A Quantitative Assay of Telomerase Activity

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Purpose. Telomerase is a ribonucleoprotein that extends telomeres at the ends of chromosome. Increased telomerase activity is associated with cellular immortality. The currently available assay for telomerase, i.e., telomeric repeat amplification protocol (TRAP), consists of 2 steps: (a) telomerase-mediated extension of an oligonucleotide primer by the enzyme-containing extracts of cells and tissues, and (b) amplification of the telomerase-extended primer products by polymerase chain reaction (PCR) and detection of the PCR products. It is generally accepted that the current TRAP assay lacks quantitative precision. The present study was to develop a quantitative telomerase assay with greater precision and sensitivity.

Methods. This new method used the primer extension method as in TRAP, plus the following modifications: (a) used a lysis buffer that yielded complete lysis of nuclei; (b) removal of PCR inhibitors by phenol/chloroform extraction after primer extension; and (c) used primers for the internal standard that were designed to reduce their competition with the telomerase products for PCR.

Results. The modified method showed a good correlation ($r^2 = 0.99$, $P < 0.001$) between telomerase amount (expressed as total protein in cell lysate) and its activity (expressed as telomerase products). Compared to the conventional TRAP, the new method (a) was more sensitive (average of 5.5-fold in cultured cancer cells and >5.9-fold in patient tumors), (b) had a lower inter- and intra-day variability (>3-fold), and (c) showed a 2 to 4-fold broader range of linearity in the standard curve. The higher assay sensitivity further enabled the use of a nonradioactive method, i.e., ethidium bromide staining of DNA, to detect the TRAP products, as opposed to the use of radioactive nucleotide and the more labor-intensive autoradiography mandated by the conventional TRAP.

Conclusion. We report here a quantitative assay for telomerase activity in cultured human cancer cells and patient tumors.

KEY WORDS: telomerase; telomerase assay; TRAP; inhibitor.

INTRODUCTION

Telomeres are located at the ends of the chromosomes in eukaryotic cells, and protect chromosomes from fusion, recombination and degradation. In somatic cells, telomeres are shortened by 50 to 200 nucleotides per cell division. Shortening of the telomere length to below a threshold value is believed to induce senescence. Most germ-line and stem cells, and about 85% of human tumors contain telomerase, a ribonucleoprotein DNA polymerase which synthesizes telomeric repeats *de novo* (reviewed in 1). The differential expression of telomerase between normal somatic cells and tumor cells makes telomerase an attractive tumor marker and a potential target for chemotherapy.

While the regulation of telomerase is unclear, this enzyme is implicated in multiple cellular processes, including cell differentiation, proliferation, cell cycle regulation, apoptosis, tumorigenesis, and possibly also DNA repair and drug resistance (1–4). Hence, quantitation of telomerase activity may have broad implications. The current standard method to measure telomerase activity is the telomeric repeat amplification protocol (TRAP) (5). The TRAP assay mainly consists of two steps, (a) telomerase-mediated extension of an oligonucleotide primer by the enzyme-containing extracts of cells and tissues, (b) amplification of the telomerase-extended primer products by polymerase chain reaction (PCR) and detection of the PCR products. Several modified TRAP assays had since been developed to increase the linearity and sensitivity of the original TRAP (6–9) or to eliminate the use of radioactive nucleotides (10,11). However, the assay reproducibility and accuracy is compromised by the presence of inhibitors in cell and tissue extracts (6,12–14). Results of the present study further show that the extraction procedures in the conventional TRAP did not result in complete lysis of cell nuclei. The goal of the present study was to develop a telomerase assay with greater reproducibility and sensitivity compared to the conventional TRAP. This was accomplished by several modifications which results in (a) complete nuclear lysis, (b) removal of the PCR inhibitors, (c) reduced competition between the internal standard primers and the telomerase products for PCR.

MATERIALS AND METHODS

Chemicals and Reagents

Bicinchoninic Acid Kit for Protein Determination was purchased from Sigma Co. (St. Louis, MO), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), gentamicin from Solo Pak Laboratories (Franklin Park, IL), Minimum Essential Medium (MEM), RPMI1640 medium, and McCoy medium from Life Technologies, Inc. (Grand Island, NY), and Advantage cDNA Polymerase Mix from Clontech (Palo Alto, CA). All chemicals and reagents were used as received.

