Inhibition of Telomerase Activity in Human Cancer Cells by RNA Interference[1]

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Abstract

Telomerase is an attractive molecular target toward which to direct cancer therapeutic agents because telomerase activity is present in most malignant cells but undetectable in most normal somatic cells. Short duplex RNA (short-interfering RNA or siRNA) has recently been shown to be an effective method for inhibiting the expression of a given gene in human cells. Accordingly, we evaluated the ability of siRNA to inhibit telomerase activity in human cancer cells. Human cancer cell lines were transfected with 21 nt double-stranded RNA homologous to either the catalytic subunit of telomerase (human telomerase reverse transcriptase) or to its template RNA [human telomerase RNA(hTR)]. Both types of agents reduced telomerase activity in a variety of human cancer cell lines representing both carcinomas and sarcomas. Inhibition was dose-dependent, although modest in degree and, as expected, transient in duration. Transfection of HeLa cells using a plasmid containing the hTR gene in both forward and reverse orientations, intended to create a duplex of the hTR transcripts endogenously, resulted in decreased telomerase activity, decreased telomerase RNA content, and decreased telomeric DNA content but no decrease in the untargeted human telomerase reverse transcriptase mRNA. Telomerase inhibition by siRNA is notable because telomerase is regarded as restricted to the nucleus, whereas RNA interference is commonly regarded as restricted to the cytoplasm.

Introduction

dsRNA[3], a hybrid consisting of a sense and antisense strand of an endogenous mRNA, can initiate a cellular response that results in the sequence-specific degradation of homologous single-stranded RNA. This occurs in a wide variety of eukaryotic organisms ranging from protozoa to mammals, including plants. The process is called PTGS in plants and RNAi in animals (reviewed in Refs. 1–3). The term posttranscriptional indicates that RNA synthesis as such is not affected. Instead, the RNA transcript gets specifically degraded so that the corresponding gene becomes silenced. PTGS and RNAi apparently represent an old and evolutionarily conserved defense mechanism against parasitic RNAs, including RNA viruses. PTGS has been extensively studied in plants where viral dsRNA initiates the host response that keeps the virus infection under control (4–7).

PTGS and RNAi share mechanistic details. The dsRNA is the key trigger, which gets processed by double-strand-specific RNase to shorter RNA fragments of both polarities observed first by Hamilton and Baulcombe (8). In *Drosophila*, the RNase-III type enzyme Dicer was identified (9). It is responsible for this processing reaction and related enzymes have been identified in other organisms (10–13). The processing product generated by Dicer is a dsRNA of ~21–22 nucleotides and identical to the short RNAs observed by Hamilton and Baulcombe (8, 14). However, the short RNA is not a plain double strand but has characteristic termini consisting of two unpaired 3′-terminal nucleotides on either side (15). It is not a simple degradation product but rather an important intermediate of the PTGS/RNAi reaction. In the further course of this process, the short dsRNA gets incorporated into a multicomponent protein complex called RISC (16). RISC can be considered a RNase, and the incorporated short RNA confers sequence-specificity upon this complex. It is believed that the incorporated short dsRNA guides the RISC to the homologous target RNA.

Because the short RNAs are not mere degradation products of the dsRNA trigger but functional intermediates, could artificially generated short dsRNAs initiate the RNAi response? Elbashir *et al.*(17) found that chemically synthesized RNAs that form a double-strand with the characteristic two unpaired nucleotides at the 3′ termini could initiate RNAi, even in a mammalian cell line (18). Similar results were obtained by Caplen *et al.* (19). Boutla *et al.* (20) demonstrated RNAi with chemically synthesized RNAs also for whole organisms such as *Drosophila* embryos. In view of the potential to initiate the RNAi response, Elbashir *et al.* (18) introduced the term "siRNA" for these short RNA duplexes. It was also shown that it is beneficial to use 5′-phosphorylated RNAs (20). Meanwhile, many examples have demonstrated the usefulness of siRNAs, which include the

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[3] The abbreviations used are: dsRNA, double-stranded RNA; PTGS, post-transcriptional gene silencing; RNAi, RNA interference; RISC, RNA-induced silencing complex; siRNA, short-interfering RNA; hTR, human telomerase RNA; hTERT, human telomerase reverse transcriptase; FBS, fetal bovine serum.

induction of resistance to viruses, *i.e.,* HeLa cell resistance to polio virus (21) and to HIV (22). The question of specificity of the RNAs has not been finally answered. Some systems were sensitive to sequence deviation (15), whereas others were tolerant (20, 23).

