doi:10.1006/mthe.2000.0196, available online at http://www.idealibrary.com on IDEAL

ARTICLE

Use of Transcriptional Regulatory Sequences of Telomerase (hTER and hTERT) for Selective Killing of Cancer Cells

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Received for publication July 11, 2000; accepted in revised form September 25, 2000; published online October 20, 2000

Telomerase (hTER and hTERT) plays a crucial role in cellular immortalization and carcinogenesis. Telomerase activity can be detected in about 85% of different malignant tumors, but is absent in most normal cells. *In situ* hybridization analysis showed that high levels of hTER and hTERT expression are present in bladder cancer, while no signal was detected in normal tissue. Therefore, in this work we propose to use hTER and hTERT transcriptional regulatory sequences to control the expression of a cytotoxic gene in bladder tumor cells, resulting in the selective destruction of this cell population. Expression vectors containing the diphtheria toxin A-chain (DT-A) gene were linked to hTER and hTERT transcriptional regulatory sequences, respectively. Inhibition of protein synthesis occurred in bladder and hepatocellular carcinoma cells transfected with the plasmids containing the DT-A gene under the control of the hTER or hTERT promoters in correlation with their activity. These studies support the feasibility of using hTER and hTERT transcriptional regulatory sequences for targeted patient-oriented gene therapy of human cancer.

Key Words: **telomerase; gene therapy; bladder cancer; diphtheria toxin.**

INTRODUCTION

One of the most crucial enzymes in cell immortality, cell aging, and cancer is telomerase. The telomerase enzyme is composed of three subunits, two of them are essential for its activity, the RNA component coded by the hTER gene and the catalytic subunit coded by the hTERT gene, both were cloned and sequenced (1–4). It has been demonstrated that in many cell lines that express hTER, telomerase activity is not detected, in contrast to hTERT expression that is generally found in telomerase positive cell lines (5). However several studies showed clear differentials in hTER expression between certain cancerous and their adjacent normal tissue (5). This difference in expression levels suggests that hTER promoter may be an interesting candidate to be used in gene therapy. Expression of hTERT is observed at high levels in malignant tumors and cancer cell lines but not in normal tissues or telomerase-negative cell lines, indicating a strong correlation between hTERT expression and telomerase activity in a variety of tumors (6, 7). These data suggest that hTERT expression, at least in some contexts, may be the limiting factor for telomerase activity. These findings and others strongly suggest an important role for telomerase in cell immortalization and tumorigenesis, making this enzyme a promising candidate for cancer diagnosis and therapy. Great efforts were invested to develop therapies by inhibiting telomerase activity in the cells of cancer tissues without causing serious harm to the healthy tissue of the patients (1, 8).

A 5′ flanking region of 341 bp in the hTER gene was identified as the minimal promoter region, containing elements responsible for activity (9). Recently, it was shown that a 181-bp fragment upstream of the transcription start site is a core functional promoter essential for transcriptional activation of hTERT gene in cancer cells (4). The differential activity of this region in mortal and immortal cells opens the possibility of utilizing the hTERT promoter for the selective expression of toxic genes only in cancer cells. The presence of a large CpG

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1525-0016/00 $35.00

island with a dense CG-rich content in the hTERT promoter suggests that DNA methylation play a role in the regulation of hTERT expression (10).

In the present study, we have shown the expression of both hTER and hTERT in human bladder cancer tissue sections using *in situ* hybridization techniques (ISH). We cloned the promoter regions of hTER and hTERT genes, and used these regulatory elements for their potential use in targeted gene therapy, by constructing expression vectors containing the toxin DT-A gene under the control of hTER or hTERT regulatory sequences.

The diphtheria toxin A chain (DT-A) catalytically ADP ribosylates the diphtamide group of eukaryotic elongation factor 2 (eEF2), inhibiting protein synthesis, and activating apoptosis (11, 12). Although diphtheria toxin is highly toxic to eukaryotic cells (13), transgenic mice containing the DT-A gene under the control of different tissue-specific promoters showed that expression of DT-A does not lead to unintended toxicity to other cells (14, 15).

