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Original Paper

Inhibition of Human Telomerase by a Retrovirus Expressing Telomeric Antisense RNA

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Human telomerase, the RNA-dependent DNA polymerase that adds TTAGGG repeats to chromosome ends, is selectively expressed in immortalised cells and most tumours, suggesting a potential role for telomerase inhibitors in cancer therapy. Replication-deficient retroviruses were used to determine whether mRNA containing UUAGGG, the complementary sequence to the template region of the hTR telomerase RNA, is sufficient to inhibit telomerase activity. Telomerase activities measured by the telomeric repeat amplification protocol (TRAP) assay in extracts prepared from immortalised mouse fibroblasts, human HeLa cells and human kidney carcinoma cells were inhibited by 75% or greater in 26 of 56 cell clones expressing UUAGGG. Telomerase activity was not inhibited by expression of mRNA containing a transposed sequence, GGGAUU. Telomerase activities *in vivo* were inferred from changes in cellular morphology, proliferation capacity, growth rate and measurement of the content of telomere DNA. Giant senescent-like cells emerged shortly after cloning mouse PA317 and human HeLa cells expressing UUAGGG. The fraction of giant cells varied from 100% at the fifth population doubling (PD) in one culture to 2–6% at 50 PD in several other cultures. Giant cells were absent in all parental cells and clones expressing GGGAUU. The average cellular content of telomere DNA was independent of telomerase activity over 50 PD. The results indicate that expression of RNA complementary to the template region of hTR is sufficient to inhibit telomerase *in vitro* and *in vivo*, but that the effect of inhibition on individual cells is highly variable.

Key words: telomerase activity, telomerase RNA, retrovirus, antisense RNA, telomerase inhibition, telomere content, cancer
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INTRODUCTION

THE CAPACITY for unlimited replication of human germ cells, immortalised cell lines and tumour cells is attributed in part to the activity of the enzyme telomerase [1–4]. Normally quiescent in somatic cells, telomerase is a ribonucleoprotein that utilises a short sequence of its integral RNA component, hTR, as a template for synthesising telomeric repeats on to chromosome ends [5, 6]. Telomere synthesis presumably counterbalances truncation of chromosomes which occurs with each replicative cycle, and thereby prevents degradation and deleterious rearrangements of chromosome ends that would otherwise lead to cell death [4, 7]. Initially identified in HeLa cell extracts [8], human telomerase has been detected in immortalised cell lines [1], precrisis and postcrisis cells following retroviral infection [9], and more than 85% of tumours [1]. Low levels of telomerase activity have also been found in activated T cells and normal somatic cells with extended proliferative capacities [10, 11].

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The seemingly essential role of telomerase in ensuring chromosome integrity [3, 12], and its nearly ubiquitous occurrence in human cancers [1], have made telomerase a potential target of anticancer therapy [3, 13]. Several successful approaches for inhibiting telomerase have been described, including inhibitors of retroviral reverse transcriptase [14], peptide nucleic acids [15], cisplatin [16], hammerhead ribozymes [17], hTR antisense RNA [18], and hTR gene deletion [19].

Although telomerase activity was inhibited in each of these studies, the biological consequences of the inhibition varied. Feng and coworkers showed that transfected HeLa cells expressing an 185 nucleotide-long antisense RNA to hTR had reduced telomerase activity, shortened telomeres, and entered crisis leading to cell death [18]. In contrast, Strahl and Blackburn reported that B and T cell lines treated with various reverse transcriptase inhibitors also had reduced telomerase activity, but no reproducible telomere shortening or decreased cell viability [14]. Similarly, Blasco and coworkers demonstrated that cell lines derived from hTR knockout mice lacked telomerase activity and had shortened telomeres. Nonetheless, these cells were tumorigenic in nude mice following oncogenic transformation [19]. Finally, approximately 25% of *in vitro* immortalised cell lines and 15% of human tumours have no detectable telomerase activity, yet many have very long and heterogeneous telomeres [20].

The ability of amphotropic replication-deficient retroviruses to transduce a wide range of mammalian cells provides a means of investigating systematically the biological effects of chronic telomerase inhibition in diverse cellular backgrounds. We describe here the construction of a retrovirus expressing RNA complementary to the template region of hTR, and its effects on telomerase activity, cellular viability and telomere maintenance in two immortalised human cell lines.

