Telomerase confers resistance to caspase-mediated apoptosis

Yira Bermudez
Diana Erasso
Nicole C Johnson
Michelle Y Alfonso
Nancy E Lowell
Patricia A Kruk

Department of Pathology, University of South Florida and the H Lee Moffitt Cancer Center, Tampa, FL, USA

Abstract: There is growing evidence that accelerated telomeric attrition and/or aberrant telomerase activity contributes to pathogenesis in a number of diseases. Likewise, there is increasing interest to develop new therapies to restore or replace dysfunctional cells characterized by short telomeric length using telomerase-positive counterparts or stem cells. While telomerase adds telomeric repeats de novo contributing to enhanced proliferative capacity and lifespan, it may also increase cellular survival by conferring resistance to apoptosis. Consequently, we sought to determine the involvement of telomerase for reduced apoptosis using ovarian surface epithelial cells. We found that expression of hTERT, the catalytic component of telomerase, was sufficient and specific to reduce caspase-mediated cellular apoptosis. Further, hTERT expression reduced activation of caspases 3, 8, and 9, reduced expression of pro-apoptotic mitochondrial proteins t-BID, BAD, and BAX and increased expression of the anti-apoptotic mitochondrial protein, Bcl-2. The ability of telomerase to suppress caspase-mediated apoptosis was p-jnk dependent since abrogation of jnk expression with jip abolished resistance to apoptosis. Consequently, these findings indicate that telomerase may promote cellular survival in epithelial cells by suppressing jnk-dependent caspase-mediated apoptosis.

Keywords: telomerase, hTERT, caspase, apoptosis, jnk, jip, epithelial cells

Introduction

Telomeric attrition is the prevailing model of a molecular clock (Harley et al 1992) proposed to explain limited cellular proliferation known as the Hayflick limit (Hayflick 1965). Telomeres, the linear ends of eukaryotic chromosomes, consist of specialized tandem $(TTAGGG)_n$ DNA repeats and associated telomeric binding proteins that serve to protect the ends of chromosomes from recombination, end-to-end fusion and degradation, thereby maintaining chromosomal integrity (Blackburn 1991). While telomeres may also be involved in nuclear organization because they attach to the nuclear matrix (deLange 1992), they are crucial in solving the 'end replication' problem (Olovnikov 1975; Harley et al 1992). That is, in normal somatic cells there is progressive telomeric loss with successive rounds of cellular replication due to the inability of conventional DNA polymerase to fully elongate linear DNA. Telomeric shortening continues until replicative senescence or cellular crisis when critically shortened telomeres no longer effectively stabilize chromosomes and most cells die. Greatest telomeric shortening has been reported in cells from patients with progeroid syndromes (Kruk et al 1995; Metcalfe et al 1996) and in epithelial cells (Counter et al 1992). However, the rates and extents of telomeric loss in vivo (7–150 base pairs/year) and in vitro (50–200 base pairs/population doubling) are highly variable among different cell types (Hastie et al 1990; Allsopp et al 1995; Kruk et al 1995; Metcalfe et al 1996), so that not all cells reach senescent crisis equally.

Correspondence: Patricia A Kruk
Department of Pathology, MDC 11, University of South Florida, 12901 Bruce B. Downs Blvd, Tampa, FL 33612, USA
Tel +1 813 974 0548
Fax +1 813 974 5536
Email pkruk@hsc.usf.edu

Occasional immortal cells emerging from crisis usually contain shortened, but stable telomeres due to the re-expression of telomerase, a ribonucleoprotein that elongates telomeric repeats de novo (Kim et al 1994). Consequently, telomerase suppresses cellular senescence and extends cellular lifespan (Meyerson et al 1997). Telomerase activity is generally limited to stem cell populations and immortalized tumor cells and is suppressed in normal cells (Kim et al 1994). Telomerase consists of an RNA component (hTR) that serves as an internal telomeric template and hTERT, a reverse transcriptase (Kim et al 1994). While both protein catalytic hTERT and hTR subunits have been identified and cloned, the protein catalytic subunit is the limiting determinant of telomerase activity (Feng et al 1995; Meyerson et al 1997; Weinrich et al 1997; Counter et al 1998). In addition to these two core components, several telomerase-associated proteins have been identified. They include the hTR RNA binding proteins TEP1, hnRNP proteins (A1, C1/C2, D), survival of motor neuron (SMN), L22, and hStau, while p23 and hsp90 bind hTERT, suggesting a role for these proteins in hTR RNA processing, telomerase assembly, and cellular localization of the telomerase complex (Nakayama et al 1997; Holt, Aisner, et al 1999; Eversole and Maizels 2000; Ford et al 2000, 2001; Le et al 2000; Fiset and Chabot 2001; Kamma et al 2001; Bachand et al 2002). While telomerase regulation is complex (Ulaner et al 1998; Cong et al 1999; Greenberg et al 1999; Takakura et al 1999), we have previously developed a culture model system in which telomerase activity can be perturbed and controlled in ovarian surface epithelial (OSE) cell lines by endogenous and exogenous stresses (Alfonso-De Matte et al 2001; Alfonso-De Matte, Moses-Soto, et al 2002; Alfonso-De Matte, Yang, et al 2002; Alfonso-De Matte and Kruk 2004).

Although the primary function of telomerase is the maintenance of structural integrity at the linear ends of chromosomes contributing to cellular immortalization, recent studies have shown an association between telomerase activity and increased chemotherapeutic resistance consistent with poor prognosis in many cancers (Asai et al 1998; Kiyozuka et al 2000; Villa et al 2000). Telomerase appears to mediate its protective effect by conferring resistance to apoptosis (Mandal and Kumar 1997; Kondo et al 1998; Fu et al 1999, 2000; Tian et al 1999; Zhang et al 1999; Herbert et al 1999; Holt, Glinsky, et al 1999; Iida et al 2000; Zhu et al 2000), suggesting that the function of telomerase is more complex than initially thought. However, the exact mechanism(s) by which telomerase confers resistance to apoptosis leading to enhanced cellular survival remains unclear.

Accelerated telomeric erosion and/or aberrant telomerase activity have been implicated in the pathogenesis of many diseases including: progeroid disorders (Kruk et al 1995; Metcalfe et al 1996; Du et al 2004); cardiovascular disease (Oh et al 2001; Serrano and Andres 2004); neurological disorders (Panossian et al 2003; Zhang et al 2003); and acquired anemia (Mitchell et al 1999; Vulliamy et al 2001; Greenwood and Lansdorp 2003; Ohshima et al 2003; Polychronopoulou and Koutroumba 2004). Given the growing interest in the potential for progenitor cell therapy to restore or repair dysfunctional cells by using telomerase-positive/stem cells to enhance cell survival, we sought to determine whether telomerase-mediated suppression of apoptosis contributes to increased cellular survival in epithelial cells.

