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DNAzyme-Based Probes for Telomerase Study in Early-Stage Cancer Detection

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Abstract

Human telomerase is a polymerase enzyme that adds tandem repeats of DNA (TTAGGG) in the telomere regions. Since telomerase can be detected in immortalized, but not normal, somatic cells, it has been considered a selective target for cancer chemotherapy. In this paper, we describe a DNAzyme-based probe method to determine telomerase in cell lysates. Telomerase elongates the primer site on the probe. Subsequent addition of the Pb^{II} cofactor activates the DNAzyme, which cleaves the elongated fragment at the RNA site, releasing the probe for repetitive cycling and signal amplification. The cleaved fragment is detected by a reporter molecular beacon. Enzymatic amplification with rapid turnover allows detection of telomerase in the range of 0.1 μg to 1 μg cell lysate, with a 5-fold increase in signal level for cancer cells over normal cells. This probe design can provide a simple, yet rapid and sensitive, measurement of telomerase activity.

Keywords

DNAzyme; probe design; intramolecular probe; telomerase; lead ion

Introduction

Over the last few decades, telomerase, also known as telomere terminal transferase, has gained increasing interest as a potentially sensitive biomarker for early cancer diagnosis and prognosis, as well as a monitor for residual disease. As an important cancer biomarker, telomerase activity has been observed in ~90% of human tumors,^[1] compared to the absence of telomerase activity in most normal somatic cells.^[2] This suggests that the immortality conferred by telomerase plays a key role in cancer development.^[3-12] Telomerase is a reverse transcriptase that transcribes single-stranded RNA into ssDNA at chromosomal ends. In order to protect chromosomal linear ends from the constant loss of important DNA during cell differentiation, telomerase uses its own RNA template to elongate telomeres with specific DNA sequence repeats (“TTAGGG” in all vertebrates) at the 3′-ends of eukaryotic chromosomes. Consequently, chromosome ends can never be compromised, irrespective of the number of cell divisions. However, since telomerase also allows cancer cells to divide in perpetuity, tumors are a likely outcome.

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To date, several commercially available assays, such as the telomeric repeat amplification protocol (TRAP), have been developed to evaluate telomerase activity. TRAP is currently the most widely used and most sensitive method for telomerase detection.^[13-18] Although quite powerful, TRAP requires the use of DNA polymerases and is therefore susceptible to PCR-derived artifacts,^[19] especially when screening compounds for telomerase inhibition. A number of PCR-free assays for telomerase activity, based either on direct probing^[20] [21] or formation of a sandwich structure containing the elongated strands,^[22] have been developed over the past decade. However, the lack of a suitable amplification mechanism has hindered most of these methods from achieving sensitivity comparable to TRAP.^[23]

Here, we study telomerase activity using a molecular sensor based on substrate cleavage by a DNAzyme.^[24] In recent years, DNAzymes have been reported for a variety of uses, including assays to detect environmental contaminants or metal ions in cell-based studies, DNA computing, nanowire production and drugs in preclinical models of cancer. However, 8-17 DNAzyme, which, in the presence of its metal ion cofactor, specifically recognizes and catalytically cleaves its complementary substrate, has attracted growing interest for its high selectivity, specificity and sensitivity for biosensor construction.

We used the Pb^{II}-dependent 8-17 DNAzyme as an active component for the analysis of telomerase activity. As shown in Figure 1, this hairpin molecule is engineered by an intramolecular assembly strategy and consists of three functional domains: 1) a DNAzyme catalytic sequence, 2) a primer sequence to allow telomere elongation in the presence of trace amounts of telomerase, and 3) a polythymine (Poly-T) linker. In the presence of telomerase the primer sequences are recognized, and the substrate fragment is generated through telomere DNA elongation. The substrate then hybridizes with the catalytic DNAzyme binding domain (Figure 1), forming a stable hairpin structure. However, if a trace amount of lead(II) ion is also present, the substrate will be cleaved by DNAzyme and dissociated from the binding domain.

Signal transduction is achieved through the molecular beacon (M9). In the absence of the substrate fragment, the molecular beacon forms a stable hairpin structure, and the reporter fluorescence is completely quenched by the proximity of the fluorophore and quencher. However, M9 is opened upon complete hybridization with the cleaved substrate, and the fluorescence signal is restored. The fluorescence signal is amplified by repeated cycling of the probe to form a large number of cleaved substrate fragments, thereby allowing determination of trace amounts of telomerase protein for application in early-stage cancer diagnosis.

In this paper, we report the design, molecular engineering and optimization of the probe in terms of primer and linker length. We characterize the performance of the optimized telomerase detection probe and compare its detection abilities to those of the conventional TRAP assay. In our design, high sensitivity is achieved without loss of selectivity of the probe, which maintains a quantifiable detection range from 0.1 μ g to 20 μ g of total protein in cell lysate and a selectivity of over 200-fold for cancer cells over normal cells. Based on these results, this probe provides a simple, yet rapid and sensitive, measurement of telomerase concentration.