MATERIALS AND METHODS

Plasmid and retroviral constructs

The parent plasmid containing the retroviral vector that was used in this study as a starting point for our constructs was a gift from Dr R. Paul (Targeted Genetics, Seattle, Washington, U.S.A.). The Moloney murine leukaemia virus-based retroviral element contains a cDNA encoding an encephalomyocarditis virus internal ribosome entry sequence (IRES), and a recombinant gene encoding a bacterial hygromycin phosphotransferase-herpes simplex virus thymidine kinase (HyTK) protein [21]. Double-stranded synthetic DNA oligonucleotides (University of New Mexico Protein Chemistry Laboratory) containing either the forward telomere sequence (FTS), 5′-AATTGA $(TTAGGG)_6$ CCATGG-3′, or the transposed telomere sequence (TTS), 5′-AATTCCATGG $(GGGATT)_6$ TC-3′, were ligated into the *Mun*I endonuclease restriction site downstream of the retroviral promoter and upstream of the IRES (Figure 1). The sequences and orientation of the recombinant constructs were confirmed by DNA sequencing [22].

Cell culture, plasmid transfections and viral transductions

HeLa, A-498 human kidney carcinoma, HS68 human foreskin, and PA317 murine embryonic fibroblast cells were purchased from the American Type Culture Collection (Rockville, Maryland, U.S.A.). HeLa and A-498 cells were cultured in minimal essential medium. The retrovirus packaging cell line PA317 and the HS68 cells were cultured in Dulbecco's modified Eagle's high glucose medium. Media were supplemented with 2 mM L-glutamine, 10% fetal calf serum, streptomycin (0.1 mg/ml), and penicillin (100 U/ml). Cells were cultured at 37°C/5% CO_2 saturated atmosphere. PA317 mouse fibroblasts were transfected with the HyTK plasmid by the calcium phosphate method [22]. HeLa and A-498 kidney carcinoma cells were stably transduced by incubation for 4 days in PA317 culture supernatants containing the retroviral particles in the presence of polybrene (5 mg/ml). Transfected PA317 and transduced HeLa and A-498 cells were selected in medium containing hygromycin B (200 U/ml, Calbiochem-Novabiochem, La Jolla, California, U.S.A.). Parental cells treated in parallel were not viable. Single cell clones were isolated by plating cells at a low density and trypsinising single colonies using glass cloning cylinders. Cell clones were cultivated until they reached subconfluency in the culture flasks prior to harvest.

Cell extracts and telomerase assays

Cell extracts were produced and telomerase activity measured by the telomeric repeat amplification protocol (TRAP assay) as described by Kim and colleagues [1]. Briefly, cells were washed in phosphate buffered saline, resuspended in cold wash buffer (10 mM Hepes–KOH (pH 7.5), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol), pelleted, and incubated for 30 min on ice in lysis buffer containing 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1 propanesulphonate (CHAPS; Sigma Chemical Co., St. Louis, Missouri, U.S.A.). Following centrifugation of the lysates for 1 h at 7°C at 120 000 ***g***, the protein concentration of the supernatants (CHAPS extract) was determined by the Bradford method [23] prior to storage at −70°C. An aliquot containing 0.5, 1.0, or 2.0 μg protein equivalents was used in each TRAP reaction. To verify the specific hTR RNA-dependent telomerase activity, control reactions were performed in the presence of 0.5 μg ribonuclease A (RNase A). Assay tubes were prepared by sequestering 0.1 μg of CX primer (5′-CCCTTACCCTTACCCTTACCCTAA-3′) under a wax barrier (AmpliWax PCR Gem 50; Perkin-Elmer Cetus, Norwalk, Connecticut, U.S.A.). Reactions were performed in 50 μl reaction mixture containing 20 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween-20, 1 mM ethyleneglycol aminoethyl-tetra-acetic acid (EGTA), 50 μM deoxynucleoside triphosphates, 0.1 μg TS oligonucleotide

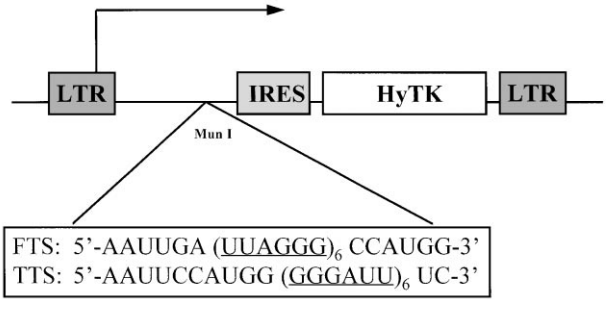

Figure 1. Schematic representation of the hygromycin phosphotransferase–thymidine kinase (HyTK) retroviral construct modified to express the forward telomere sequence (FTS) $(UUAGGG)_6$, or the transposed telomere sequence (TTS) $(GGGAUU)_6$ under the retroviral long terminal repeat (LTR) promoter. The arrow denotes the direction of transcription. IRES, internal ribosome entry site.