Materials and methods

Cell culture and transfections

Normal ovarian surface epithelial cell lines (IOSE-80, FHIOSE 1816-686, IMCC3, IMCC5) were used as previously described (Kruk et al 1990, 1999). All cell lines were previously determined to be telomerase-negative (Kruk et al 1999). Cells were maintained in Medium 199/MCDB 105 (1:1; Sigma Scientific, St Louis, MO, USA) supplemented with 10 μg gentamicin (GIBCO BRL, Grand Island, NY, USA) and 5%–10% fetal bovine serum (FBS; Hyclone, Logan UT, USA) in a humidified 5% CO_2/95% air atmosphere. Cisplatin (CP), staurosporine (STS), and tumor necrosis factor-α (TNF-α) were employed to induce apoptosis and were obtained from Sigma Scientific.

Cells were transfected with plasmid constructs of wild-type (wt) hTERT, dominant-negative (DN) hTERT, jip, or jnk using the previously described lipofectamine transfection protocol (Alfonso-De Matte, Yang, et al 2002). Transfections were confirmed via Western blot analysis. Transfection efficiency was measured in parallel cultures transfected with green fluorescent protein (GFP) cDNA under identical conditions.

Telomerase assay

To quantitatively detect changes in telomerase, cells were assayed for telomerase activity using the telomerase polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA; Roche Molecular Biochemicals, Indianapolis, IN, USA) as described previously (Alfonso-De Matte et al 2001). Briefly, cells were washed with

Dulbecco's phosphate buffered saline (DPBS), trypsinized and centrifuged at 500 *g* for 5 min. Pellets were washed twice in DPBS, then resuspended in 200 µl of CHAPS lysis buffer and kept on ice for 30 min, after which time the lysates were centrifuged at 100 000 *g* for 60 min at 4°C. Lysates were assayed using the Bio-Rad detergent corrected (DC) Protein Assay (Bio-Rad, Hercules, CA, USA) for determination of protein concentration following detergent solubilization. In order to perform the telomerase PCR-ELISA assay within the linear range, cell extracts equivalent to 2 µg of protein were used. Following PCR-ELISA, telomerase activity was detected using a Dynex-MRX plate reader (Dynex Technologies, Chantilly, VA, USA) and recorded as absorbance units. The values were expressed as relative absorbance at 450 nm ± SE.

Telomeric length determination

Telomeric lengths were determined by Southern blot analysis as described previously (Kruk et al 1995). Briefly, cells were washed with phosphate buffered saline (PBS), lysed in 10 mM TrisHCl/1mM EDTA/0.5% SDS/0.1 mg of proteinase K per ml (Sigma Scientific) at 37°C. DNA was isolated by standard high salt extraction, treated with 100 µg RNase A per ml, and resuspended in 10 mM TrisHCl/ 1 mM EDTA, pH 7.2. The DNA concentration was determined by the absorbance at 260 nm. The DNA was digested to completion with HinfI (1 unit per µg DNA; New England Biolabs, Beverly, MA, USA). DNA (1–2 µg) was loaded onto 0.5% alkaline agarose gels, resolved by electrophoresis, transferred to nylon membranes in 1 N NaOH, and hybridized in Church's hybridization buffer at 42°C with (γ-^{32}P) adenosine triphosphate (ATP) (Dupont/NEN, Wilmington, DE, USA) end-labeled $(TTAGGG)_4$ (Midland Certified Reagent Company, Midland, TX, USA). Membranes were washed in 0.5X saline–sodium citrate (SSC)/0.1% Sodium dodecyl sulfate (SDS) at 42°C, and the telomeres visualized by autoradiography. Mean telomeric lengths were calculated using ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA) based on center of mass calculations and expressed in kb ± SE. Mean telomeric lengths were determined from at least two autoradiograms from each of three biological experiments.

Cell growth

Cell growth was determined by the MTS colorimetric assay (Promega, Madison, WI, USA). The assay was performed in 96 well microtiter plates as described previously (Johnson and Kruk 2002) and is based on soluble formazan production by dehydrogenase enzymes found in metabolically active cells. Cells were plated at 2.5 x 10^3 cells per well. Absorbance was determined at 490 nm using a Dynex MRX plate reader and the results expressed as the mean absorbance ± SE.

DNA ladders

Cellular apoptosis, as performed previously (Johnson et al 2004), was examined via DNA laddering by electrophoresis of 1 µg of high salt-extracted DNA on a 2% agarose gels followed by SYBR green I staining (FMC Bioproducts, Rockland, ME, USA).

SDS-PAGE and Western blot analysis

As described previously, Western blot analysis was performed to observe changes in members of the apoptotic cascade associated with telomerase expression (Alfonso-De Matte, Moses-Soto, et al 2002; Johnson and Kruk 2002; Johnson et al 2004). Cells were trypsinized, pelleted at 500 *g* for 5 min, lysed in ice cold CHAPS lysis buffer and 15 µg protein were added to 4X loading buffer (250 mM Tris pH 6.8, 8% SDS, 20% glycerol, 0.012% bromophenol blue, 4% β-mercaptoethanol), electrophoresed in 12.5 % SDS-polyacrylamide gels, transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA) via semi-dry or wet transfer. All membranes were blocked for 1 hour with 5% non-fat milk Tris Buffered Saline plus 0.1% Tween-20 (T-TBS) and incubated overnight at 4°C in primary antibody. Membranes were incubated and developed according to the Enhanced Chemiluminescent Protocol (Amersham Pharmacia Biotech). After initial blotting, membranes were reprobed for actin to ensure even loading. All antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA).

Statistical analysis

Samples for telomerase PCR-ELISA, MTL, and growth assays were run in triplicate and the data subjected to Student *t* test analysis for determination of statistical significance between treated and untreated samples.