Tumors and Cultures

Specimens of human head and neck cancers were obtained via the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Human cancer cells, including pharynx FaDu cells, prostate PC3 cells, ovarian SKOV-3 and breast MCF-7 cells, were purchased from American Type Culture Collection (Manassas, VA). FaDu and MCF7 cells were maintained in MEM, PC3 cells in RPMI1640, and SKOV-3 in McCoy medium. Culture medium was supplemented with 9% heat-inactivated FBS, 0.1 mM

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ABBREVIATIONS: MEM, minimum essential medium; SDS, sodium dodecyl sulfate; TRAP, telomeric repeat amplification protocol.

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non-essential amino acids, 2 mM L-glutamine, 90 μg/ml gentamicin and 90 μg/ml cefotaxime sodium. Cells in exponential growth phase were harvested at ~70% confluence.

Preparation of Cell Lysate

In the conventional TRAP (5), cells were harvested by scraping and washed sequentially with PBS and ice-cold washing buffer (10 mM Hepes-KOH (pH 7.5), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol). The cell pellet was resuspended (about 5,000 cells/μl) in ice-cold CHAPS-based lysis buffer (10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol, 0.5% CHAPS, 10% glycerol), on ice for 30 min. After centrifugation at 18,000 g for 15 min at 4°C, the supernatant was used as the cell lysate for the telomerase analysis. To attain complete lysis of the nuclei and to enhance the extraction efficiency, cells were incubated with detergents on ice for 10 min. We evaluated several detergents including SDS, Triton X-100, and Tween 20. The final selected method used SDS-based lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol, 0.1% SDS, and 10% glycerol) to lyze cells (10,000–20,000 cells/μl). To the cell lysate, three volumes of the CHAPS-based buffer were added, mixed, and kept on ice for 5 min. The large volume of the CHAPS-based buffer was to reduce the viscosity and to precipitate genomic DNA. The mixture was centrifuged at 18,000 g for 15 min at 4°C, and the resulting supernatant was stored at −70°C. Protein concentration in extracts was determined using the Bicinchoninic Acid Kit (Sigma, St Louis, MO), and adjusted to 0.5 μg/μl with the CHAPS-based buffer. Samples with equal amounts of protein (1–2 μg) were analyzed for telomerase activity.

To measure the telomerase activity in solid tumors, tumor tissues (~2 mm^3 in size) were minced into smaller fragments (less than 0.5 mm^3) and transferred to 1.5 ml Eppendorf tubes. The tissues were homogenized in the SDS-based lysis buffer, using a Kontes pellet pestle (Fisher, Pittsburgh, PA). Three volumes of the CHAPS-based buffer were then added. The mixture was centrifuged and the supernatant was analyzed as above.

Primer Extension

Telomerase activity is measured as the synthesis of repeating telomere hexamers (TTAGGG) on an upstream primer (5′-AATCCGTCGAGCAGAGTT-3′, or TS primer, see ref. 5). The reaction mixture (final volume of 50 μl) contained 2–4 μl of cell extract, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.005% Tween-20, 1 mM EGTA, 50 μM dNTPs, 0.05 μg of TS primer, and 0.1 mg/ml bovine serum albumin. The mixture was incubated at 30°C for 30 min for primer extension, then at 90°C for 2 min to terminate the reaction.

Internal Standard Primers and Template

We initially used internal standard primers which shared the same sequences as the TS and CX primers (i.e., the upstream and downstream primers used to amplify the telomerase-extended telomeric repeats, see ref. 5). These internal standard primers, i.e., 5′-AATCCGTCGAGCAGAGTTGCTCAACAGTATGGGC-3′ (upstream primer) and 5′-CCCTAACCCTAACCCTAACCCTAATCCGTTTTAGAATCCATG-3′ (downstream primer), amplified a 161 bp segment of pGL3 vector (Promega, Madison, WI) which was used as the template. The use of these primers resulted in a competition with the telomerase-extended telomeric repeats. The competition could not be eliminated by using excess primers (see Results). Hence, we selected another set of internal standard primers which did not share the sequences of the TS and CX primers. These primers, i.e., 5′-ACACAACATACGAGCCGGAA-3′ (upstream primer) and 5′-TTAATGCAGCTGGCACGACA-3′ (downstream primer), amplified a 130 bp segment of pGEM-T Easy vector (Promega, Madison, WI). The amplified template for the internal standard was purified by spin columns (QIAquick PCR purification kit, Qiagen, Valencia, CA). The concentration was determined by a spectrophotometer.