(5′-AATCCGTCGAGCAGAGTT-3′) or 62 base oligonucleotide (5′-AATCCGTCGAGCAGAGTT $(TTAGGG)_7$ TT-3′), 0.1 mg/ml bovine serum albumin, 2 U Taq DNA polymerase (Perkin-Elmer Cetus), and 0.5 µl [α-^{32}P]dCTP (3000 Ci/mmol, DuPont NEN, Boston, Massachusetts, U.S.A.). The elongation reaction of the TRAP assay was performed at 23°C for 10 min; polymerase chain reaction (PCR) amplification included 30 cycles at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1.5 min. Aliquots of 20 µl of reaction mixture were analysed in 7.5 or 15% polyacrylamide gels and the gels were dried and analysed by autoradiography. PolyA$^+$RNA included in the TRAP assays where noted was isolated using the Micro-FastTrack mRNA Isolation Kit (Invitrogen, San Diego, California, U.S.A.).

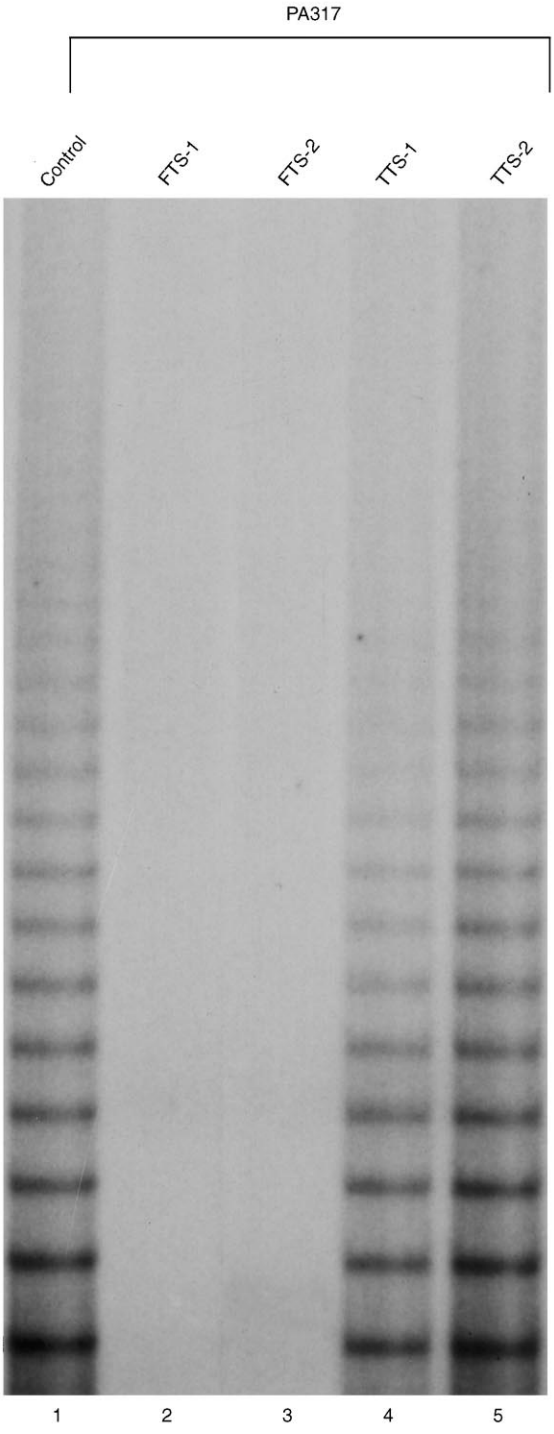

Figure 2. Telomerase activity in PA317 murine embryonic fibroblasts. Telomeric repeat amplification protocol (TRAP) reactions on 3-[(3-cholamidopropyl)dimethylammonio]-1 propane sulphonate (CHAPS) extracts [1] were performed using aliquots of 1.0 µg total cellular protein. Control, non-transfected parental PA317 cells (lane 1); transfected cell clones FTS-1 and FTS-2 (lanes 2 and 3); transfected cell clones TTS-1 and TTS-2 (lanes 4 and 5).

Quantification of TRAP assays

Multiple TRAP assays were performed on each extract to ensure reproducibility of the observed results. For a number of samples, the TRAP-eze telomerase detection kit (Oncor, Gaithersburg, Maryland, U.S.A.) was used in tandem to assay for telomerase activity and identical results were obtained. For quantification, the dried polyacrylamide gels were exposed to a PhosphorImager screen, and individual bands of the TRAP reaction were quantified using the ImageQuant software (Molecular Dynamics, Sunnyvale, California, U.S.A.). Results were corrected for background and a standard value of 100% telomerase activity was given to the signal obtained in paired non-transfected/non-transduced control cells. Signal intensities of TTS and FTS clones were expressed as fractions of the control activity measured in each experiment. Fisher's exact test was used to compare the relative telomerase activities of the cell clones and to determine the correlation between transduction with the FTS retrovirus and telomerase activities < 25%.