From findings described above, it is evident that two ways of inducing RNAi are available, first using long dsRNA and second using siRNA. Long dsRNA is capable of inhibiting specific gene expression in undifferentiated mammalian cells but produces nonspecific inhibition in differentiated cells (24, 25). In differentiated cells, long dsRNA induces the expression of a variety of genes involved in host defense against foreign nucleic acids, including protein kinase PKR, IFNs, 2′-5′-oligoadenylate (2–5A) synthetase, and sequence-nonspecific RNase L (26–28). Hence, the discovery that short dsRNA efficiently silences genes was a welcome discovery because it implied that a short duplex might be useful for gene silencing in mammalian cells while avoiding the induction of the IFN response.

Here, we present data showing that RNAi technology can be harnessed to down-regulate telomerase components, resulting in decreased telomerase activity in human cancer cells. This is of interest because telomerase is a promising target for cancer chemotherapy. We used two types of RNA triggers: first, chemically synthesized siRNA; and second, a long dsRNA that was expressed in the target cells from a hairpin construct. Hairpin DNA contains sense and antisense sequences so that an RNA transcript will form an intramolecular double-strand. Similar hairpin constructs have been shown to initiate RNAi for various targets in diverse biological systems such as plants, trypanosomes (29–32), Drosophila (33, 34), and mouse (25). Unlike an ordinary enzyme, telomerase has an RNA and a protein component that are both necessary for telomerase activity. We targeted each component: (*a*) the telomerase RNA (hTR), which contains the template for the telomere sequence; and (*b*) the mRNA encoding the catalytically active protein component, the telomerase reverse transcriptase hTERT.

Materials and Methods

siRNAs. dsRNA was synthesized by Xeragon (Huntsville AL). For telomerase RNA, hTR no. 1 siRNA targeted the region containing the telomere repeat template sequence (35), shown in boldface: 5′-UUGU**CUAACCCUAAC**UGAG-TT-3′ and 3′-TT-AACAGAUUGGGAUUGACUC-5′.

hTR no. 2 siRNA targeted a 19-bp sequence centered in the 26-bp L6 loop, the longest single-stranded region in hTR according to the secondary structure proposed by Chen *et al.* (36): 5′-GGCTTCTCCGGAGGCACCCTT-3′ and 3′-TT-CCGA AGA GGC CTC CGT GGG-5′.

In the case of the mRNA for telomerase's protein catalytic subunit, hTERT, the target was the region containing the site of the dominant negative mutation (bolded) used to inactivate the gene by Hahn *et al.* (Ref. 37; shown is the normal sequence): 5′-CAAGGUG**GAUGUG**ACGGGCTT-3′ and 3′-TTGUUGCACCUACACUGCCCG-5′.

siRNA Transfection. Cell lines were obtained from American Type Culture Collection and maintained in the media recommended by them. Cells were transfected by the method of Tuschl (18). Cells in 0.5-ml aliquots were plated in a 24-well plate at a concentration estimated to provide 30–40% confluence 16 h later. At that time, dsRNA for either hTR or hTERT (0.25, 0.5, 1, or 2 μg) was diluted with 125 μl of Optimem medium (Invitrogen). In a separate tube, 7.5 μl of oligofectamine (Invitrogen) was diluted with 30 μl of Optimem. The two solutions were mixed gently by inversion and incubated at room temperature for 7–10 min. The contents of the two tubes were then combined, mixed gently by inversion, and incubated at room temperature for 20–25 min. One hundred μl containing the liposome complexes was added to the culture medium in each well and mixed by gentle rocking for 30 s. HeLa cells were maintained in serum throughout, but for other cell types, serum was removed for the first 4 h of transfection. At 22 or 42 h, cells were trypsinized, counted, and 2000 cells removed for assay of telomerase activity.

Telomerase Activity. Telomerase activity was measured by the TRAPeze assay (Serologicals, formerly Intergen) based on the work of Kim *et al.* (38), Piatyszek *et al.* (39), and Wright *et al.* (40). Pilot experiments demonstrated that siRNA, when added to the telomerase activity reaction mixture in the amounts that could be introduced by cell lysate, did not alter telomerase activity. Some variability was noted in telomerase activity in replicate experiments; in such cases, we have provided the data from a typical experiment.