Removal of Inhibitors of PCR

Cell and tissue extracts are known to contain inhibitors of PCR (6,12–14). To remove the inhibitors, the reaction mixture after the primer extension step was extracted with phenol/chloroform, as follows. One μg of tRNA and 0.02 ng of the internal standard template were added to the primer extension products. The total volume of the mixture was brought to 100 μl with water, mixed with an equal volume of buffer-saturated phenol (Life Technology) and then centrifuged at 18,000 g for 3 min, at room temperature. The top aqueous layer (90 μl) was carefully transferred to a new tube and mixed with an equal volume of chloroform: isoamyl alcohol (24:1) solution and centrifuged at 18,000 g for 3 min, at room temperature. The top aqueous layer (80 μl) was again transferred to a new tube and the nucleotides were precipitated with 0.2 volume of 3 M sodium acetate (pH 5.3) and 3 volumes of 100% ethanol at −70°C for at least 2 hr. The mixture was centrifuged at 18,000 g for 15 min at 4°C. After carefully removing the supernatant using suction at room temperature, the pellet was washed once with cold (−20°C) 100% ethanol. The mixture was centrifuged and the ethanol layer was removed using suction. The washed pellet was allowed to completely air-dry and was then resuspended in 20 μl 1× PCR buffer which contained 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 50 mM KCl.

PCR Amplification

The resuspended nucleotide pellet, obtained after phenol/chloroform extraction and ethanol precipitation, was transferred to a MicroAmp tube (0.2 ml thin wall tube, Perkin Elmer, Norwalk, CT) on ice, followed by the addition of 20 μl of a Master II solution (1 ×PCR buffer, 0.1 μg CX primer $(5'\text{-CCCTTA-}3')_4$ 0.06 μg TS primer, 5 ng each of the upstream and downstream primers (see above), 50 μM dNTP, 0.8 μl Advantage cDNA Polymerase Mix (50 ×)). For the radioactive TRAP, 4 μCi ^{32}P-dCTP (ICN, Costa Mesa, CA) was added in the Master II mixture.

PCR was performed on the GeneAmp PCR system 2400 (Perkin Elmer) and initiated by 1 cycle at 94°C for 3 min, 50°C for 4 min, and 68°C for 2 min, then 26 cycles at 94°C for 30 sec, 50°C for 30 sec, and 68°C for 90 sec, followed by 68°C

for 10 min. Ten μl loading buffer (0.25% bromophenol blue, 30% glycerol) was then added to the PCR solution.

Quantitation of TRAP Products

For the nonradioactive TRAP, the PCR products (50 μl total) were loaded on a 10% polyacrylamide gel (Protein II xi Vertical Electrophoresis Cells, Bio-Rad, Hercules, CA). For the radioactive TRAP, 20 μl of PCR product was loaded on a gel.

For both radioactive and nonradioactive TRAP, the internal standard was used to correct for the recovery of DNA after phenol/chloroform extraction, ethanol precipitation and transfer, and to correct for the PCR amplification efficiency. A negative control which used lysis buffer instead of cell/tissue extract was included to correct for the background signal. To quantify the amount of the nonradioactive PCR products, we added 0.75 μg DNA molecular markers (pBR322/HaeIII, Molecular Marker V from Roche, Indianapolis, IN) to each gel. The intensities of the three DNA marker bands at 51, 57, 64 bp with the respective amounts of 8.78, 9.83, and 11.03 ng, or 1.17, 1.31, and 1.47% of the total amount of DNA markers, were used to calculate the amount of the nonradioactive TRAP products.