Telomere content

Genomic DNA was isolated from cultured cells using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota, U.S.A.) according to the manufacturer's protocol. Telomere content was determined by the telomere slot blot assay recently described by Bryant and colleagues [24]. Genomic DNAs from clonal isolates, typically containing 50, 150 and 300 ng DNA, were denatured for 30 min at 55°C in 0.33 M NaOH and 0.5 M NaCl. Each sample was divided

Table 1. *Telomerase activities of transposed telomere sequence (TTS) and forward telomere sequence (FTS) clones*

	Telomerase activity range*		
	< 25%	25–75%	> 75%
TTS (12 clones analysed)			
HeLa	0	5	1
A-498	1	1	2
PA317	0	0	2
Total	1	6	5
FTS (56 clones analysed)			
HeLa	19	16	9
A-498	5	3	2
PA317	2	0	0
Total	26	19	11

*Telomerase activity was measured by the PhosphorImager method described in Materials and Methods. Non-transduced control cells were assigned a telomerase activity of 100%. Numbers represent the number of clones in each activity range. Clones were tested for telomerase activity in at least two independent experiments. Transduction with hygromycin phosphotransferase-thymidine kinase–FTS is significantly correlated with telomerase activities of < 25% ($P = 0.01$).

into two equal volumes and loaded on to duplicate Hybond-N^+ nylon membranes. The membranes were hybridised to ^{32}P-end-labelled telomere-specific probe, 5-′(TTAGGG)$_3$-3′, or centromere-specific probe, 5′-GTTTTGAAACACTCT-TTTTGTAGAATCTGC-3′ [25], and exposed to a phosphor screen for 24–72 h, depending upon the signal strength. The relative amount of telomere or centromere probe hybridising with each DNA sample was quantified by integrating the volume of the hybridisation signal ratios. The content of centromere DNA is directly proportional to the mass of total DNA and independent of telomere length and is used to normalise for differences in DNA content in different dilutions and in different DNA samples. The relative content of telomere DNA in each sample was determined by dividing the volume of the telomere hybridisation signal T by the volume of the centromere hybridisation signal C and is expressed as the T/C ratio. Telomere lengths were inferred from the T/C ratios as described previously [24]. Terminal restriction fragment (TRF) lengths were also measured by the Southern blot method as described previously [24].

RESULTS

Telomerase inhibition by retroviral vectors

A retroviral vector based on the HyTK virus [21] was designed to express an mRNA containing a repetitive hexamer complementary to the template region of the RNA component of human telomerase (designated FTS) (Figure 1). The HyTK–FTS vector expresses a single transcript that contains the FTS sequence and the coding sequence for a HyTK fusion protein separated by an IRES. Thus, selection for resistance to hygromycin ensures co-expression of the colinear FTS transcript. An identical vector designed to express a transposed sequence (designated TTS) was used as a control (Figure 1).

The HyTK–FTS and HyTK–TTS constructs were introduced into the murine retrovirus packaging cell line, PA317 and transfected cells were selected by resistance to hygromycin B at 200 U/ml. Telomerase activity was measured in non-transfected cells and transfected clones expressing FTS or TTS. As shown in Figure 2, telomerase activity was readily detected by the TRAP assay in both non-transfected PA317 cells (lane 1) and clones expressing TTS (lanes 4 and 5). In striking contrast, telomerase activity was not detected in clones expressing FTS (lanes 2 and 3).

The PA317 cell line produces retroviral virions with an amphotropic host range of mammalian cells [26]. HeLa and A-498 human kidney carcinoma cells were exposed to the virus-containing supernatants, and transduced cells were selected by resistance to hygromycin B. The structural integrity of the integrated retrovirus in all clones described subsequently was confirmed by Southern blotting and the presence of the FTS and TTS sequences was verified by DNA sequencing. Transduced clones were maintained in the presence of hygromycin B and viral expression was also directly