Quantitation of Telomerase RNA. Total RNA was purified using the SV Total RNA Isolation System (Promega). Telomerase RNA was quantified by a reverse transcriptase-PCR assay. Fifty or 100 ng of total RNA was incubated in 5 μM random hexamers (Pharmacia-LKB), 0.5 mM deoxyribonucleoside triphosphates $\times$ 4, 0.5 unit/μl RNAsin (Promega), 1 mM DTT, and 2.5 units/ml Moloney leukemia virus reverse transcriptase (Invitrogen) in 50 mM KCl, 10 mM Tris-Cl (pH 9.0), and 0.1% Triton X-100 in 20 μl for 45 min at 37°C. The reaction was then heated to 95°C for 10 min to denature the reverse transcriptase.

Each PCR reaction contained 10 μl of the reverse transcriptase reaction mixture, 0.5 μM primers, 10 mM deoxyribonucleoside triphosphates $\times$ 4, 2.5 μCi (α-^{32}P)dCTP, 3000 Ci/mmol, in 2.0 mM $MgCl_2$, 40 mM KCl, 8 mM Tris-Cl (pH 9.0), and 0.1% Triton X-100 in 50 μl. The products of the PCR reaction were electrophoresed in 10% nondenaturing polyacrylamide gel in 1$\times$ TBE at 40 V for 18 h. Radioactivity was quantified by phosphorimaging. The value of the no RNA control was subtracted from each experimental value.

The primers used were 5′-CTGGGAGGGGTGGTGGC-CATTT-3′ and 5′-CGAACGGGCCAGCAGCTGACAT-3′. Reaction parameters were 94°C for 20 s, 50°C for 20 s, and 72°C for 30 s for 25 cycles.

Quantitation of hTERT mRNA. hTERT mRNA was quantified by a reverse transcriptase-PCR method similar to that used to quantify telomerase RNA, except that the Mg^{2+} concentration in the PCR reaction was 1.0 mM, and the primers were 5′-GCCAGAACGTTCCGCAGAGAAAA-3′ and 5′-AAT-CATCCACCAAACGCAGGAGC-3′. Reaction parameters were 94°C for 20 s, 48°C for 30 s, and 72°C for 30 s for 30 cycles.

Hairpin Construction. The pSPT BM20 plasmid was purchased from Boehringer Mannheim, now Roche Molecular Biochemicals. The SP6 promoter was replaced with the T3 promoter. The *Acc*I 1440-bp fragment from bacteriophage

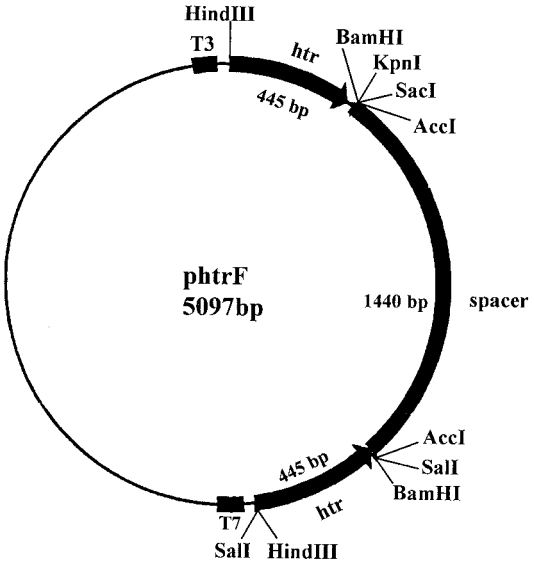

Fig. 1. Map of phtrF plasmid showing insert (*bolded*) containing forward and reverse orientations of the human telomerase RNA gene (hairpin). The *Hin*dIII fragment of this plasmid was subsequently inserted into the mammalian expression vector pZeoSV2.

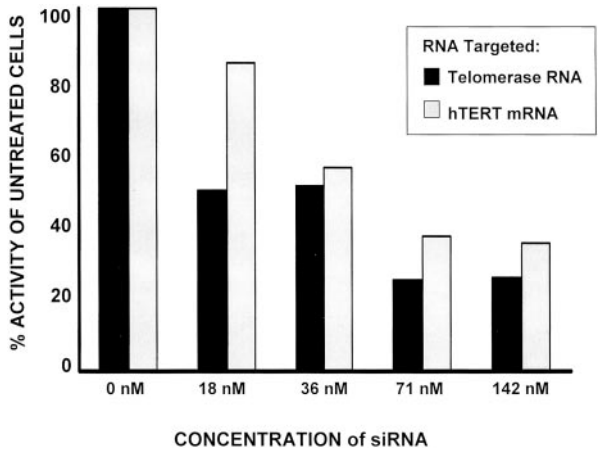

Fig. 2. siRNAs decrease telomerase activity in HCT-15 human colon carcinoma cells. HCT-15 cells were transfected with siRNA at the concentrations indicated in the figure, as described in "Materials and Methods." At 42 h, cells were harvested for telomerase assay.