In this study, we present evidence that transfection of bladder and hepatocarcinoma cell lines with DT-A expression plasmids under the control of hTER or hTERT regulatory elements resulted in the killing of the cell in accordance with the relative activity of these promoter elements in the above mentioned cell lines.

MATERIALS AND METHODS

Cell culture. Human bladder carcinoma cell lines T24P and RT 112, hepatocellular carcinoma cell lines HepG2 and Hep3B, primary fibroblast cell line IMR-90, osteosarcoma cell lines U-2-OS and SAOS-2, and embryonic kidney cell line 293T were all obtained from the American Type Culture Collection USA (ATCC). The cells were grown as previously described (16). The 293 Diphtheria toxin resistant cells were obtained from Dr. Giladi (Hadassah Medical Hospital, Jerusalem), were grown in the presence of 1 μg/ml Diphtheria toxin (Calbiochem), their characterization will be published elsewhere (paper in preparation).

Luciferase reporter gene constructs. All the luciferase gene reporter vectors were constructed from the pGL3 basic (Luc-1) vector (Promega) which lacks both eukaryotic promoter and enhancer sequences. A region of 505 bp from the telomerase RNA (hTER) promoter (AF047386) was amplified from nucleotide –436 to +69 using primers 5′-ATATGGTACCACTGAGCCGAGACAAGATTCTGCT-3′ (upstream) and 5′-ATATAAGCTTTACGCCCTTCTCAGTTAGGGTTAGAC-3′ (downstream). A region of 773 bp from the telomerase catalytic unit (hTERT) promoter (AF097365) from nucleotide –419 to +354 was amplified using PCR primers 5′-ATATGGTACCATTCGACCTCTCTCCGCTGG-3′ (upstream) and 5′-ATATAAGCTTTCGGGCCACCAGCTCCTT-3′ (downstream). The restriction sites of *Kpn*I and *Hin*dIII were incorporated in the primer design. The resulting PCR products were digested by these enzymes and ligated into the Luc-1 basic vector yielding Luc–TER and Luc–TERT, respectively. Both Luc–TER and Luc–TERT plasmids were digested with *Xba*I and *Nco*I and the insert of luciferase gene (luc) was replaced by Diphtheria toxin A chain (DT-A) coding region to yield DTA–TER and DTA–TERT.

Transfection and luciferase assay. A total of 0.4×10^6 cells were plated in a six-well Nunc multidish (30 mm). Transient transfection using 7 μg of plasmid was carried out using the calcium phosphate method as previously described (16), the incubation time of the precipitate formation was modified to 15 min. Cells were harvested after 48 h and luciferase activity was measured using the Promega kit "Luciferse Assay System" (E-1500-PROMEGA, U.S.A.). Light output was detected using a Lumac Biocounter apparatus. Protein content was measured for each cell lysate by the Bio-Rad protein assay reagent, and the results were expressed as light units/μg protein.

DIG-labeled RNA probes. A region of 598 bp of the human telomerase RNA (hTER) gene (U85256) was amplified using PCR primers 5′-GGGTTGCGGAGGGTGGGC-3′ (upstream) and 5′-CGACTTTGGAGGTGCCTTCA-3′ (downstream). A region of 376 bp of the human telomerase reverse transcriptase (hTERT) gene (AF015950) was amplified using PCR primers HT1 5′-AAGTTCCTGCACTGGCTGATGAG-3′ (upstream) and HT5 5′-TCGTAGTTGAGCACGCTGAACAG-3′ (downstream). These regions were cloned into the T-easy vector (Promega), the plasmid was linearized by *Sal*I for antisense RNA transcripts. RNA transcripts generated by T7 were labeled with DIG-11–UTP by using the Amersham kit (Boehringer Mannheim) according to manufacturer's instructions.

Sequencing. Sequencing of the plasmids was carried out on both strands of DNA using the automated DNA sequencing according to Ye terminator cycle sequencing procedure using ABT Prism 379, Perkin–Elmer DNA sequencer.

In situ hybridization. Sections of bladder cancer and normal bladder were treated and prepared as previously described (17). Each section was covered with 30 μl of the hybridization solution containing 30–100 ng of DIG-labeled hTER and hTERT probes.