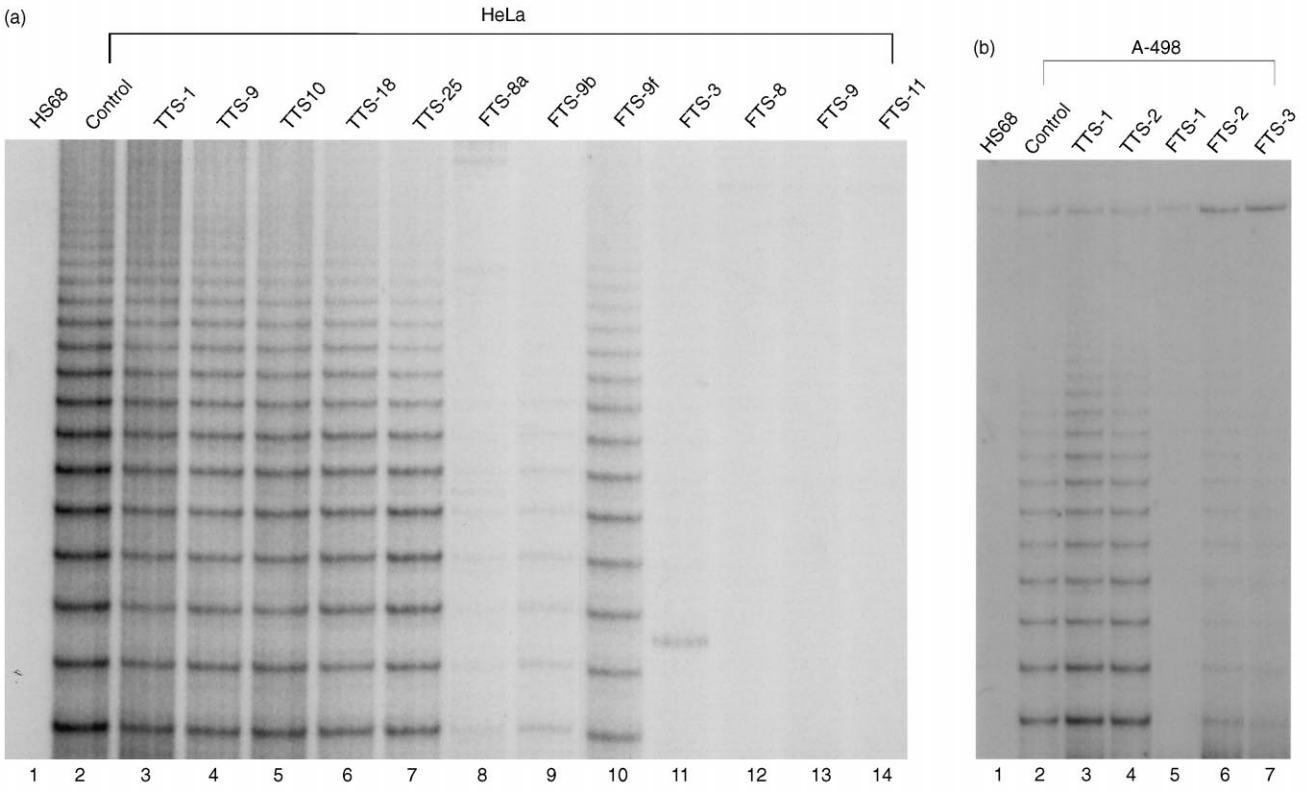

Figure 3. Telomerase activity in forward telomere sequence (FTS) and transposed telomere sequence (TTS) transduced HeLa and A-498 human kidney carcinoma cell clones. The human foreskin cell line, HS68, served as a telomerase-negative control (lane 1). (a) HeLa cells. Non-transduced control parental HeLa (lane 2); transduced TTS clones 1, 9, 10, 18, 25 (lanes 3–7); transduced FTS clones 8a, 9b, 9f, 3, 8, 9, 11 (lanes 8–14); an aliquot of 3-[(3-cholamidopropyl)dimethylammonio]-1 propane sulphonate (CHAPS) extract equivalent to 1.0 μg total cellular protein was used per telomeric repeat amplification protocol (TRAP) reaction. (b) A-498 cells. Non-transduced control A-498 (lane 2); transduced TTS clones 1 and 2 (lanes 3 and 4); transduced FTS clones 1, 2 and 3 (lanes 5–7); an aliquot of CHAPS extract equivalent to 2.0 μg total cellular protein was used per TRAP reaction.

confirmed by hybridisation of mRNA to viral-specific probes (data not shown).

HeLa cell clones expressing FTS exhibited a wide range of telomerase activities (Figure 3a). Telomerase activities of several FTS clones were greatly reduced (lanes 8 and 9) and in some instances were indistinguishable (lanes 11–14) from that of the HS68 cell line (lane 1) that lacks detectable telomerase activity. In another clone (lane 10), telomerase activity was comparable to that of non-transduced control cells (lane 2). In contrast, telomerase activities were not inhibited in clones expressing TTS (lanes 3–7). Similar results (Figure 3b) were obtained in A-498 human kidney carcinoma cells: telomerase activity was variably inhibited in clones expressing FTS (lanes 5–7), but was unaffected in clones expressing TTS (lanes 3 and 4).

Telomerase activities were measured in a total of 56 clones expressing FTS and 12 clones expressing TTS (Table 1). Using a PhosphorImager, the intensity of each TRAP reaction was quantified and assigned to one of three ranges of control activity (< 25%, 25–75%, > 75%). There was a statistically significant correlation ($P = 0.01$) between FTS expression and telomerase activities less than 25% of control. In contrast, no correlation between TTS expression and telomerase activity was observed.