lambda was inserted at the *Acc*I site. The pGRN164 plasmid containing the human telomerase (hTR) gene was kindly provided by Dr. Gregg Morin of Geron Corporation (Menlo Park, CA). The hTR gene was extracted as a *Hin*dIII/*Sac*I fragment and inserted into the equivalent site of the modified pSPT BM20 containing the λ fragment, henceforth called pT3htr. The hTR gene was extracted from the pGRN164 plasmid this time as a *Hin*dIII/*Bam*HI fragment and inserted into the equivalent site of the pBluescript II KS plasmid (Stratagene). The hTR gene was then extracted from the Bluescript vector as a *Kpn*I/*Bam*HI fragment and inserted into the equivalent site of a pT3T7 vector (Boehringer Mannheim). *Sal*I was used to extract the fragment from pT3T7 because it cuts once before the hTR gene by the site brought from pBluescript (near the *Kpn*I end) and once after the *Bam*HI site at the preexisting *Sal*I site of pSPT BM20. This *Sal*I fragment containing the hTR gene was then inserted at the *Sal*I site recreated at the end of the lambda-spacer insertion of pT3htr, henceforth called phtrF (Fig. 1). The orientation of this second hTR insertion was selected, using *Bam*HI digestion, to be opposite to that of the original hTR insertion and hence created a hairpin, which could be excised as a simple *Hin*dIII fragment. The excised *Hin*dIII fragment was inserted into the equivalent site of the mammalian expression vector pZeoSV2/lac2(+) (Invitrogen) to make pZeoSV2-hTR.

Plasmid Transfection. HeLa cells were transfected with the pZeoSV2-hTR construct. Briefly, 1.2×10^6 cells in 1 ml of medium-10% FBS were cultured overnight in 100-mm Petri dishes to 50–60% confluency. The next day, the serum-containing medium was exchanged for serum-free medium. Eighty μl of rehydrated X-treme Gene Q2 transfection reagent (Roche Molecular Biochemicals) diluted to l ml with SFM-A was mixed with 40 μg of phtrF DNA in 0.5 ml of DNA dilution buffer (Roche Molecular Biochemicals) and incubated 5–10 min at room temperature. This mixture was then added to each dish containing cells and incubated 4 h in 5% CO_2. Then 6 ml of medium containing 20% FBS were added. After overnight culture, the medium was replaced with 10 ml of medium containing 10% FBS and Zeocin (0.2 mg/ml). Additional cultures were prepared using the pZeoSV2 vector lacking the hairpin insert. Cultures were fed every 3–4 days. When colonies appeared, they were harvested using cloning rings and transferred initially to a 96-well plate. By 75 days of selection in Zeocin, sufficient cells of each clone had accumulated for the assays described below and for preparation of frozen stocks.

Quantitation of Telomeric DNA. Telomeric DNA content was measured by the method of Bryant *et al.* (41). This method quantitates telomeric DNA using a slot blot and a telomere-specific probe. It also quantitates centromeric DNA by a separate slot blot of an identical sample using a centromere-specific probe. The ratio of telomeric DNA to centromeric DNA is then compared between cell samples, in our case, between hairpin-transfected cells and control cells. Thus telomeric shortening is measured as a reduction in the ratio of telomeric DNA to centromeric DNA.

Results

Effect of siRNAs

On Telomerase Activity. SiRNAs for hTR and hTERT depressed the telomerase activity of HCT-15 human colon carcinoma cells in a dose-dependent manner. Fig. 2 shows the effect at 44 h. Results throughout are reported as a percentage of telomerase activity of cells treated with the lipid transfecting agent only (*i.e.,* as a percentage of activity of untreated cells). The maximum effect observed with HCT-15 cells was 25% of untreated cell activity for hTR siRNA and 35% of untreated cell activity for hTERT siRNA.

