). In addition, induction of necrosis was confirmed by measuring the release of DNA into the media by necrotic cells (Fig. 10).

UV irradiation induced primarily an apoptotic response (Fig. 9A), and a concomitant accumulation of p53 following treatment (Fig. 5C). UV is known to be a strong inducer of apoptosis (31, 33), and we have here detected a low level of necrosis. Although there was a significant variation between the four cell types in their resistance to stress, and the ratio between apoptosis and necrosis, expression of hTERT reduced cell death, both apoptotic and necrotic, in all the cell lines.

γ-Irradiation induced a very low level of apoptosis (Fig. 9B), consistent with a previous report (8) on the lack of γ-irradiation-induced apoptosis in fibroblasts. The major type of cell death that we observed was necrosis (Figs. 9C and 10). To our knowledge, this is the first demonstration of γ-irradiation-induced necrosis in fibroblasts. As was the case with UV treatment, cells containing telomerase activity were significantly more resistant to both types of cell death after γ-irradiation.

Induction of necrosis was observed after H2O2 treatment, with no difference between the cells with or without telomerase (Figs. 9C and 10). The differences in resistance to H2O2 between the different cell lines had a pattern similar to UV resistance, with LF1 and HCA2 being more resistant to stress than WI-38 and IMR-90. Necrosis has been reported previously...
to occur at the higher doses of $H_2O_2$ (more that 5 mM); however, our method of detection of necrosis is much more sensitive than the methods used in earlier studies (55). In summary, telomerase activity protected cells from apoptosis and necrosis induced by UV and $\gamma$-irradiation but not by $H_2O_2$.

**DISCUSSION**

**Telomerase and Stress-induced Senescence**—We have demonstrated that expression of catalytically active telomerase does not prevent stress-induced senescence. This was observed in different cell lines of normal human fibroblasts following UV and $\gamma$-irradiation and $H_2O_2$ treatments. This allows us to rule out cell line-specific variations, which frequently cause discrepancies between different studies. Cells undergoing SIPS displayed the characteristics of replicative senescence such as SA-\(\beta\)-gal activity, lack of DNA synthesis, and $p21$, $p53$, and $Rb$ status. The level of $p16$ was not up-regulated in the cells undergoing SIPS. Although some authors have reported (34) a transient accumulation of $p16$ mRNA during SIPS, accumulation of $p16$ protein, as seen in replicatively senescent cells, has not been observed in SIPS (18, 35).

The signal that triggers the induction of SIPS is poorly understood and most likely involves DNA damage. Nonspecific DNA damage together with damage-induced telomere shortening has been proposed to be the major mechanism of SIPS. However, if damage-induced telomere shortening had a significant contribution to SIPS, we would expect to observe, at least, a partial protective effect of telomerase. Therefore, our results suggest that telomere shortening does not play a significant role in SIPS and that nonspecific DNA damage is capable of inducing the senescent phenotype.

One possible argument against this interpretation could be that telomerase does not protect the cells from SIPS, because the commitment to undergo SIPS occurs before telomerase has had time to elongate the broken telomeres or because telomerase does not have access to the broken telomere ends. This does not seem to be the case, however, because (i) we were able to see the protective effect of telomerase on induction of apoptosis, which is a much more rapid process than induction of senescence, and (ii) telomerase has been reported to efficiently synthesize telomeric repeats at the ends of broken DNA in vivo (36–40). Furthermore, transient expression of telomerase in pre-senescent cells was reported to delay senescence (41). Because we assayed the induction of SIPS 9–10 days after stress, telomerase should have had sufficient time to elongate the broken telomeres and allow the cells to resume proliferation.

The identification of four complementation groups for immortalization has suggested that senescence may be regulated by multiple pathways (42). At present, there is a growing number of reports of various treatments that can induce premature senescent phenotype. These treatments include various types of DNA damage, overexpression of oncogenes (activated Ras or Raf) (43, 44) or mitogenic signals (overexpression of E2F) (45), and various treatments that affect chromatin structure (14, 46). It has been demonstrated that Ras-induced senescence cannot be rescued by expression of telomerase (20). Oncogene-induced senescence and senescence induced by changes in chromatin structure directly affect gene expression and are likely to utilize different signaling pathways in SIPS and replicative senescence. The current model for the induction of replicative senescence is that critically short telomeres are recognized as broken DNA, which in turn trigger cell cycle arrest and senescence rather than apoptosis. Our results suggest that DNA breaks at nonspecific genomic sites can also lead to induction of senescence. Therefore, we can point out two important differences between SIPS and replicative senescence: SIPS is not affected by telomerase and is not associated with accumulation of $p16$.

**The Role of $p21$ in Stress-induced Senescence**—In human fibroblasts, $p21$ is up-regulated in all types of senescence such as replicative senescence (47), Ras-induced senescence (21), and SIPS (see Ref. 7 and this communication). $p21$ plays a central role in replicative senescence, because $p21^{-/-}$ cells bypass senescence and continue to proliferate until a crisis (22). However, $p21$ was found to be dispensable for Ras-induced senescence (20).

We present the first report where the requirement of $p21$ for SIPS has been tested directly by using $p21^{-/-}$ human fibroblasts. SIPS was reduced —2-fold in the $p21^{-/-}$ cells, indicating that $p21$ plays a role in SIPS but, in contrast to replicative senescence, is not absolutely required. This suggests that SIPS can be triggered by multiple pathways. It has been shown that $p16$ accumulates in keratinocytes grown in an inadequate culture environment (48), which has led to the proposal that $p16$ may be a general mediator of stress response. It is unlikely, however, that $p21$ plays a role in the rapid induction of SIPS in fibroblasts, because it is not accumulated in the wild type cells undergoing SIPS and is constitutively up-regulated in $p21^{-/-}$ cells. We have observed a change in $Rb$ status in $p21^{-/-}$ cells undergoing SIPS, which suggests that some factors, other than $p16$ and $p21$, may trigger the $Rb$ response and growth arrest during SIPS.