To exclude the possibility that the antisense RNA in extracts of FTS clones interfered with the amplification of elongated products generated in the TRAP assay, the reactions were repeated using a 62 base mock extended oligonucleotide designed to mimic the TS primer extended by seven telomere repeats (Figure 4a). The control extract from non-transduced HeLa cells extended the 62 base oligonucleotide and yielded a full-length ladder similar to the ladders obtained by extension of the TS oligonucleotide (lane 1). The telomerase-negative extract from a cell clone expressing FTS supported amplification, but not elongation of the mock product (lane 2).

The possibility that soluble factors in the telomerase-negative extracts inhibit the elongation reaction of the TRAP assay was also excluded when telomerase-positive (Figure 4b, lane 1) and telomerase-negative extracts (lane 3) were mixed at equivalent protein concentrations (lane 4). The same result was obtained with a 10-fold excess of negative extract. Likewise, inhibition was not observed if the telomerase-positive HeLa control extract was mixed with the poly A^+RNA isolated from an equivalent number of telomerase-negative, FTS-transduced cells (data not shown).

Cellular effects of telomerase inhibition

The effects of FTS and TTS expression on telomerase activity were constant for at least 50 population doublings (PD) in the presence of hygromycin B, enabling analysis of biological effects of chronic telomerase inhibition. Multinucleated giant cells resembling the senescent cells induced by telomerase inhibition in the study of Yegorov and colleagues [27] developed during the isolation of 23/43 FTS-expressing HeLa clones. Several attempts to clone these giant cells were unsuccessful, indicating that they had limited proliferative potential. These giant cells were observed in HeLa and PA317 clones expressing FTS, but not in parental cells or clones expressing TTS (Figure 5 and Table 2). Giant non-viable cells comprised nearly 100% of the cells in HeLa clone FTS-3 at PD 5. The incidence of giant cells also increased with continued passage. In clones FTS-9b and FTS-9c, giant cells increased from approximately 2% at PD 0 to approximately 6–8% at PD 65. No giant cells were observed initially in clones FTS-4i, FTS-8a or FTS-9f. However, the incidence of giant cells increased to 2–6% at PD 65 (Table 2). With the exception of clone FTS-4i, the presence of giant cells in these clones was correlated with telomerase activities of < 25%. Giant cells were never observed in any non-transduced or TTS-expressing cell clones (Table 2 and data not shown).

Initial telomere lengths in 16 FTS-expressing HeLa cells, calculated from telomere DNA content [24], were highly variable, ranging from 2.1 to 12.4 kb. Telomere lengths of parental HeLa cells and five HeLa clones, FTS-4i, FTS-8a, FTS-9b, FTS-9c and FTS-9f, were monitored over 50 PD (Figure 6). There was no consistent correlation between telomerase activity and changes in telomere content. Results obtained by Southern analysis mirrored the changes observed in telomere DNA content (data not shown).