Both agents depressed telomerase activity also in HeLa human cervical carcinoma cells in a dose-dependent manner. Fig. 3 shows the effect at 42 h. In both cell types, the siRNA for hTR was more inhibitory than the siRNA for hTERT

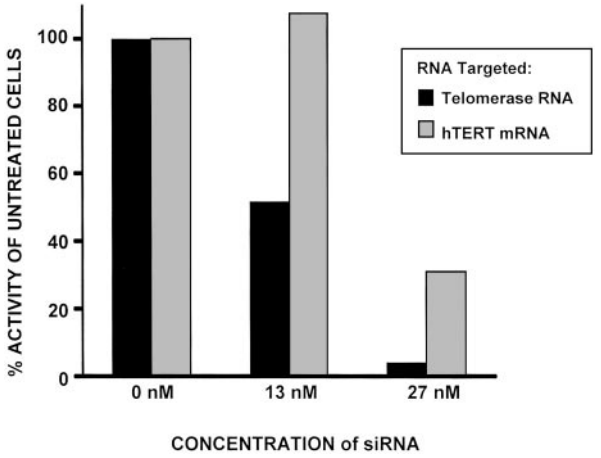

Fig. 3. siRNAs decrease telomerase activity in HeLa human cervical carcinoma cells. HeLa cells were transfected with siRNA at the concentrations indicated in the figure, as described in "Materials and Methods." At 42 h, cells were harvested for telomerase assay.

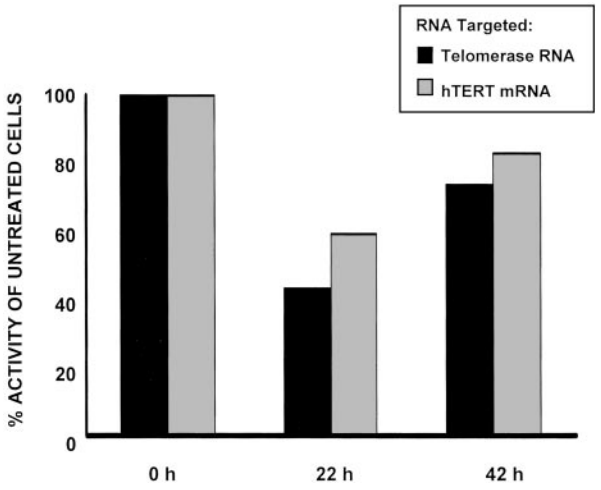

Fig. 4. siRNAs decrease telomerase activity in HT1080 human fibrosarcoma cells. HT1080 cells were transfected with siRNA at a concentration of 142 nM, as described in "Materials and Methods." At 22 and 42 h, cells were harvested for telomerase assay.

at a given concentration. In dose-response experiments of similar design, telomerase inhibition was seen also with other types of carcinoma cells, *i.e.,* NCI H23 human lung carcinoma cells and A431 human epidermoid carcinoma cells (data not shown).

Each agent depressed telomerase activity also in cells of mesodermal origin, *i.e.,* HT-1080 human fibrosarcoma cells (Fig. 4) and CCL121 human osteosarcoma cells (data not shown). However in both these cell lines, inhibition was greater at 22 h than at 46 h, unlike the results of the carcinoma cell lines tested.

Using HeLa cells, four strategies were used in an effort to demonstrate more complete inhibition of telomerase activity. First, cells were treated with higher concentrations of siRNA for hTR, up to 1136 nM but inhibition was not additionally