Determination of hTER and hTERT mRNA. Reverse-transcriptase polymerase chain reaction (RT–PCR) was performed to synthesize the cDNA of the hTER and hTERT genes from 1 μg total RNA. The cDNA hTERT was amplified using PCR primers 5′-CGGAAGAGTGTCTGGAGCAA-3′ (upstream) and 5′-GGATGAAGCGGAGTCTGGA-3′ (downstream) (2), and the reaction was carried out for 28 cycles (94°C for 45 s, 60°C for 45 s, and 72°C for 90 s). The cDNA of hTER was amplified using primers 5′-ACCCTAACTGAGAAGGGCGT-3′ (upstream) and 5′-GCCAGCAGCTGACATTTTTT-3′ (downstream) (18), and the reaction was carried out for 29 cycles (96°C for 15 s, 55°C for 30 s and 72°C for 30 s). The efficiency of cDNA synthesis from each sample was estimated by PCR with β-actin-specific primers (Stratagene). The PCR products were separated by electrophoresis in TAE buffer on 2% agarose gel and stained with ethidium bromide.

In vitro methylation. In vitro methylation of the constructs Luc–TER and Luc–TERT was carried out using universal CpG, *Hae*III, *Pst*I, *Eco*RI, and dam methylases (New England Biolabs, Beverly, MA), completeness of methylation was tested by treating the methylated plasmid with the correspondent restriction enzymes.

RESULTS AND DISCUSSION

Expression of hTERT and hTER

It was previously reported that most bladder tissue cancers express hTERT and this expression is associated with telomerase activity, which is absent in normal bladder tissues (19). Lately a new diagnostic kit for detection of telomerase activity in tissue and in exfoliated cells was developed (Boehringer). Although this method is highly sensitive for the detection of telomerase activity, it cannot examine the expression of each of the telomerase components (hTER and hTERT) separately, at the single cell level. Therefore, in this investigation we measured the expression of hTER and hTERT by ISH in tissue sections from patients with bladder cancer, allowing us to examine the histological distribution of the hTER and hTERT expression. Figure 1A shows a high level of hTER expression in papillary bladder carcinoma, while no signal was detected in normal tissue (Fig. 1B). Figure 1C reveals a high signal of hTERT expression in solid tumor,

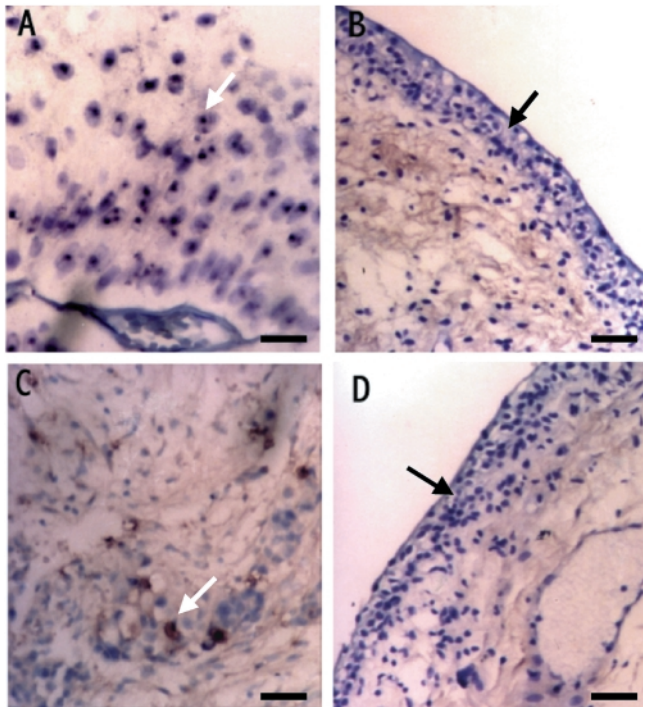

FIG. 1. Expression of hTER and hTERT in tissue sections of bladder cancer-papillary transitional cell carcinoma hybridized with probe for hTER (A); normal bladder epithelial mucosa hybridized with probe for hTER (B); invasive transitional cell carcinoma hybridized with hTERT probe (C); normal bladder epithelial nonneoplastic bladder mucosa hybridized with hTERT probe (D). hTER and hTERT expression is indicated by strong signals (white arrows) in bladder cancer cells (A and C), respectively. Expression of hTER and hTERT is absent in normal bladder mucosa (B and D), respectively (black arrows). Scale bars: A, 5 μm; C, 10 μm; B and D, 50 μm. Sections of bladder cancer were treated and prepared as described previously (17).

while normal bladder cells show only a very faint signal (Fig. 1D).