Results

Telomerase increases mean telomeric length and cell growth

In agreement with others (Kim et al 1994; Meyerson et al 1997; Weinrich et al 1997; Counter et al 1998; Luiten et al 2003), we found that transfection with hTERT cDNA was

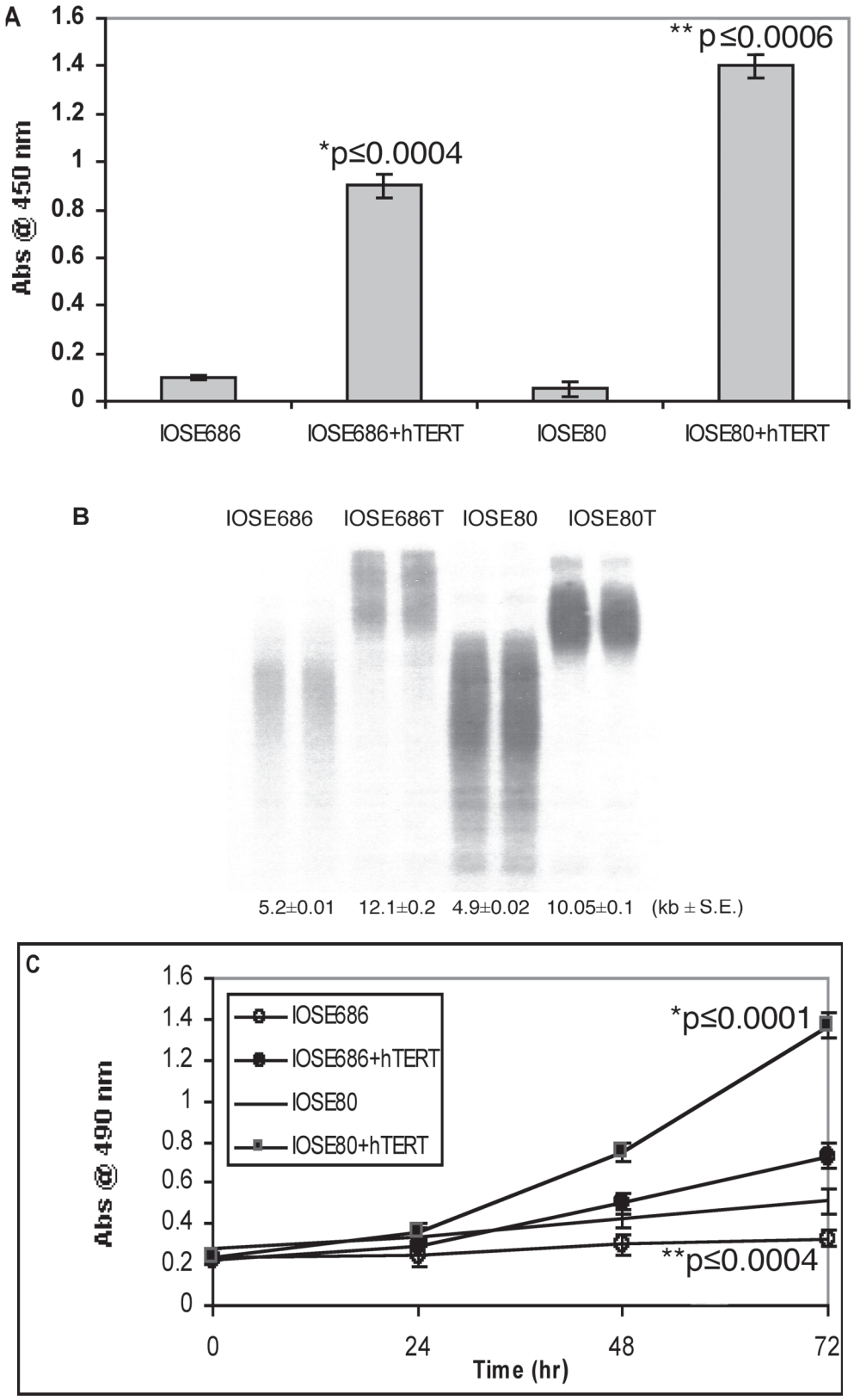
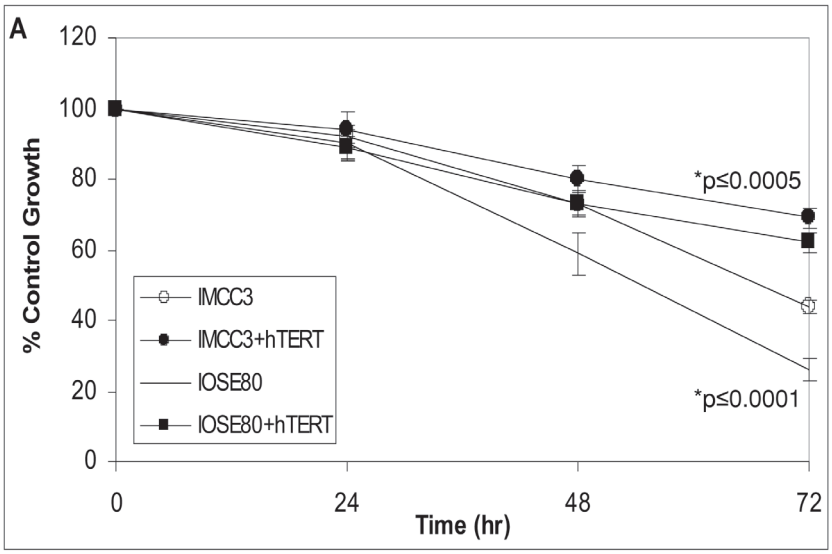


Figure 1 hTERT expression extends mean telomeric length and increases cell growth.
IOSE686 and IOSE80 cells were transfected ± hTERT cDNA and assayed for telomerase activity by PCR-ELISA (A), mean telomeric length by Southern blot analysis (B), and short term growth in cell culture by MTS assay (C). Telomerase activity and cell growth are expressed as the absorbance at 450 nm and 490 nm, respectively, ± SE while mean telomeric length is expressed as average kb ± SE.
Abbreviations: cDNA, single-stranded complementary deoxyribonucleic acid; hTERT, catalytic component of telomerase; IOSE, SV-40 large T-antigen transfected ovarian surface epithelial cell; PCR-ELISA, telomerase polymerase chain reaction-enzyme-linked immunosorbent assay; SE, standard error of mean.

sufficient to activate telomerase expression in ovarian surface epithelial cells (Figure 1A). Likewise, long-term expression of hTERT resulted in increased mean telomeric lengths to levels more than double their telomerase-negative counterparts (Figure 1B) and indicative of functional telomerase activity with hTERT transfection. In addition, cells transfected with hTERT demonstrated increased growth capacity (Figure 1C). That is, cell yield in hTERT-positive cells was found to be three times greater than their telomerase-negative counterparts in short term culture assays.