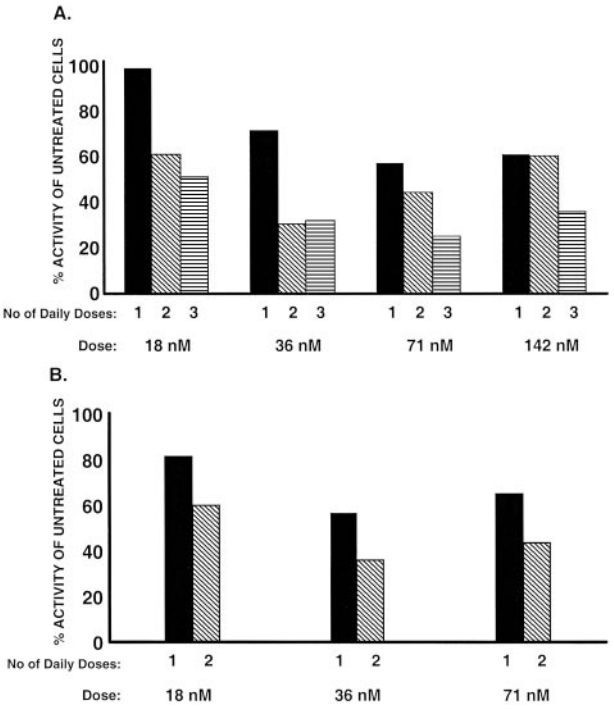

Fig. 5. Effect of daily administration of hTR siRNA on HeLa cell telomerase activity. *A*, HeLa cells were transfected with hTR no. 1 siRNA at the concentrations indicated in the figure as described in "Materials and Methods." Cultures receiving one daily dose were assayed at 24 h. Cultures receiving two daily doses were assayed at 48 h. Cultures receiving three daily doses were assayed at 72 h. *B*, HeLa cells were transfected with hTR no. 1 siRNA at the concentrations indicated in the figure as described in "Materials and Methods." Both cultures receiving the agent at 0 h only and cultures receiving the agent at both 0 and 24 h were assayed at 48 h.

increased (data not shown). Second, cells were treated with siRNA for hTR on a daily basis. Fig. 5*A* shows the results of treatment using various concentrations. The bars marked 1, 2, and 3 represent the telomerase activity after 1, 2, and 3 days of treatment, each assayed 24 h after the last dose. There was progressive inhibition for the 72-h period investigated. However, the lowest value reached was only 35% of the untreated. Additions of 142 nM did not produce appreciably more inhibition than those of 71 nM. To address the question as to whether multiple transfections decrease the telomerase activity more than a single initial transfection, cells were transfected either at 0 h only or at both 0 and 24 h, and both sets were assayed at 48 h. As shown in Fig. 5*B*, two transfections resulted in lower telomerase activity than a single one. Third, cells were treated with siRNAs for both hTR and hTERT simultaneously. However inhibition did not exceed that seen with each separately (data not shown). Fourth, cells were treated with siRNA targeting hTR but at a different site. On the assumption that internally hybridized regions would not be accessible to siRNAs, we chose a 19-bp sequence centered in the 26-bp L6 loop, the longest single-stranded region of the hTR secondary structure proposed by Chen *et al.* (36). However, at 51 h, this second generation siRNA for hTR was less inhibitory than the first (Fig. 6).

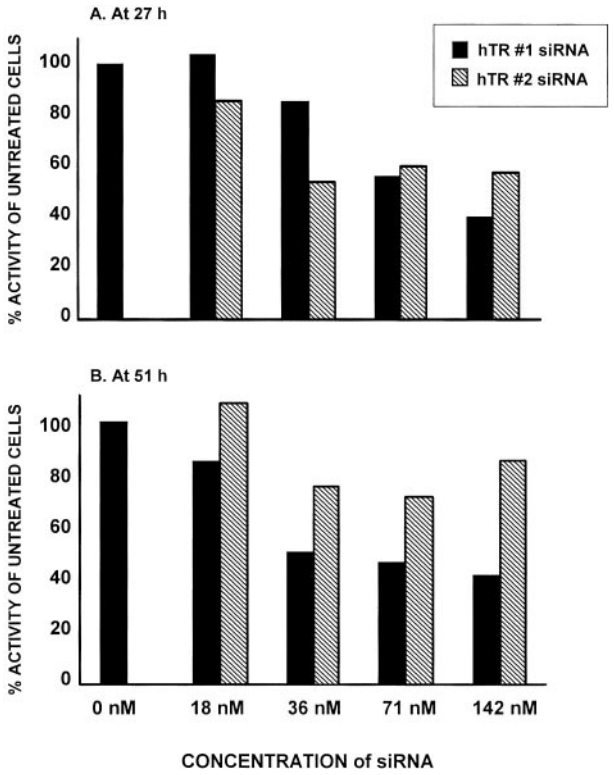

Fig. 6. Comparison of siRNAs for two different sites in hTR on telomerase activity. HeLa cells were transfected with siRNAs for hTR at the concentrations indicated in the figure, as described in "Materials and Methods," assayed at 27 or 51 h.