We measured telomerase activity by the telomerase PCR ELISA kit in T24P, RT112, HepG2, Hep3B, IMR-90, U-2-OS, and Saos-2 cell lines (data not shown). As expected the bladder and hepatocarcinoma cell lines tested showed high telomerase activity while no activity was detected in the IMR-90 cell line and in the osteosarcoma cell lines, known as telomerase-negative cells (20, 21).

Expression of hTER and hTERT in Various Cell Lines by RT–PCR Analysis

We analyzed hTER and hTERT expression in various bladder and hepatocarcinoma cell lines by RT–PCR analysis. Figure 2 shows significant levels of hTER RNA in the cell lines of HepG2, Hep3B, T24P, and RT112. The telomerase-negative primary fibroblasts cell line IMR-90, and the immortalized osteosarcoma cells also expressed hTER, this result is consistent with previously published data, that showed hTER expression in various normal tissues, which lack detectable amounts of telomerase activity (22). Hepatocellular carcinoma (HepG2, Hep3B) and bladder carcinoma cell lines (T24P and RT112) display high levels of hTERT, while the telomerase negative cell line IMR-90 cell line does not express hTERT, as examined by RT–PCR analysis (Fig. 2). These results confirm the close correlation between hTERT expression and telomerase activity. hTERT is highly expressed in malignant cells (telomerase-positive) such as hepatocellular carcinoma, bladder carcinoma cells but not in adjacent normal tissues (7, 23). It is interesting to note that the osteosarcoma cells show a very low but still detectable expression of hTERT (Fig. 2). On the other hand it was previously reported that although hTER plays an essential role in the maintenance of telomeres and the level of its expression increases with tumor progression, there is not a consistent correlation between telomerase activity and hTER expression (22). Thus, although hTER function is absolutely required for telomerase activity, its expression is not sufficient by itself for enzymatic activity. It was previously reported that since telomerase is a multicomponent enzyme (RNA and catalytic subunit), genetic events leading to reactivation of the hTER element may occur prior to reactivation of the protein elements (24). Our proposed strategy is to use the hTERT and hTER transcriptional regulatory sequences to control the expression of a target cytotoxic gene in telomerase-positive tumor cells and thus kill these cells. Thus, it is important to determine in each tumor the expression of both hTERT and hTER. Our novel approach is to use patient-tailored gene therapy based on the gene expression profile of the individual patients.

Determination of hTER and hTERT Promoter Activity in Various Cell Lines

We have cloned the promoter regions of hTER and hTERT genes into luciferase reporter vectors to assay for promoter activity. The plasmids Luc-TER and Luc-TERT

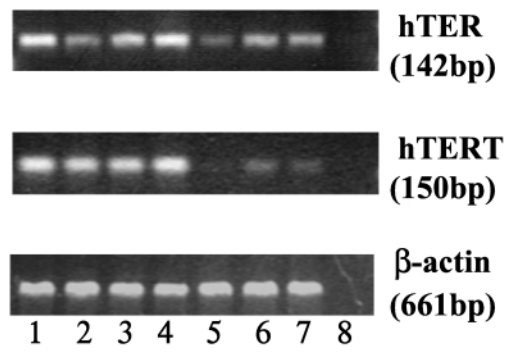

FIG. 2. RT–PCR analysis for the expression of hTER and hTERT in different cell lines. RT–PCR analysis was performed on cDNA of the cell lines: 1, HepG2; 2, Hep3B; 3, T24P; 4, RT112; 5, IMR-90; 6, Saos-2; 7, U-2-OS; 8, negative control. The RT–PCR was carried as described under Materials and Methods. The efficiency of the RT–PCR analysis was tested using β-actin-specific primers (Stratagene). The PCR products were separated by electrophoresis in TAE buffer on 2.5% agarose gel and stained with ethidium bromide.