Gel electrophoresis was performed at a constant voltage of 200 volts for 5 hr. For the detection of nonradiolabeled TRAP products, a gel was stained with 0.25 μg/ml ethedium bromide in water for 20 min, and then washed with water for 30 min. For the detection of radiolabeled TRAP products, a gel was dried on Whatman filter paper by using a gel dryer and processed for autoradiography by exposure to Hyperfilm (Amersham Pharmacia Biotech, Piscataway, NJ) overnight. For both radioactive and nonradioactive methods, the image was captured by a gel documentation system (Gel Print 2000i, Biophotonics, Ann Arbor, MI). The band intensity was analyzed using the GPTools software. To calculate the amount of the nonradioactive TRAP products in sample-of-interest, we used Equation 1 to calculate the actual amount of TRAP products (i.e., bands greater than 50 bp). We further used Equation 2 to normalize for the sample-to-sample variation in the DNA recovery steps and the PCR amplification efficiency.

$$A_{TR} = \frac{I_{TR} - I_C}{I_M - I_{MC}} \cdot A_M \quad (1)$$

$$N_{TR} = A_{TR} \cdot \frac{I_{ISC} - I_C}{I_{ISS} - I_C} \quad (2)$$

Where A_{TR} is the amount of TRAP products; A_M is the amount of the 51, 57, 64 bp marker bands (total 29.6 ng in our assay); I_{TR} is the total intensity of the bands (>50 bp) of TRAP products; I_M is the total intensity of the three DNA marker bands; I_{ISC} and I_{ISS} are the respective intensity of the internal standard in the control and a sample lane; I_{MC} and I_C are the respective background signals in the marker and control lanes; N_{TR} is the normalized amount of TRAP products.

RESULTS

Cell Extraction

The cell extraction method used in the conventional TRAP and the commercially available TRAPeze kit involved the use of a CHAPS-based buffer. This extraction method resulted in a large pellet containing intact cell nuclei (Figure 1A). The use of the detergent SDS resulted in complete lysis of cell nuclei (Figure 1B) and a 12% higher protein concentration in the supernatant (Table I). However, detergents inhibited the telomerase activity, in the rank order of SDS > Triton X-100 > Tween 20 > CHAPS (data not shown). In the primer extension reaction, SDS at concentrations ≥0.005% w/v was found to reduce the telomerase activity by 10 to 20%. The telomerase-inhibiting effect of SDS was minimized by using lower SDS concentration in a small volume of lysis buffer to lyze the cells, followed by the addition of a 3-fold volume of a CHAPS-based buffer to reduce the SDS concentration and viscosity of the lysate. The final concentration of SDS in a typical primer extension assay was <0.002%, or more than 2.5 fold lower than the concentration that caused telomerase inhibition.

Removal of PCR Inhibitors

In the conventional TRAPeze method, the amount of TRAP products (indicated by the band intensities) decreased as the total protein in the cell extracts increased above 1.5 μg (data not shown). This inverse relationship is unexpected and the presence of Taq polymerase inhibitor(s) in cell extracts has been suggested as the cause of the reduction of the TRAP products (6,12–14). We evaluated several methods for removing the inhibitors and for purifying the telomeric products. These methods include proteinase K digestion, heating samples at 95°C, phenol/chloroform extraction, and use of the QIAquick nucleotide removal kit (QIAGEN). Phenol/chloroform extraction followed by ethanol precipitation, which is often used to purify nucleotides, was the most effective method, enhanced the amount of the TRAP products by 5.5 folds (Table I), and yielded a linear relationship between the protein concentration and intensity of the TRAP product bands (Figures 2, 3). We found that the addition of tRNA for co-precipitation, incubation at low temperature (–70°C) for at least 2 hr, high speed centrifugation, and minimal disturbance to the pellets after centrifugation were crucial for complete recovery of the nucleotides during ethanol precipitation.