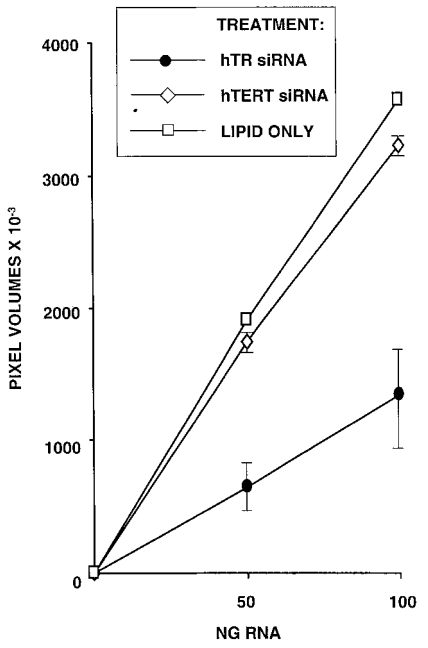

Fig. 7. Telomerase RNA content in siRNA-treated HeLa cells. HeLa cells were treated with hTR no.1 siRNA or hTERT siRNA, each at 142 nM, or oligofectamine alone and harvested 42 h later. Total RNA was isolated, and telomerase RNA content quantitated by RT-PCR, as described in "Materials and Methods." Shown are means ± SE for two experiments.

On Telomerase RNA Content. The effect of siRNAs on telomerase RNA content is shown in Fig. 7. Compared with HeLa cells treated with the lipid transfecting agent oligofectamine alone, cells treated with hTR siRNA had decreased telomerase RNA content in the reverse transcriptase-PCR assay by >50%. In contrast, cells treated with hTERT siRNA had no decrease in telomerase RNA.

Effects of Hairpin Construct

To investigate cellular effects over a longer term, we used a DNA construct containing a hairpin structure targeting telomerase RNA. It contained the hTR sequence in both sense and antisense orientations separated by a space. The expected transcription product is a stem-loop RNA with the double-stranded portion representing the hTR sequence.

On Telomerase Activity. The telomerase activity of the clones isolated is shown in Table 1. Of the five surviving clones, three clones (nos. 5, 9, and 10) had deficient telomerase activity (57, 13, and 47% of the average of the vector-only controls) and two (nos. 3 and 4) did not.

On Telomerase RNA Content. The telomerase RNA content of these clones is shown in Fig. 8. The three with deficient telomerase activity (nos. 5, 9, and 10) had low telomerase RNA content. The two with normal telomerase activity (nos. 3 and 4) had normal telomerase RNA content.

On hTERT mRNA Content. The clones were assayed for hTERT mRNA content also, using a similar reverse transcriptase-PCR assay. None of the five clones was deficient in hTERT mRNA content (results not shown).

Table 1 Telomerase activity of clones

Clone	Activity
Vector only	100%
Vector plus telomerase hairpin insert	
No. 3	>100%
No. 4	>100%
No. 5	57%
No. 9	13%
No. 10	47%

On Telomeric DNA Content. Four of five clones had a reduced telomeric DNA content at 75 days and one did not. The reduction in the four cases was 45% (±18% SD) compared with the average value for untreated cells and for cells transfected with the vector without the hairpin insert.

Discussion

Our results show that telomerase activity in human cancer cells can be inhibited by short dsRNAs (siRNAs) targeting telomerase components. Inhibition was shown in a variety of carcinoma cell lines (HCT-15 colon carcinoma, HeLa cervical carcinoma, NCI H23 lung carcinoma, and A431 epidermoid carcinoma). Inhibition was shown also in cell lines of mesodermal origin (CCL121 osteosarcoma and HT-1080 fibrosarcoma), although inhibition appeared to be of shorter duration than in the carcinoma cell lines tested. Inhibition was dose-dependent. hTR or hTERT were both susceptible targets.

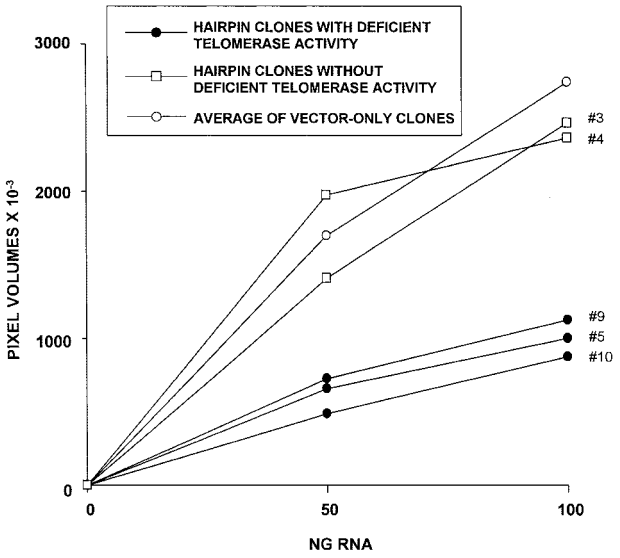

Fig. 8. Telomerase RNA assay of clones transfected with pZeoSV2-hTR plasmid after culture for 75 days. The telomerase RNA content was determined by a reverse transcriptase-PCR assay as described in "Materials and Methods" using either 50 or 100 ng RNA.