Telomerase enhances cell survival

To determine whether telomerase conferred increased cell survival, IOSE cells ± hTERT cDNA were treated with 25 μM cisplatin (CP) for 2 hours and assayed for cell growth. Cisplatin suppressed cell growth in parental cell lines 50%–70% of untreated controls (Figure 2A). In contrast, CP was only able to suppress cell growth in hTERT-positive cells by approximately 30% (Figure 2A). While increased growth rates in telomerase-positive cells may contribute to reduced cytotoxicity, parallel cultures were examined for DNA



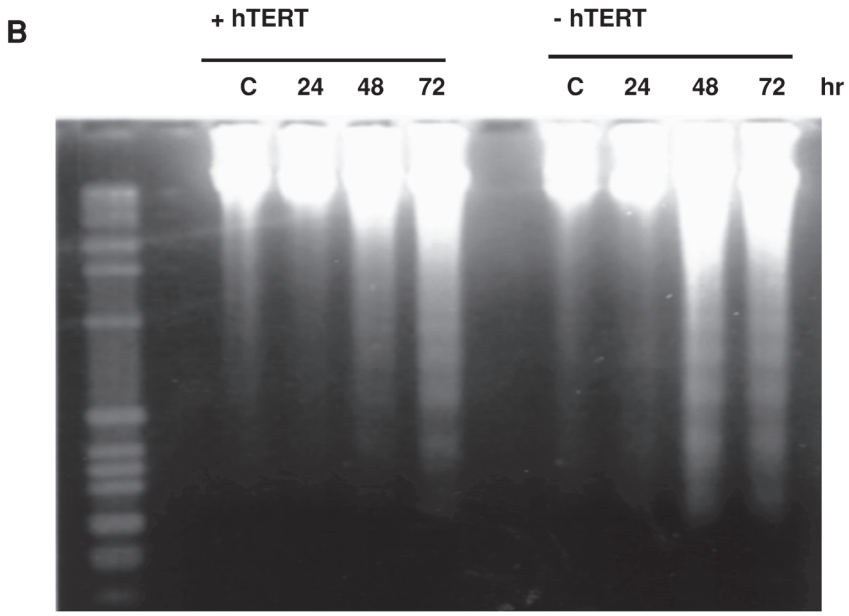


Figure 2 Telomerase enhances cell survival.
IMCC3 and IOSE80 cells were transfected ± hTERT cDNA, treated with 25 μM CP for 2 hr and then assayed for cell growth by MTS (A) or apoptosis by DNA laddering (B). Cell growth is expressed as the absorbance at 490 nm ± SE.
Abbreviations: cDNA, single-stranded complementary deoxyribonucleic acid; CP, cisplatin; hTERT, catalytic component of telomerase; IOSE, SV-40 large T-antigen transfected ovarian surface epithelial cell; SE, standard error of mean.

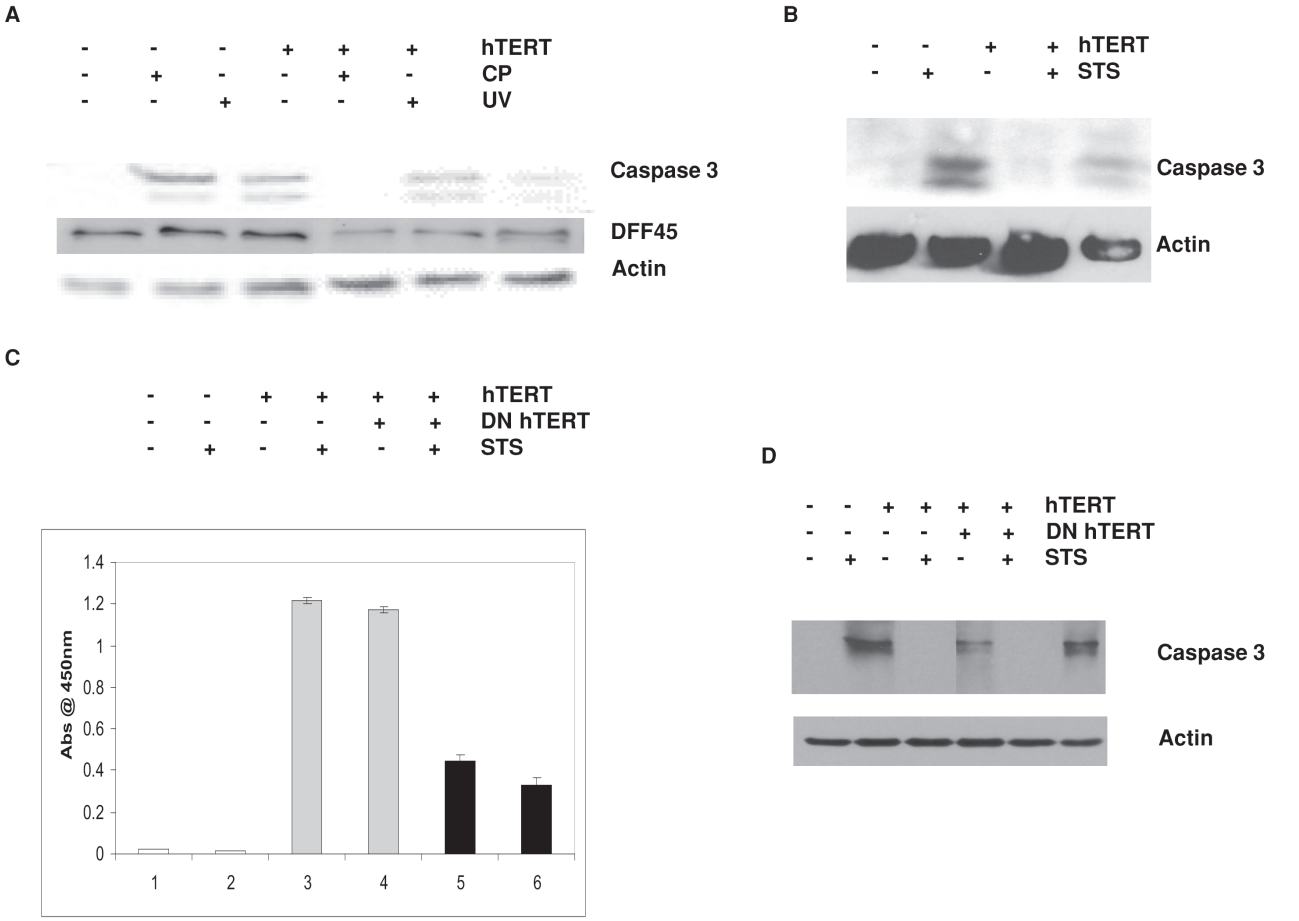


Figure 3 Telomerase confers resistance to caspase 3-dependent apoptosis.
IOSE cells transfected ± hTERT cDNA were treated with 25 μM CP for 2 hours, 20J/m^2 (A), or 1 μM STS for 4 hours (B), and assayed for activated caspase 3 (17 kDa and 19 kDa bands) or DFF45 cleavage (45 kDa band) by Western blot analysis. (C) Control IOSE cells, IOSE cells transfected with hTERT, and IOSE cells transfected with both hTERT and DN hTERT cDNA were treated ± 1 μM STS and examined for telomerase activity by PCR-ELISA. Telomerase activity was expressed as the absorbance at 450 nm ± SE. Samples from (C) were analyzed by Western immunoblot for activated caspase 3 (D). Actin served as a loading control for all Western immunoblots.
Abbreviations: cDNA, single-stranded complementary deoxyribonucleic acid; CP, cisplatin; DFF, DNA fragmentation factor; DN, dominant negative; hTERT, catalytic component of telomerase; IOSE, SV-40 large T-antigen transfected ovarian surface epithelial cell; MTS, ; PCR-ELISA, telomerase polymerase chain reaction-enzyme-linked immunosorbent assay; SE, standard error of mean, STS, staurosporine.

laddering to determine whether reduced apoptosis contributed to reduced cytotoxicity in telomerase-positive cells. In agreement with the cytotoxicity reported above, IOSE cells demonstrated significant DNA laddering and fragmentation by 48 hours following treatment with CP (Figure 2B). In contrast, telomerase-positive cells showed only marginal DNA laddering by 72 hours following CP, indicative of reduced apoptosis in telomerase-positive cells.