Internal Standard

The internal standard in TRAP is used to correct for differences in the PCR amplification efficiency. We initially designed an internal standard which shared the same primers

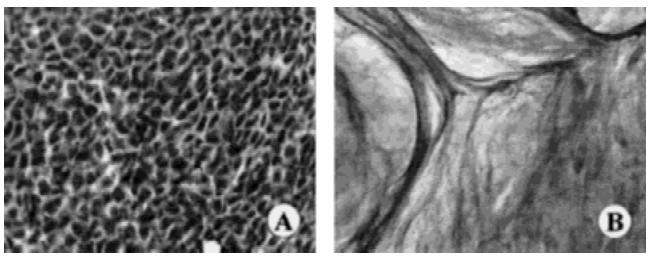

Fig. 1. Comparison of efficiency of different lysis procedures. Lysis of human pharynx cancer FaDu cells. (A) CHAPS-based lysis buffer, which showed intact nuclei in the pellet (hematoxylin and eosin staining). (B) SDS-based lysis buffer, which showed only hematoxylin-stained DNA in the pellet with no residual intact nuclei. 400x magnification.

Table I. Comparison of Sensitivity of Conventional and Modified TRAP

Cell	Extracted protein (ng per cell)			Telomerase product (ng per μg protein)			Overall improvement ratio
	Modified	Conventional	Ratio	Modified	Conventional	Ratio	
FaDu	0.19	0.17	1.14	64.4	16.0	4.0	4.6
MCF-7	0.40	0.37	1.08	137.7	14.9	9.2	9.9
PC-3	0.33	0.31	1.08	14.6	4.7	3.1	3.4
SKOV-3	0.22	0.19	1.16	53.9	15.5	3.5	4.1
Average	0.29	0.26	1.12	67.7	12.8	5.0	5.5

with the telomerase-extended telomeric repeats. Because PCR preferentially amplifies short sequences over longer sequences, an increase in the amount of the shorter telomeric repeats led to a decrease in the amplification product of the internal standard band. For example, no internal standard band was observed at high levels of TRAP products generated by using high protein levels of 8 and 12 μg (Figure 3A). Increasing the primer amount by 3-fold could not reduce the competition and smearing background occurred with the increased concentration of the primers. To overcome this problem, we designed independent primers for amplification of a 130 bp internal standard. This method resulted in a relatively constant intensity of the internal standard band irrespective of the level of TRAP products (see results of the modified method in Figures 2 and 3). However, the intensity of the internal standard band did change, as expected and desired, proportionally with the efficiency of PCR amplification. An example is the data obtained in the presence of different amount of inhibitors of the PCR amplification (Figure 4, $r^2 = 0.98$, $P < 0.001$).