To evaluate siRNA against telomerase as an anticancer agent, it will be necessary to achieve more complete inhibition of activity than demonstrated here. Surprisingly, above a certain concentration, increases in siRNA concentration did not increase inhibition. Repeated doses also failed to completely inhibit, as did administration of siRNAs for hTR and hTERT together. The exact site targeted in the RNA may be a key factor, as has proved to be the case with antisense inhibition because some sites are bonded internally or to neighboring molecules, but the cost of the reagents prevented us from exploring many sites.

The effect of hTR siRNA in decreasing telomerase RNA was a sequence-specific one. siRNA targeting hTERT mRNA did not decrease telomerase RNA.

To investigate longer term effects, we used a hairpin structure. A DNA construct containing a hairpin structure targeting telomerase RNA transfected into HeLa cells decreased the content of telomerase RNA. Two pieces of evidence suggest a causal relationship. First, the clones deficient in telomerase activity were also deficient in telomerase RNA content, whereas the clones without a deficiency in telomerase activity were not deficient in telomerase RNA content. Second, the effect was a specific one in the sense that none of the clones had a deficiency in hTERT mRNA, an RNA species that was not targeted.

Across the clones at 75 days, the telomerase activity was roughly correlated with telomerase RNA content, but neither was well correlated with telomeric DNA content. Culture of the clones is continuing in order to follow telomere content and to determine whether, as telomeres shorten further, impaired clonogenicity, chromosomal fusions, and apoptosis develop (42, 43).

RNAi is regarded as a primarily cytoplasmic process as suggested by two previous observations. In *Drosophila* cells, the RISC copurifies with ribosomes (44). In *Caenorhabditis elegans*, siRNAs targeting intronic sequences are not effective inducers of RNAi (45, 46). Zeng and Cullen (47) have recently presented evidence that, in human cells, RNAi is restricted to the cytoplasm.

Hence, telomerase, which obviously acts in the nucleus, might be expected to be exempt from RNAi. hTR contains a nucleolar localization signal, *i.e.,* a conserved H/ACA box found in its 3′ portion (48, 49). hTERT also contains a nucleolar localization signal in its 15 NH_2-terminal amino acids; mutations in this region prevent complex formation between hTERT and hTR (50–52).

The apparent susceptibility of telomerase to siRNA as shown by the above data has several possible explanations. hTERT mRNA is presumably translated in the cytoplasm, but the susceptibility of hTR to siRNA attack is not readily explained. One possibility is that telomerase components occur in the cytoplasm transiently (53) or are exposed to cytoplasmic components during mitosis. Restriction of RNAi to the cytoplasm and the transiency of telomere components in the cytoplasm might together explain our inability to more completely inhibit telomerase activity using RNAi. Alternatively, there may be a difference in this regard between the 293T human embryonic kidney cell line of nonmalignant origin used by Zeng and Cullen (47) and the multiple types of cell lines used in our work, all of malignant origin. In addition, there may be a difference in RNAi induced by endogenously produced RNA as in the Zeng and Cullen work and in RNAi induced by transfected duplex RNA as in our work. Finally, there are observations that suggest some parts of the RNAi process may be nuclear. For example, siRNA can be observed in the case of viroids, which are subviral RNA pathogens that replicate in the nucleus via an RNA double strand (54). Matzke *et al.* (55) have pointed out that one of the two Dicer homologues in *Arabidopsis* contains two bipartite nuclear localization signals. The recent finding that small RNAs resembling siRNAs function to silence chromatin in yeast (56) and to rearrange the genome in Tetrahymena (57) is consistent with siRNAs operating in the nucleus of mammalian cells. The use of the U6 snRNA promoter cassettes in inducing RNAi in human cells, shown by many groups, may provide additional evidence that RNAi can occur in the nucleus (58) because the transcripts of such cassettes are primarily nucleoplasmic (59).

Telomerase remains an attractive target for cancer therapy because telomerase is present in most malignant cells but undetectable in most normal somatic cells (60). Thus an agent effectively inhibiting telomerase could be active against many forms of malignancy yet spare most types of normal cells (61). Nucleic acid agents hold the promise of a degree of specificity that may be difficult to achieve with small molecules. Although nucleic acid agents in clinical trials currently are DNA rather than RNA, dsRNA may have the stability required for clinical use.

Acknowledgments

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