TABLE 1
Activity of Telomerase Promoters in Various Cell Lines

Cell line	Relative luciferase activity	
	Luc–TER	Luc–TERT
HepG2	34.0	10.4
Hep3B	22.0	3.6
T24P	21.0	2.4
RT112	33.0	6.0
U2OS	28.0	0.2
Saos-2	12.0	0.28
IMR-90	10.0	0.9

Note. The various cell lines were transfected with the indicated expression vectors carrying the hTER or hTERT promoter sequences described under Materials and Methods. Luciferase activity in each plasmid is expressed as percentage of the positive control plasmid (Luc-4) activity. The values represent the averages of three independent experiments each performed in triplicate determinations with a SD of 10%.

which contain the luciferase gene under the control of the hTER and hTERT promoters, respectively, were transfected into various carcinoma cell lines to examine their promoter activity relative to the Luc-4 (SV40 promoter-enhancer as control plasmid). The results described in Table 1 indicate that while the hTER promoter is differentially but highly activated in all the cell lines tested, the activity detected in IMR-90, and Saos-2 cell lines is lower than that detected in the other telomerase-positive cell lines. These results are in good correlation with the level of mRNA tested by RT–PCR (Fig. 2). Transfection of telomerase-negative IMR-90, Saos-2, and U2OS cells with the expression plasmid Luc–TERT results only in background levels of luciferase activity, whereas substantial levels of activity were produced by the same construct in the telomerase-positive cell lines (Table 1).

Effect of Methylation on hTER and hTERT Promoter Activities

A strong role for regulation of hTERT expression and telomerase activity by methylation is suggested by the presence of a dense CpG-rich region in the promoter of hTERT (25). In the present study we have tested the effect of different methylation enzymes on the hTER and hTERT promoters activity. The reporter construct Luc–TER was premethylated *in vitro* by the methylases *Pst*I and *Eco*RI, and transfected into various cell lines (Table 2). The two mentioned enzymes can methylate only the hTER promoter sequence, since the pGL3 vector does not harbor any *Pst*I or *Eco*RI site. The results outlined in Table 2 show that when Luc–TER was *in vitro* methylated with either *Pst*I and *Eco*RI methylases, luciferase activity was significantly reduced in all the cell lines tested. It is interesting to note that following *Eco*RI methylation the luciferase activity is less inhibited in the bladder carcinoma cell lines than in the other cell lines tested. Thus, the effect of methylation on the promoter activity may depend on the cell line used in the transfection experiment. The reporter construct Luc–TERT was premethylated *in vitro* with either universal CpG Sss, dam and *Hae*III methylases and transfected into different cell lines (Table 2). The results described in Table 2 show that while the luciferase activity was significantly reduced following methylation with universal CpG methylase, no effect was detected on the luciferase activity following methylation with dam methylase in all the cell lines tested. *In vitro* methylation of the construct Luc–TERT with *Hae*III methylase caused a moderately reduction of luciferase activity when transfected into hepatocarcinoma Hep3B and bladder carcinoma RT112 cell lines, but no significant effect was detected when transfected into bladder carcinoma T24P and embryonic kidney 293 T cell lines. The above-mentioned results indicate that methylation may play a significant role in hTER promoter activity and are also in accordance with previous publication, indicating that hTERT promoter

TABLE 2
Effect of Methylation on the hTER and hTERT Promoter Activity

System	% inhibition from unmethylated control			
	Hep3B	RT112	T24P	293T
Luc–TER + *PST*I Me	50	48	49	45
Luc–TER + *Eco*RI Me	62	30	33	81
Luc–TERT + Sss Me	57	79	77	nd
Luc–TERT + dam Me	0	0	0	nd
Luc–TERT + *Hae*III Me	35	38	11	0

Note. In vitro methylation of the constructs Luc–TER and luc–TERT was carried out using the indicated methylases, as described under Materials and Methods. The methylated and unmethylated constructs were transfected into the various cell lines and the luciferase assays were carried out as described under Materials Methods. The results are expressed as percentages of inhibition of luciferase activity to that observed following transfection with the unmethylated construct. nd, not detected.