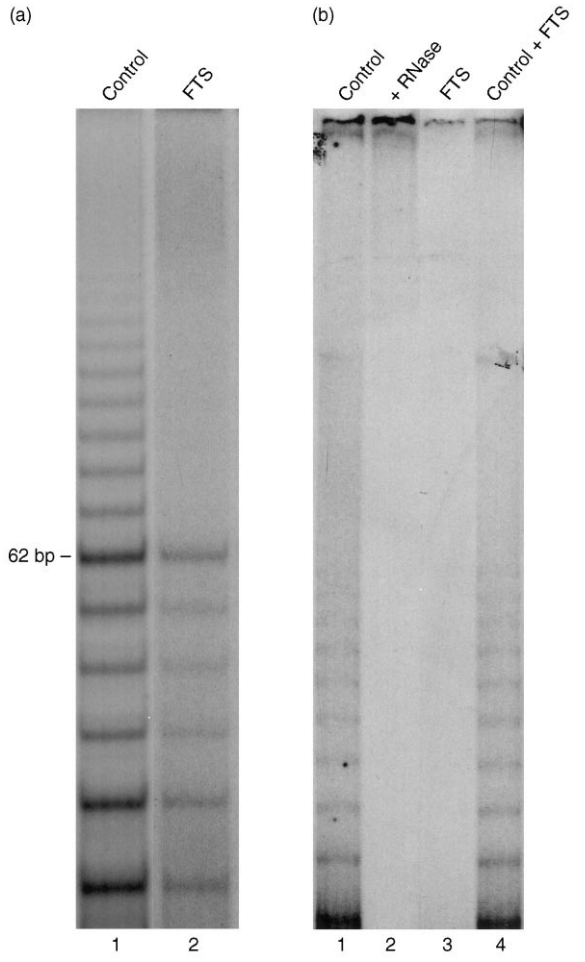

Figure 4. Inhibition studies of telomerase-negative HeLa extracts on amplification (a) and extension (b) of the telomeric repeat amplification protocol (TRAP) assay. (a) The TRAP assays were performed with substitution of the TS oligonucleotide by a 62 base mock extension product on aliquots containing 0.5 μg total cellular protein. Non-transduced control HeLa (lane 1); telomerase-negative forward telomere sequence (FTS) clone (lane 2). (b) The TRAP assays were performed on a mixture (lane 4) of telomerase-positive (lane 1) and telomerase-negative (lane 3) extracts, each at 1.0 μg total cellular protein. The TRAP reaction of the telomerase-positive HeLa control in the presence of RNase confirms the RNA dependence of telomerase activity (lane 2).

DISCUSSION

The two most significant conclusions drawn from this investigation are that human telomerase can be inhibited by a retrovirus expressing mRNA containing a sequence element complementary to only the template region of hTR, and that the viability of cells is reduced when telomerase is inhibited by this method.

Although telomerase activity was inhibited by 75% or more in 46% of clones expressing FTS, there was little or no inhibition of telomerase in the remaining clones expressing FTS. One explanation for this variability is that the site of integration of the retroviral genome influences the expression of FTS [28]. Alternatively, the endogenous levels of telomerase activity may differ between cell clones [20]. However, since telomerase inhibition occurred only in clones expressing FTS ($P = 0.01$) and did not fluctuate over the course of 50 PD, the possibility that the telomerase inhibition was due to periodic fluctuations in the expression of telomerase and not FTS expression is unlikely.

Inhibition of telomerase by FTS expression resulted in the appearance of multinucleated giant cells (Table 2 and Figure 5) that resembled the senescent-like cells appearing

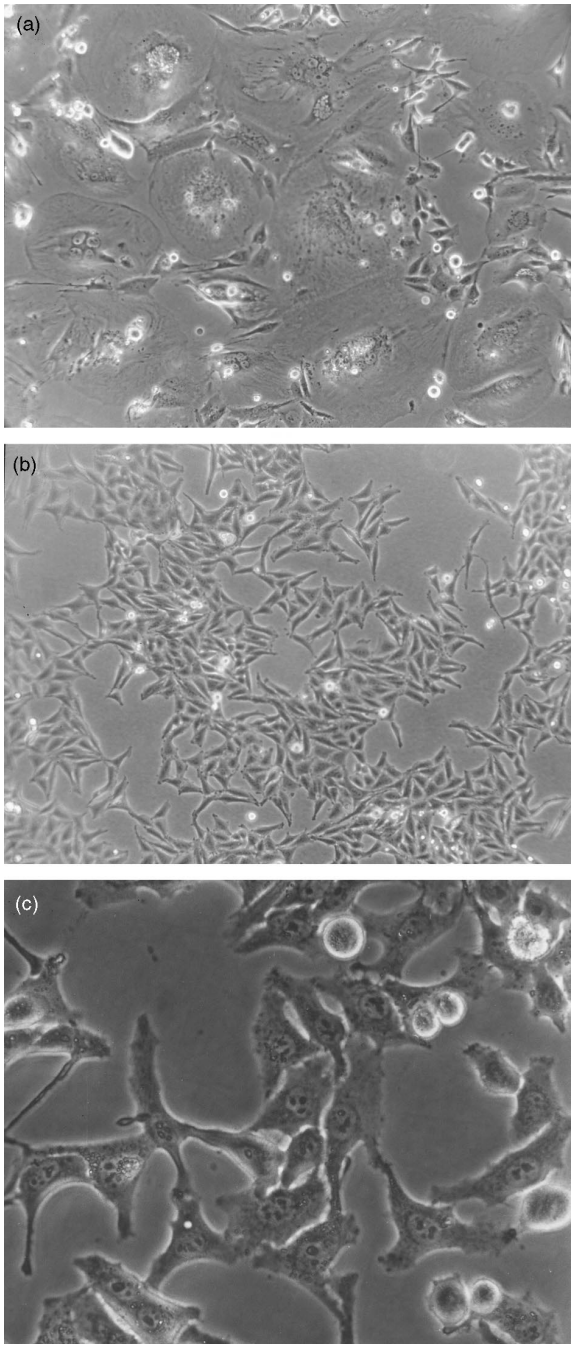

Figure 5. Phase contrast photomicrographs of (a) non-viable, multinucleated giant cells in HeLa clone FTS-3 (magnification 100×), and normally growing cells in (b) HeLa clone TTS-1 (magnification 100×) and (c) non-transduced HeLa control cells (magnification 200×).