Telomerase reduces caspase 3-mediated apoptosis

To determine whether reduced apoptosis in telomerase-positive cells was caspase 3-dependent, we treated telomerase-positive and negative IOSE cells with 25 μM CP for 2 hours, 20J/m^2 ultraviolet (UV) or 1 μM STS for 4 hours and examined cell lysates for levels of activated (cleaved) caspase 3 (Figures 3A, 3B). All three agents were able to induce significant activation of caspase 3 cleavage in telomerase-negative cells. However, we noted reduced caspase 3 cleavage by all three apoptosis-inducing agents in telomerase-positive cells. In addition, some samples were examined for DFF45, a known cleavage target of caspase 3 to ensure caspase 3 activity (Figure 3A). We found increased cleavage of DFF45 in telomerase-negative cells compared with telomerase-positive cells. To ensure that telomerase was sufficient and specific to reduce caspase 3-mediated apoptosis, we co-transfected telomerase-positive cells with a dominant negative (DN) hTERT cDNA to determine whether inhibition of telomerase restored caspase 3 activation. As expected, DN hTERT suppressed telomerase

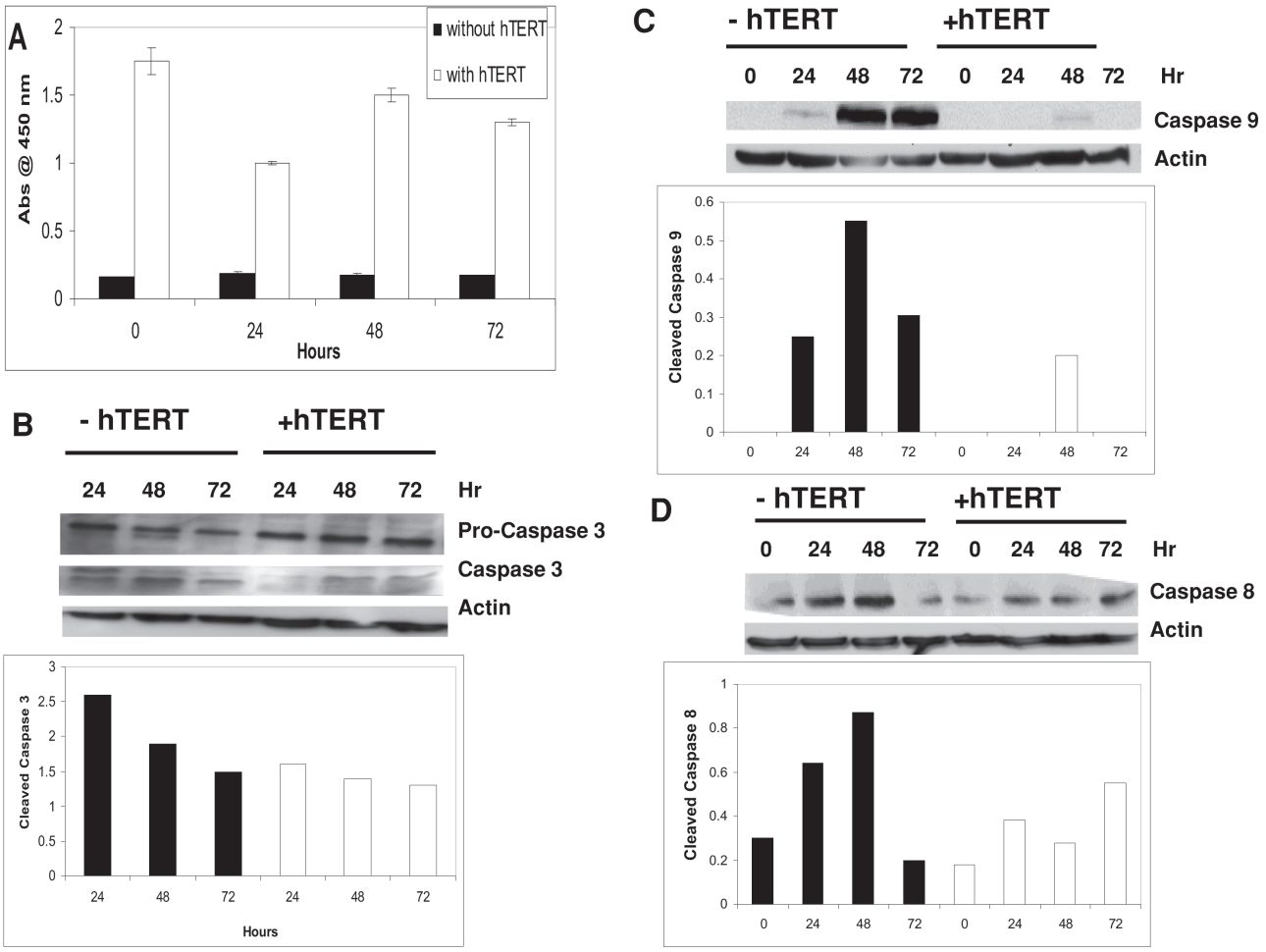


Figure 4 Telomerase-mediated anti-apoptosis is caspase-dependent.
IOSE cells transfected ± hTERT cDNA were treated with 1ng/ml TNF-α for 24 hours and assayed for telomerase activity by PCR-ELISA for up to 72 hours following treatment (A). Telomerase activity was expressed as the absorbance at 450 nm ± SE. Samples from (A) were also analyzed by Western immunoblot for procaspase 3, activated caspase 3 (B), cleaved caspase 9 (C), and cleaved caspase 8 (D). Actin served as a loading control for all Western immunoblots. Densitometric analysis of Western blots is provided in graphical presentation below respective blots.
Abbreviations: cDNA, single-stranded complementary deoxyribonucleic acid; hTERT, catalytic component of telomerase; IOSE, SV-40 large T-antigen transfected ovarian surface epithelial cell; PCR-ELISA, telomerase polymerase chain reaction-enzyme-linked immunosorbent assay; SE, standard error of mean, STS, staurosporine; TNF-α, tumor necrosis factor-α.

activity (Figure 3C), but levels of telomerase were unaffected by STS. When these cultures were examined for caspase 3 activation, DN hTERT was able to restore STS-induced caspase 3 activation to levels found in control cells while hTERT alone suppressed caspase 3 activation (Figure 3D).