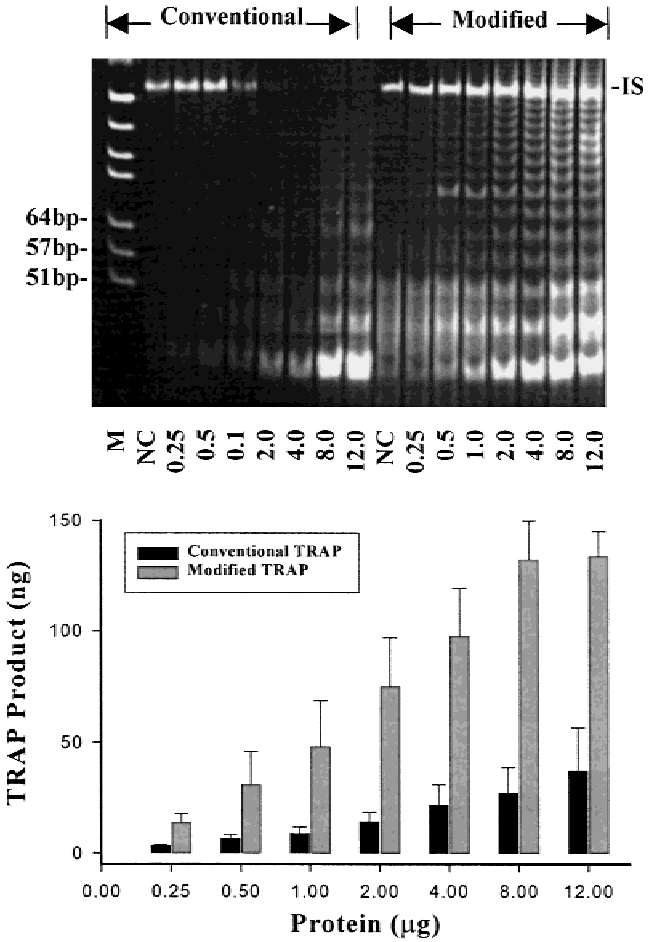

Fig. 2. Comparison of the sensitivity of modified TRAP and conventional TRAP in cultured cells. Human pharynx cancer FaDu cells were used. The conventional TRAP followed the procedures described by Kim et al (5). The modified method was performed as described in Materials and Methods. TRAP products were detected by a nonradioactive method using ethidium bromide to stain DNA. Top: Ethidium bromide-stained gel. M: DNA molecular markers (PBR322/HeaIII). NC: negative control. The other lanes are FaDu cell extracts containing different amounts of proteins in μg, as indicated. The laddering bands indicate TRAP products. IS is the 130 bp internal standard. Bottom: Relationship between telomerase activity and protein concentration. The data are the mean ± SD of three experiments. Note the higher band intensity ($P < 0.01$ for all of the data bars) for the modified TRAP.

Evaluation of the Modified TRAP

Compared to the conventional TRAP, the modified method showed a higher sensitivity (average 5.5 folds, Table I) and greater reproducibility (CV of 43 % for conventional method *vs* 12% for the modified method, data not shown). The improvement in assay sensitivity was almost entirely derived from the use of phenol/chloroform extraction to remove PCR inhibitors (Table I). The higher variability of the conventional TRAP, compared to the modified TRAP, partly resulted from incomplete and variable DNA extraction, and partly from the use of wax beads to hot-start the PCR. Wax beads were used to separate the CX primer (bottom layer) from the telomerase-extended nucleotides (top layer). We found that the uneven surface of wax beads upon melting resulted in trapping of some of the solution and consequently in uneven mixing of the two solutions. This problem was solved by using Advantage cDNA polymerase mix (Clontech), which contains TaqStart antibody that binds and inhibits KlenTag-1 DNA polymerase. Denaturation of the antibody at high temperature during the first cycle of PCR released KlenTag-1 DNA polymerase and thereby initiated the PCR reaction. A similar method using AmpliTaq Gold DNA polymerase has been reported (9).

The modified method showed a good correlation between band intensity of TRAP products and total protein concentrations ($r^2 = 0.99$, $P < 0.001$) with a linear range from 0.25 μg to 8 μg total protein concentration for the nonradioactive TRAP and from 0.25 μg to 4 μg for the radioactive TRAP (Figure 3). The higher sensitivity of the modified method enabled the detection of TRAP product by a nonradioactive method, i.e., staining DNA with ethidium bromide. We compared the telomerase activity measured by the con-