TABLE 3
Response of Various Carcinoma Cell Lines to Cotransfection with the Luciferase Control Vector Luc-4 and the Expression Vectors DTA-TER and DTA-TERT

System	Relative luciferase activity % versus control				
	HepG2	RT112	IMR-90	U2OS	Saos-2
Luc-4 + DTA–TER 0.025 μg	80.0	86.0			
Luc-4 + DTA–TER 0.05 μg	48.0	60.4	43	14	43
Luc-4 + DTA–TER 0.1 μg	48.0	59.0	nd	9.6	33
Luc-4 + DTA–TER 0.25 μg	36.5	35.0	nd	3.6	19
Luc-4 + DTA–TER 0.5 μg	32.0	33.0	32	1.7	14
Luc-4 + DTA–TER 1.0 μg	20.0	16.5	25	nd	nd
Luc-4 + DTA–TERT 0.025 μg	70.0	80.0	—	—	—
Luc-4 + DTA–TERT 0.05 μg	44.0	45.0	85	61	65
Luc-4 + DTA–TERT 0.1 μg	45.0	76.0	—	32	66
Luc-4 + DTA–TERT 0.25 μg	25.0	38.0	—	24	43
Luc-4 + DTA–TERT 0.5 μg	12.3	41.0	80	24	44
Luc-4 + DTA–TERT 1.0 μg	18.0	16.0	80	nd	nd

Note. Various amounts of the constructs DTA–TER and DTA–TERT were cotransfected with 7 μg of Luc-4, respectively (containing SV40 promoter and SV40 enhancer). The results are expressed as percentages of inhibition of luciferase activity relative to that observed following transfection with Luc-4 alone. The values represent the averages of three independent experiments each performed in triplicate.

methylation is involved in the regulation of hTERT expression at least in some cells (10). These data may be useful for the adjustment of the hTER/hTERT promoter activities to be used in gene therapy trials.

Expression of the DT-A Chain Coding Region under Control of hTER and hTERT Regulatory Sequences

Our studies (Figs. 1A–1D) and previous reports showed great differences in hTER and hTERT expression between malignant and normal tissue, supporting the possibility of effective gene therapy based on the promoters of telomerase components (22). Thus the hTER and hTERT promoters were used to drive the expression of a toxic gene (suicide gene) in malignant cells. The transcriptional regulatory elements of a gene that is highly expressed in malignant cells but not in normal cells can be used to drive the expression of the toxin gene, ensuring that only the malignant cells will produce the toxin. It was previously reported that directed expression of the gene that encodes the DT-A chain resulted in selective destruction of a targeted cell population when this encoding region is linked to tissue-specific transcriptional regulatory elements (26–28).

Cotransfection experiments were performed with various cell lines using 7 μg of the control vector Luc-4 and different amounts of the expression construct DTA–TER or DTA–TERT, which contains the DT-A gene downstream of the regulatory regions of hTER and hTERT, respectively (Table 3). DTA–TER strongly inhibited luciferase expression at already 0.05 μg of the plasmid, getting to 80% inhibition at 1 μg of the expression vector in the telomerase positive cell lines, also in the telomerase negative IMR-90 and osteosarcoma cell lines the luciferase activity is significantly inhibited. The construct DTA–TERT shows similar low levels of luciferase activity, comparable to those seen with DTA–TER in the telomerase-positive cells tested (Table 3). Even when the DTA–TERT at the concentration of 1 μg was used in the cotransfection experiment in IMR-90 cell line (telomerase-negative cells) almost no inhibition was detected. It is interesting to note that the low level of the hTERT gene expression in U2OS and Saos-2 cell lines (Fig. 2) is enough to drive the expression of the DT-A gene. These results strongly suggest that while the bladder and hepatocellular carcinoma cells transactivate the hTERT promoter, in the IMR-90 primary fibroblast cells these promoter regions had a very low activity. These results also indicated the safety of the toxin expression vector, since it did not cause damage to cell lines that do not express the hTERT gene.