Table 2. Telomerase activities and occurrence of giant cells in HeLa forward telomere sequence (FTS) and transposed telomere sequence (TTS) clones

	Telomerase activity* (% of control)	Giant cells† (PD)			
		0	5	16	33
Experiment 1					
HeLa clone					
Control	100	–	–	–	–
TTS-1	>75	–	–	–	–
TTS-9	25–75	–	–	–	–
FTS-3	<25	–	nv		
FTS-11	<25	–	na	+++	na
		0	25	50	65
Experiment 2					
HeLa clone					
Control	100	–	–	–	–
FTS-4i	>75	–	–	+	+
FTS-8a	<25	–	–	+	+
FTS-9b	<25	+	+	++	+++
FTS-9c	<25	+	+	++	+++
FTS-9f	<25	–	+	++	+++

*Telomerase activity was measured by the telomeric repeat amplification protocol (TRAP) assay described in Materials and Methods. Non-transduced HeLa cells were assigned 100% activity. Activities are given in ranges as described in Table 1. †The incidence of giant cells was investigated by microscopic analysis. Each '+' represents approximately 2% of the cell culture. PD, population doublings; na, not analysed; nv, non-viable cell culture, *c.* 100% giant cells.

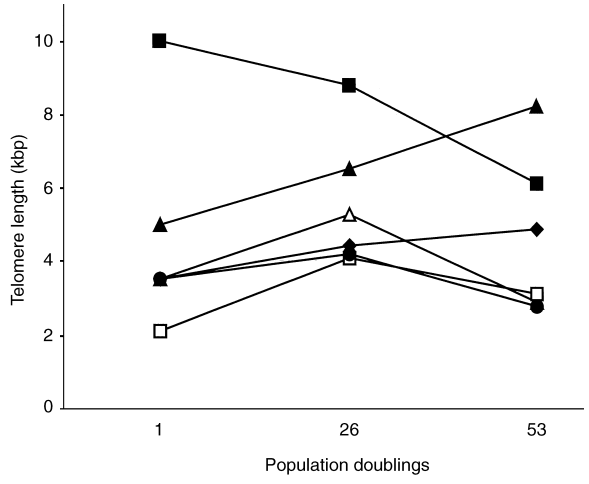

Figure 6. Telomere lengths of HeLa control cells (◆), and clones FTS-4i (■), FTS-8a (▲), FTS-9b (△), FTS-9c (□), and FTS-9f (●) in kbp, calculated from data obtained using the slot blot assay described in Materials and Methods [24]. Telomere lengths were measured for over 50 population doublings.

after the inhibition of telomerase by reverse transcriptase inhibitors [27]. Although 'giant cells' occur occasionally in human tumour cell lines [29], we observed their presence only in cells expressing FTS; giant cells were not observed in either parental or TTS-expressing PA317 or HeLa cells. One HeLa FTS clone that contained giant cells had a significantly reduced growth rate and another ceased to grow after 5 PD. Other FTS-expressing clones either contained giant cells initially or developed them with subsequent passage. Thus, by these criteria, FTS expression reduced the viability of a subset of cells in every FTS clone studied. We speculate that these non-viable giant cells derive from a subpopulation containing one or more critically short telomeres, which in the absence of telomerase activity, enter crisis and die.

Despite the absence of telomerase activity, and the gradual increase in the frequency of non-proliferating giant cells, all but one FTS-expressing, telomerase-negative cell clone remained viable and grew similarly to the non-transduced and TTS-expressing control cells during the 65 PD covered by the present study. There was no consistent correlation between FTS expression, telomerase inhibition or telomere shortening in the several clones examined. Even cells whose telomeres were only 2 kbp in length at PD 0 grew for an additional 65 PD in the absence of telomerase. This observation is in agreement with results recently reported by Blasco and colleagues [19], showing that telomerase-negative murine fibroblasts with critically shortened telomeres derived from mice deleted for the gene encoding mTR (the mouse homologue to hTR) had undiminished proliferative capacities. These findings are consistent with the existence of redundant, telomerase-independent mechanisms of telomere maintenance, such as those proposed to be active in immortalised cells lacking telomerase activity [20].

The molecular mechanism by which the FTS RNA confers the observed reduction of telomerase activity in the HeLa and A-498 clones is not known. However, because telomerase inhibition was FTS-specific, and expression of a 185 nucleotide-long RNA complementary to hTR has previously been shown to inhibit telomerase, it is reasonable to speculate that there is a specific interaction between FTS and the template domain of hTR. Although the proposed interaction of FTS and hTR would involve much smaller target sequences than those in the investigation of Feng and coworkers [18], inhibition of telomerase activity by small telomeric oligonucleotides and ribonucleolytic domains have been reported [17, 30]. Detailed investigations on the mechanism underlying the observed inhibition of telomerase activity by the FTS retrovirus are the subject of our ongoing research.

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