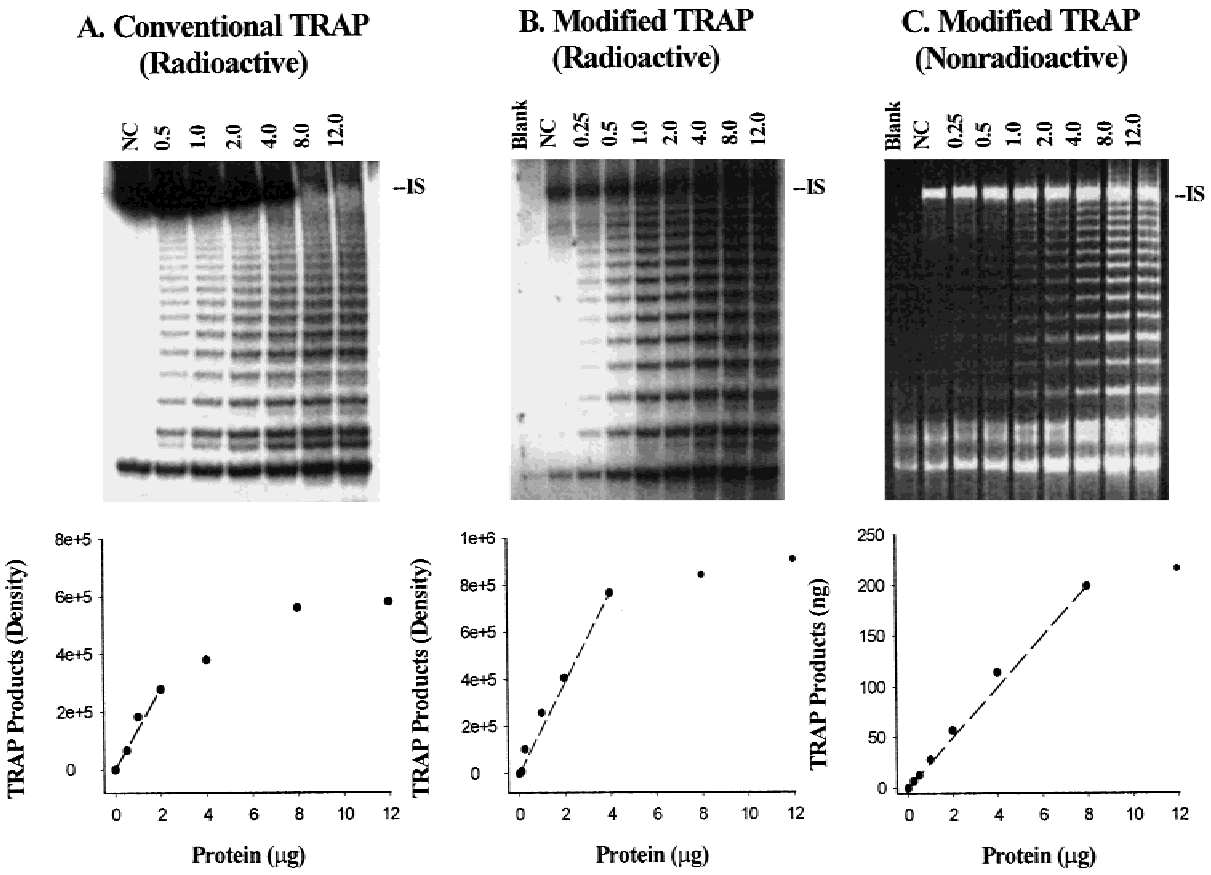

Fig. 3. Comparison of the linearity of modified TRAP and conventional TRAP. Telomerase activity in FaDu cells was detected by both conventional and modified TRAP. The conventional TRAP followed the procedures described by Kim et al (5). The modified method was performed as described in Materials and Methods. For radioactive TRAP, the products were recorded as density values. For nonradioactive TRAP, the density values of TRAP products were converted to actual amount using known amount of DNA marker as references. Blank: reaction mixture without cell extract and internal standard. NC: negative control (using lysis buffer instead of cell extract). The other lanes are FaDu cell extracts containing different amounts of proteins in μg, as indicated. IS is the 130 bp internal standard.

ventional and modified TRAP in head and neck tumors obtained from 17 patients, using the nonradioactive method to measure TRAP products. Figure 5 shows that telomerase activity was detectable in all 17 tumors by the modified method but in only 14 tumors by the conventional TRAP. The ratio of the amount of TRAP products detected by the modified method to that by the conventional method ranged from 2.9 to >9.9 (average of >5.9-fold), indicating an 5.9-fold higher sensitivity for the modified method in solid tumors.

DISCUSSION

The present study established an improved TRAP for measuring telomerase activity. The three major modifications were: (a) using a SDS-based lysis buffer to lyze cell or tissues completely; (b) removal of PCR inhibitors by phenol/chloroform extraction after TS primer extension, (c) using internal standard primers that were designed to reduce their competition with the telomerase-extended telomeric repeats. Other minor modifications include substituting the wax bead method with a hot-start Taq DNA polymerase to reduce variability, and decreasing the number of PCR cycles to 27 to remain in the exponential phase of PCR amplification. The improved method yielded a significantly higher sensitivity and

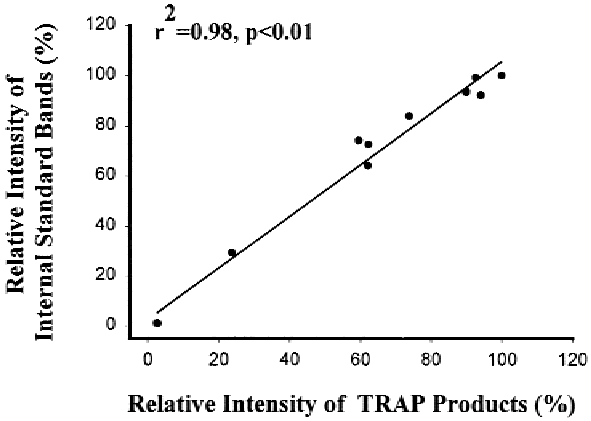

Fig. 4. Correlation between internal standard and PCR efficiency. After primer extension, phenol/chloroform extraction, and ethanol precipitation, different concentrations of Taq DNA polymerase inhibitor (suramin, from 0.1 to 100 μM) was used to reduce the PCR efficiency. The band intensity obtained in the absence of suramin was used as 100%, and results of other samples were expressed as percentages thereof.