Effect of the Toxin Expression Vectors DTA–TER and DTA–TERT on the DT-Resistant-293 Cell Line

To obtain additional evidence for toxicity resulting from expression of the DT-A gene, we cotransfected 293 T and 293-DT resistant cell lines with the vectors containing the DT-A coding region under the control of TER or TERT promoters, respectively, and with the control vector Luc-4. Figures 3A and 3B show that there is a complete inhibition of the luciferase expression following cotransfection with the toxin expression construct and Luc-4 in the 293 T cell line, but no inhibition was detected in the DT-resistant cells. The explanation for the increase in luciferase activity detected in the DT-resistant cells following cotransfection with the DT-A construct is still under investigation. In parallel we have determined the killing effect of the DT-A constructs by measuring the protein content of the cells which survived after the cotransfection (Fig. 3C). Figure 3C shows that there is also a complete inhibition of protein synthesis in the 293 T cell line following cotransfection with the DT-A vectors, while no inhibition was detected in the DT-resistant cell line. These results confirmed that the protein inhibition obtained in cells expressing the DT-A gene is caused only by the action of the diphtheria toxin and not by any other factor. Since the initial protein concentration in the 293-DT-resistant cell line was lower than the concentration measured in the 293 T cell line (Fig. 3C), we decided to investigate the cells growth curve in both cell lines. The data obtained from the growth curve of both cell lines showed similar growth curves, while the only difference is the initial cell num-

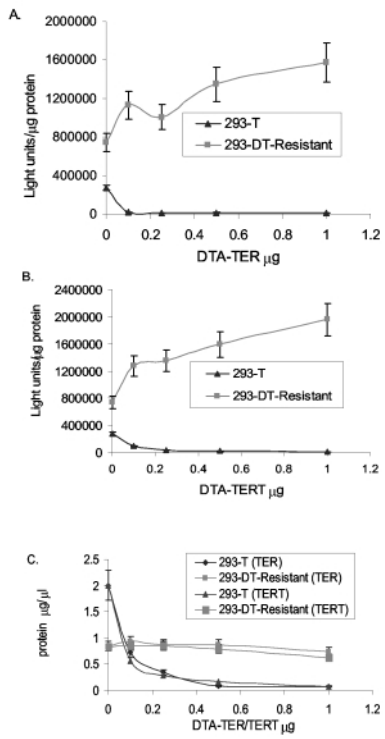

FIG. 3. Effect of the therapeutic vectors DTA–TER and DTA–TERT on DT-resistant 293 cell line compared to that on 293-T cell line. The 293T and DT-resistant 293T cell lines were cotransfected with 2 μg of the control vector (Luc-4) and various concentrations of DTA–TER (A) or DTA–TERT (B) as indicated. The protein concentration was determined for each cell lysate by the Bio-Rad protein assay reagent (C).

ber after the 24 h prior to the transfection (data not shown).

These studies demonstrate that the transcriptional regulatory sequences of hTER and hTERT can be exploited to achieve highly cell-specific expression of exogenous toxin genes *in vitro*. The advantages of using DT-A produced intracellulary for gene therapy was previously discussed in the literature (26, 29). The use of the gene only encoding diphtheria toxin-A chain, does not exhibit any bystander effect because the B chain is responsible for cell penetration, this might be desirable in many cases, since it prevents harmful effects to surrounding normal cells. The potent activity of the toxin can be attenuated by using mutated DT-A chain gene (30). Using a plasmid with an expression cassette of the toxin, no immune response will be encountered, moreover the whole western population is immunized against this toxin. In addition our system will not be affected by multidrug resistance effects, a major problem in chemotherapy.

In the second stage of our study DT-A toxin expression will be evaluated in an animal model system of bladder cancer. There is precedent for delivering proteins intravesically; a phase I clinical trial of the recombinant oncotoxin TP40 involved the intravesical delivery of the recombinant toxin to treat superficial human bladder cancer in phosphate-buffered saline for four to six treatments, and no significant toxicity was observed (31). In the present study we have designed expression vectors that will be tested for the treatment of bladder cancer. One advantage associated with bladder cancer is its easy accessibility and relative isolation from other areas of the body. It has been shown that normal urothelium cells do not express telomerase activity, while 92% of bladder carcinoma (including all stages) have active telomerase (22). Thus, these vectors may be effective for the treatment of most bladder cancers.

ACKNOWLEDGMENTS

This work was supported by the German–Israeli–Palestinian Authority Trilateral grant sponsored by the Deuthsche Forschungs Gemeinschaft (DFG) and by NIH Grant 1RO1CA69646-01A1.

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