Telomerase reduces caspase and mitochondrial apoptotic activation

To identify additional targets of telomerase-mediated anti-apoptosis, IOSE cells ± hTERT were treated with 1 ng/ml TNF-α, which induces apoptosis by both the intrinsic and extrinsic apoptotic pathways. TNF-α did not significantly alter telomerase activity in telomerase-positive cells (Figure 4A). However, activation of caspases 3, 8, and 9 in telomerase-positive cells was reduced by up to 50% compared with their telomerase-negative counterparts following treatment with TNF-α (Figures 4B–4D).

Likewise, telomerase altered pro- and anti-apoptotic mitochondrial protein expression. Densitometric analysis revealed that treatment with TNF-α resulted in up to an 84% reduction in Bcl-2 levels in telomerase-negative cells, but up to a 172% increase in Bcl-2 expression in telomerase-positive cells (Figure 5A), suggesting an anti-apoptotic tendency in the latter cells. When we examined the samples for levels of pro-apoptotic proteins, we found that activation of tBID (by cleavage of BID) was more pronounced in telomerase-negative cells (Figure 5B). Further, when the levels of t-BID to Bcl-2 were compared, the ratio of t-BID/

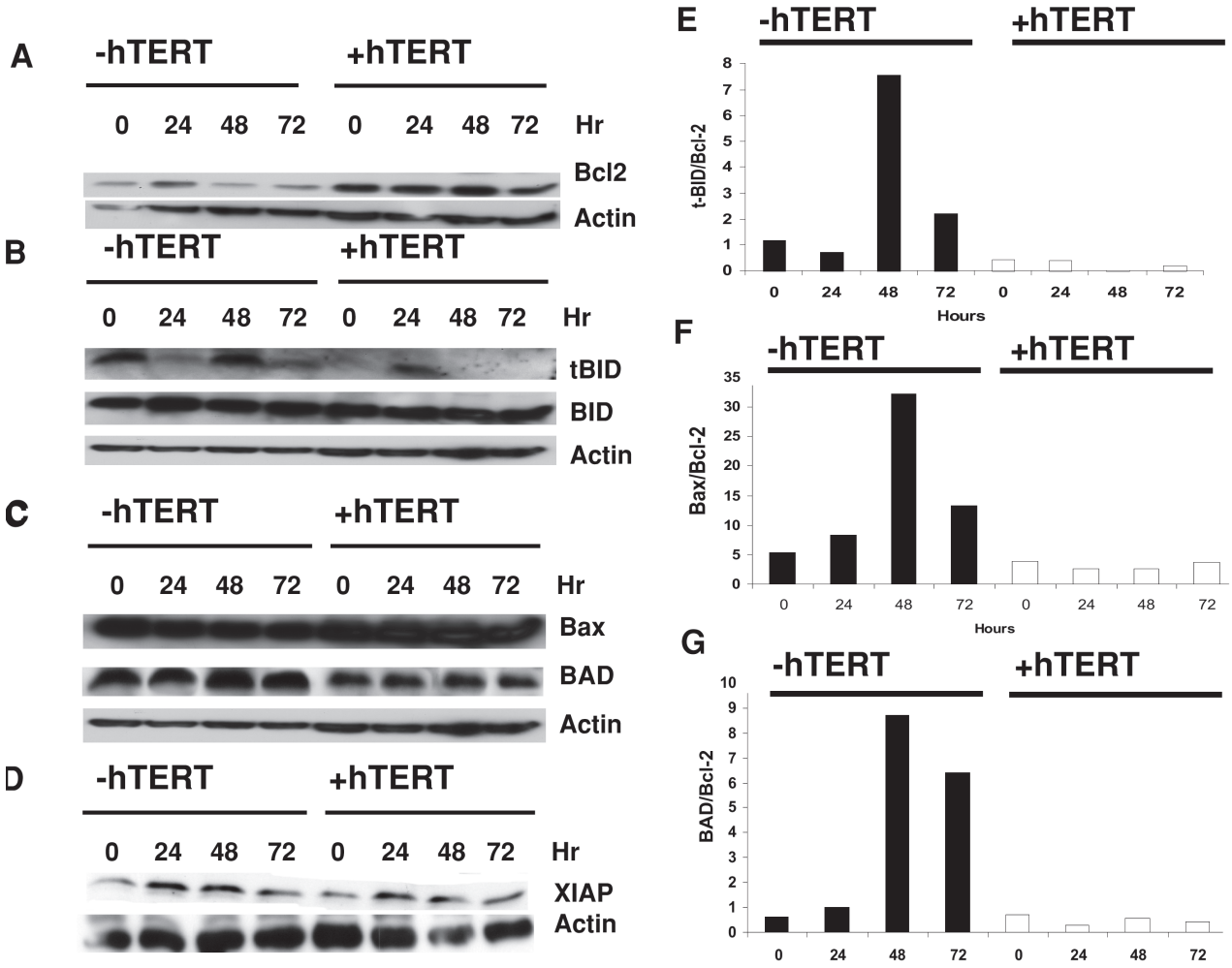


Figure 5 Telomerase alters pro- and anti-apoptotic mitochondrial protein expression.
IOSE cells transfected ± hTERT cDNA were treated with 1 mg/ml TNF-α for 24 hours and analyzed by Western immunoblot for Bcl-2 (A), tBID and BID (B), BAX (C), and XIAP (D). Actin served as a loading control for all Western immunoblots. Densitometric analysis of Western 2blots for the ratios of t-BID/Bcl-2, BAX/Bcl-2 and BAD/Bcl-2 are provided in graphical representation in (E–G), respectively.
Abbreviations: cDNA, single-stranded complementary deoxyribonucleic acid; hTERT, catalytic component of telomerase; IOSE, SV-40 large T-antigen transfected ovarian surface epithelial cell; TNF-α, tumor necrosis factor-α.