**Effect of Telomerase on Programmed Cell Death**—We have shown that telomerase protects human fibroblasts from stress-induced apoptosis and necrosis. It has been known that inhibition of telomerase in tumor cell lines may induce apoptosis (49, 50) and the effect that is possibly mediated by appearance of short telomeres. In addition, it has been demonstrated that inhibition of telomerase during brain development can induce neurons to undergo apoptosis (24). Several recent reports (23–27) have demonstrated that expression of telomerase protects the cells from stress-induced apoptosis. The mechanism of the protective effect of hTERT in the case of stress-induced apoptosis is less clear. It has been suggested that hTERT may promote survival by a mechanism other than telomere maintenance (24). It has also been suggested that telomerase may inhibit an early event in the apoptotic cascade (23).

We have observed inhibition of apoptosis/necrosis induced by UV and $\gamma$-irradiation but not by $H_2O_2$. The mechanism of
FIG. 8. Detection of apoptotic and necrotic cells by acridine orange staining followed by FACS analysis. A, density plot of normal cell cycle distribution of untreated fibroblasts. Cell populations at G1 and G2 stages are indicated. Gating for detection of apoptotic and necrotic cells was set up using actinomycin D-treated young normal fibroblasts (B) and actinomycin D-treated senescent normal fibroblasts (C) that have been shown to undergo apoptosis and necrosis, respectively (31). D, representative density plot showing induction of apoptosis and necrosis in WI-38 normal fibroblasts following UV irradiation. E, representative density plot showing induction of apoptosis and necrosis in hTERT-immortalized WI-38 fibroblasts. Reduction in the amount of cells undergoing cell death in hTERT-immortalized WI-38 fibroblasts can be seen compared with normal WI-38 cells.
H₂O₂-induced cytotoxicity is controversial. Some authors (51) implicate DNA as a primary target, whereas others explain the killing effect of H₂O₂ by its action on cell membranes (52) or by induction of lysosomal rupture (53). It therefore appears that H₂O₂ targets multiple cellular compartments. In contrast, the cytotoxic effect of UV and γ-irradiation is mediated by DNA.
UV irradiation induces pyrimidine dimers and DNA breaks, and H9253-irradiation is primarily a double-strand break inducer. It is known that telomerase may add telomeric repeats to the broken DNA ends, the process called "healing" (36–40). We hypothesize that healing of the broken DNA ends by telomerase may be the mechanism that protects the cells from induction of programmed cell death.

**Relationship between Apoptosis and Senescence**

The reason why in some cases DNA damage induces senescence whereas in others apoptosis occurs is not well understood. One important factor could be the amount of damage, because lower doses tend to induce senescence and higher doses induce apoptosis. However, this is not always the case; for example, γ-irradiation does not induce apoptosis in fibroblasts even at a high dose. Another popular model suggests that DNA damage in telomeres induces senescence and that DNA damage to the other genomic sites induces apoptosis. The results presented in this report argue against this model. Additional evidence against this is that cells lacking TRF2, and therefore experiencing rapid telomere shortening, undergo apoptosis but not senescence (54).

Why telomerase activity protects the cells from apoptosis but not from SIPS is unclear. One possible explanation is that the healing process mediated by telomerase is able to partially mask DNA damage from the apoptotic machinery but is unable to provide legitimate repair. Therefore, in the presence of telomerase activity cells that would otherwise undergo apoptosis or necrosis enter the senescence pathway (Fig. 11). The healing process cannot, however, help the cells to repair their DNA to avoid senescence.

**Stress-induced Senescence and Aging**

In the past several years, it has become apparent that cell proliferation and telomere shortening are not the only inducers of a senescent-like phenotype. Senescence can be induced by overexpression of oncogenes, changes in chromatin, oxidative stress, and DNA damage. How do all these processes contribute to organismal aging? Replicative senescence is likely to play a role in aging of highly proliferative tissues such as endothelium and lymphoid tissue. However, there are many tissues, such as brain, muscle, and many others, in which the cells do not divide at all or divide minimally. The function of these tissues and their cells also deteriorates with aging. This functional decay cannot be attributed to replicative exhaustion. Oxidative stress and DNA damage are likely to be the major contributors in age-associated changes of these cells. Our finding that senescence caused by stress is telomere-independent and can be induced by DNA damage at any genomic location facilitates this view.

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**FIG. 10.** *Induction of necrosis in normal and hTERT-immortalized fibroblast cell lines after stress treatments.* Necrosis was analyzed by the release of DNA from necrotic cells into the medium, and DNA was quantified with a cellular DNA fragmentation enzyme-linked immunosorbent assay kit (Roche Molecular Biochemicals) as described under "Experimental Procedures." The percentage of DNA released from the total DNA is shown. Experiments were repeated three times, and S.D. values are indicated.

**FIG. 11.** *Interplay between cell death (apoptosis/necrosis) and senescence pathways induced by DNA damage.* Telomerase can heal broken DNA ends, and cells that would otherwise undergo apoptosis/necrosis are shifted to senescence pathway. The healing, however, does not provide legitimate repair and does not protect the cells from senescence.
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