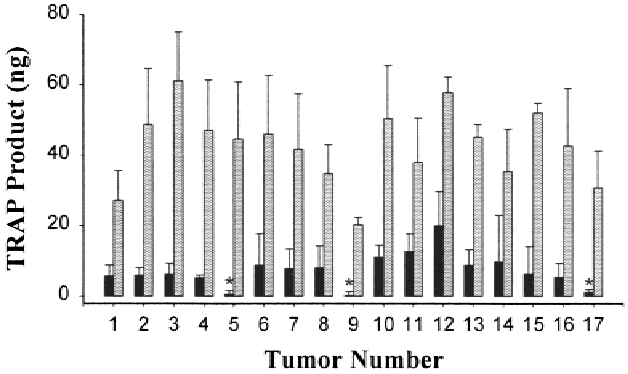

Fig. 5. Comparison of sensitivity of modified TRAP and conventional TRAP in patient tumor tissues. Head and neck tumors were obtained from 17 patients and analyzed for telomerase activity. The conventional TRAP followed the procedures described by Kim et al (5). The modified method was performed as described in Materials and Methods. Dark bars: conventional TRAP. Light bars: modified TRAP. Note the higher amounts of TRAP products for the modified TRAP (average of >5.9-fold, $P < 0.01$ for all of the tumors). *: Tumors did not show TRAP products above the background level.

reproducibility compared to the conventional TRAP. The higher sensitivity and the broader linear range of the modified method further enabled the use of ethidium bromide staining to detect the TRAP products, thus eliminating the need of using radioactivity. In comparison, the conventional TRAP, because of its lower sensitivity, mandates the use of radioactive nucleotide or DNA fluorescent dye such as the PicoGreen that requires specialized equipment for detection (13,14). The more narrow linear range for the radioactivity-based assay may be due to the saturation of the x-ray film at high TRAP products.

The presence of TRAP inhibitor(s) in cell or tissue extracts is widely known (6,12–14). These inhibitors interfere with the PCR amplification efficiency, and thereby reduce the accuracy and precision of the telomerase activity determination. One method to avoid the inhibitor(s) is to use a concentration of proteins that is below the threshold for PCR inhibition (6,13); but this may compromise the assay sensitivity. Sun et al. recently reported a non-TRAP based assay where a biotinylated primer was used for telomeric extension and the product was then isolated by streptoavidin-coated beads and analyzed by electrophoresis and autoradiography (15). Because the telomeric products were not amplified, this non-TRAP based method has a lower sensitivity compared to a TRAP-based method. However, this non-TRAP based method may serve as an alternative to phenol/chloroform extraction for purification of the telomerase-elongated primer and to eliminate the inhibitors in the TRAP assay. The inhibitors of the TRAP method may originate from exogenous and intracellular sources (14). Hemoglobin was found to be an exogenous inhibitor, whereas the identity of the intracellular inhibitors is not known. Our results showed that the inhibitor(s) was removed by phenol/chloroform extraction but not by heating nor proteinase K digestion, suggesting that the inhibitor(s) may not be proteinaceous.

In molecular medicine, many therapeutic targets are molecules that require biological assays. For telomerase, its activity in cells is measured by its ability to extend the telomere. The telomerase-extended telomeric repeats, because of their low quantity, are then measured after PCR amplification. These biological methods, because of their inherent complexity (e.g., the presence of PCR inhibitors in the primer extension products and the competition between the internal standard primers and the primers for the telomerase-extended telomeric repeats), are often less quantitative than chemical methods such as high pressure liquid chromatography. The present study exemplifies an approach to improve the quantitative ability of an assay of a potentially important molecular target.

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