Bcl-2 was more than 7 times greater in telomerase-negative cells (Figure 5E). Likewise, while BAX levels did not change significantly during TNF-α treatment course (Figure 5C), densitometric analysis revealed that BAX levels were consistently 20%–25% lower in telomerase-positive cells. When the levels of BAX or BAD to Bcl-2 were compared, the ratios of BAX/Bcl-2 and BAD/Bcl-2 were up to 30 times and 8 times greater, respectively, in telomerase-negative cells indicating an increased propensity for apoptosis in these cells compared with telomerase-positive cells (Figures 5F, 5G). In contrast, telomerase did not appear to alter the levels of the inhibitor of apoptosis protein (IAP), XIAP (Figure 5D).

Telomerase-mediated anti-apoptosis is jnk-dependent

To identify a molecular signaling pathway involved in telomerase-mediated suppression of apoptosis, we assessed the contribution of jnk to suppress apoptosis since we found that induction of apoptosis in telomerase-positive cells was associated with a 2-fold increase in the levels of phosphorylated (p)-jnk compared with telomerase-negative cells (Figure 6A). In contrast, transfection with GFP as a procedural control was not associated with significant increases in p-jnk expression since p-jnk levels in GFP-transfected cells did not differ from untreated cells by more

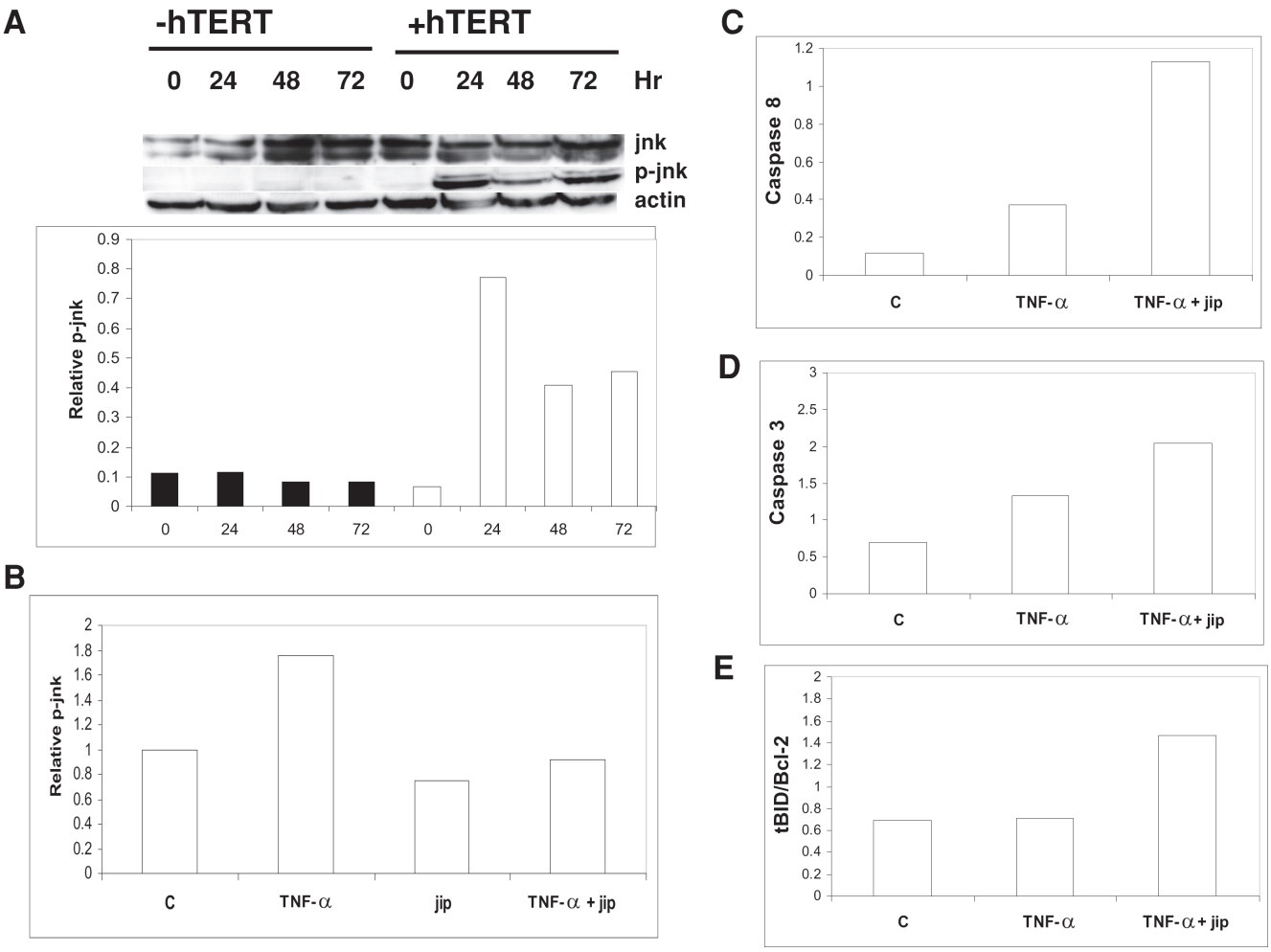


Figure 6 Telomerase-mediated anti-apoptosis is jnk-dependent.
(A) IOSE cells transfected ± hTERT cDNA were treated with 1 ng/ml TNF-α and analyzed by Western immunoblot for p-jnk and jnk. Densitometric analysis of Western blots for the levels of p-jnk is provided in graphical representation below the Western immunoblot. IOSE cells, transfected with hTERT cDNA ± jip cDNA, were treated with 1 ng/ml TNF-α for 24 hours and analyzed by Western immunoblot for p-jnk (B), cleaved caspase 8 (C), cleaved caspase 3 (D), and levels of tBID/Bcl-2 (E). The results are presented as densitometric analyses of Western blots in graphical presentation. Actin served as a loading control for all Western immunoblots.
Abbreviations: cDNA, single-stranded complementary deoxyribonucleic acid; hTERT, catalytic component of telomerase; IOSE, SV-40 large T-antigen transfected ovarian surface epithelial cell; TNF-α, tumor necrosis factor-α.

than 10% (data not shown). Co-transfection with jip, an inhibitor of jnk, not only reduced levels of p-jnk in telomerase-positive cells by 25%, but also abolished TNF-α-mediated induction of p-jnk in these cells (Figure 6B). Likewise, inhibition of jnk with jip in telomerase-positive cells restored apoptotic sensitivity as indicated by increased caspase 3 and 8 cleavage as well as increased levels of tBID/Bcl-2 following treatment with TNF-α (Figures 6C–6E).

Discussion

Apoptosis, programmed cell death, plays an important role in many normal and pathophysiological processes and is triggered when cellular death factors outweigh cellular survival factors. Aspartic acid-specific cysteine proteases (caspases), synthesized as zymogens, are the critical mediators of apoptosis (Cohen 1997). Whether activated by membrane-bound death receptors (Walczak and Krammer 2000) or by stress-induced mitochondrial perturbation with subsequent cytochrome c release (Bratton et al 2000; Loeffler and Kroemer 2000), the respective initiator caspases 8 and 9 function to activate downstream caspases 3 and 7 (Nicholson 1999). Once triggered, caspases lead to stepwise cellular destruction by disrupting the cytoskeleton, shutting down DNA replication and repair, degrading chromosomal

DNA, and, finally, disintegrating the cell into apoptotic bodies (Nagata 2000).

In the present study, several lines of evidence suggest that telomerase confers resistance to epithelial cell apoptosis. First, activation of telomerase by transfection with hTERT resulted in reduced cisplatin-induced cytotoxicity as well as reduced DNA ladder formation. This is consistent with previous reports where telomerase activity in primary cultures of cancer cells was inversely related to chemosensitivity (Faraoni et al 1999). Similarly, in a pilot study, Takahashi et al (2000) showed that ovarian cancer patients responding to platinum therapy had low levels of telomerase whereas 50% of non-responders demonstrated elevated telomerase activity. Likewise, reduced apoptosis in pancreatic cancer cells after exposure to etoposide was associated with elevated telomerase activity (Sato et al 2000).

Second, ectopic expression of telomerase reduced apoptosis by suppressing caspase 3 cleavage in OSE cells. This appears to be the major pathway for telomerase-mediated resistance to apoptosis and has likewise been reported by Yamada et al (2003) who showed that overexpression of hTERT suppressed caspase 3 activity in K562 hematopoietic cells and Luiten et al (2003) who showed reduced caspase 3 activation following ectopic expression of hTERT in T cells. However, there have been reports of telomerase-mediated resistance to apoptosis through caspase 3-independent means (Ren et al 2001), which serves to highlight the potential complexity of telomerase-mediated resistance to apoptosis. Interestingly, we also found that telomerase-mediated suppression of caspase 3 activation occurred in response to a number of apoptotic insults including CP, UV, and STS. Consequently, it appears that telomerase may confer global resistance to apoptosis and may be related to the propensity of many DNA-damaging agents to target the telomeric TTAGGG DNA sequence (Ramirez et al 2003).

Third, hTERT expression was sufficient and specific to confer resistance to apoptosis since co-transfection with dominant negative (DN) hTERT inhibited telomerase activity and abolished resistance to apoptosis. Likewise, we and others have shown that inhibition of telomerase in a variety of cancer cell types resulted in enhanced drug-induced apoptosis associated with increased caspase 3 activity (Kondo et al 1998; Kraemer et al 2004; Shoup et al 2004). Consequently, since telomerase re-activation is a crucial step for cellular immortalization and malignant transformation, inhibition of telomerase may act as a tumor-suppressive mechanism and have clinical utility as adjuvant therapy for enhanced chemosensitization.

Fourth, our data also indicate that telomerase suppresses an early step in the apoptotic cascade since, in addition to caspase 3 cleavage, telomerase re-activation suppressed cleavage of the upstream caspases 8 and 9, but did not affect levels of the caspase 3 inhibitor, XIAP. Further, we found that telomerase-mediated resistance to apoptosis was jnk-dependent because apoptosis associated with ectopic expression of hTERT resulted in elevated levels of p-jnk while suppression of jnk expression by jip abolished resistance to apoptosis. Interestingly, jnk promotes both pro- and anti-apoptotic functions, dependent upon differential phosphorylation of pro- and anti-apoptotic Bcl-2 family members. That is, jnk-mediated cleavage of BID to truncated (t)-BID results in mitochondrial release of cytochrome C whereas its phosphorylation of BAD at theronine 201 inhibits inactivation of the anti-apoptotic protein, BCL-X_l, by BAD (Deng et al 2003; Yu et al 2004). In agreement, we found that re-expression of telomerase resulted in increased Bcl-2 expression consistent with studies showing that telomerase activity is higher in colorectal and cervical cancers expressing high levels of Bcl-2 (Mandal and Kumar 1997; Iida et al 2000). Further, the ratio of pro-apoptotic proteins tBID, BAD, or BAX/Bcl-2 remained low in telomerase-positive cells favoring resistance to apoptosis.

Age-dependent telomeric attrition in normal somatic cells, including endothelial cells, smooth muscle cells, cardiomyocytes, lymphocytes, and neurons, impairs cellular function so that telomeric shortening is thought to contribute to the pathogenesis of chronic diseases of aging including heart disease, diabetes, and Alzheimer's disease (Jeanclos et al 1998; Halvorsen et al 2000; Oh et al 2001, 2003; Obana et al 2003; Panossian et al 2003; Zhang et al 2003; Nakashima et al 2004; Serrano and Andres 2004). Accelerated telomeric shortening associated with progeroid and DNA damage repair syndromes, including Werner's syndrome, Xeroderma pigmentosum, Ataxia telangiectasia, and Bloom's syndrome (Du et al 2004; Kruk et al 1995; Metcalfe et al 1996; Mondello et al 1997), similarly contributes to accelerated cellular senescence and reduced viability. Likewise, aberrant telomerase regulation has been reported in developmental abnormalities, cancer, and disorders of the hematopoietic system. Specifically, in aplastic anemia and Fanconi's anemia, the mean telomeric length of peripheral blood cells is considerably shorter than normal counterparts despite increased levels of telomerase,

which is considered a result of high turnover in hematopoietic stem cells and high rates of telomere breaks (Greenwood and Lansdorp 2003; Ohshima et al 2003; Polychronopoulou and Koutroumba 2004). In addition, autosomal dominant dyskeratosis congenita is associated with mutations in the hTR component of telomerase while X-linked dyskeratosis congenita is due to mutations of dyskerin, a protein involved in RNA processing and that also resides within the telomerase complex (Mitchell et al 1999; Vulliamy et al 2001). Telomerase dysfunction in either form of dyskeratosis congenita results in progressive bone marrow failure. The potential clinical use of telomerase-positive cells or stem cells to expand proliferative capacity and replace damaged somatic cells could reverse or prevent symptoms associated with progeroid disorders, age-related diseases, or diseases associated with dysfunctional telomerase regulation and telomere maintenance. Indeed, Roy et al (2004) showed that when fetal neuroepithelial cells were transfected with hTERT and transplanted at sites of spinal cord injury, they differentiated into functional neurons and became incorporated into the tissue at the damaged site. Using TERT transgenic mice, Oh et al (2003) showed that telomerase expression could delay cardiac myocyte cell cycle exit and stimulate cardiac hypertrophy. Though further studies are warranted, our data indicate that restoration of hTERT expression and telomerase activity in epithelial cells increases cell survival possibly by conferring resistance to apoptotic insults important in the pathogenesis of disease.

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