Experimental Section

Chemicals and Reagents

All DNA synthesis reagents, including 6-Fluorescein (FAM) phosphoramidite, 5'-4-(4-dimethylaminophenylazo) benzoic acid, (Dabcyl) phosphoramidite, and 2'-O-Triisopropylsilyloxymethyl-protected RNA monomers, were purchased from Glen Research.

Lead acetate and all reagents for buffer preparation and HPLC purification were acquired from Fisher Scientific. The buffer used for the experiment contained 50 mM Tris-acetate at pH 7.2 and 100 mM NaCl.

Synthesis and Purification of Oligonucleotides

To optimize the design of the hairpin probe, multiple candidates were designed and prepared (Table 1). All were synthesized using an ABI 3400 DNA/RNA synthesizer (Applied Biosystems) at the 1- μ mol scale via standard phosphoramidite chemistry. HPLC was performed on a ProStar HPLC Station (Varian Medical Systems) equipped with a fluorescence detector and a photodiode array detector. A C₁₈ reverse-phase column (Alltech, C18, 5 μ m, 250 $\times$ 4.6 mm) with acetonitrile and 0.10 M aqueous TEAA buffer, pH 7.0, as the mobile phase.

Determination of the Melting Temperature

The melting temperature measurement for the designed sequences TeloD9, TeloD12, and TeloD6 were performed using a Bio-Rad thermal cycler at a heating rate of 1°C/min, with the fluorescence intensity at 520 nm recorded as a function of temperature every 30 s. The cooling curve of the molecule was also obtained by decreasing the temperature at a rate of 3°C/min and recording the fluorescence intensity. Fluorescence-temperature profiles of the three molecules were recorded in 50 nM Tris-acetate buffer, pH 7.2, with 100 mM NaCl. *T_m* values were taken as the temperatures corresponding to half-dissociation of the intramolecular duplex.

Cell Extraction

One normal cell line, HBE135, and two different liver carcinoma cell lines, M7617 and LH86, were used in experiments. LH 86, a human hepatoma cell line, is from a well-differentiated hepatocellular carcinoma tissue. HBE135, LH86 and M7617 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (heat-inactivated) and 100 IU/mL penicillin-streptomycin at 37°C in a humid atmosphere with 5% CO₂. Cells were harvested and washed twice with ice-cold phosphate-buffered saline (PBS) (10 mM KPO₄, pH 7.5, 140 mM NaCl). Then cells were lysed in NP-40 lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-HCl, pH 8.0, and 5 mM EDTA) with a proteinase inhibitor cocktail (Sigma) for 30 min on ice. Lysates were centrifuged at 14,000 rpm for 20 min at 4°C. The supernatant was stored at -80 °C in 20% glycerol and 100 mM NaCl. The protein content of the supernatant was measured using the Bio-Rad protein assay.

Telomerase-Mediated Primer Elongation

The reaction buffer was modified from the protocol published by Kim et al.^[25], consisting of 20 mM Tris-HCl, 1.5 mM MgCl₂, 60 mM KCl, 1 mM EGTA, 0.05% Tween, and 50 μ M nucleotide. To the reaction buffer, 0.15 nmol of probe and 1 μ g of cell extract (protein) were added to generate the final reaction mixture. This reaction mixture was then diluted to a final volume of 50 μ L and incubated for 1 h at 37°C in a PCR thermal cycler. Then, the temperature was increased to 94°C for 5 min to deactivate telomerase and to terminate the elongation.

TRAP Assay

Telomerase extracts were prepared and analyzed as described.^[26] Briefly, cells were washed in PBS and then lysed in ice-cold lysis buffer (10 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM 2-mercaptoethanol, .5% CHAPS and 10% glycerol). All cell extracts were immediately frozen and stored at -80°C. Usually,

the whole-cell extracts were thawed immediately prior to the TRAP assay. First, extracts were centrifuged at 14000g for 30 min at 4°C. Supernatants were transferred to clean tubes, and protein concentrations were measured using the Bio-Rad protein assay kit. Finally, TRAP, which is a one-tube PCR-based assay, was performed^[2] to validate telomerase activity.

Hybridization Assay

The working principle underlying the application of TeloD9 to monitor telomerase is shown schematically in Figure 1. TeloD9 was annealed at a final concentration of 100nM, using the same procedure performed in the melting temperature determination. The annealed sample was then cooled to room temperature for subsequent assays. An 80-μL aliquot containing 100 nM hybridized DNAzyme/substrate solution and 10X molecular beacon M9 was loaded into wells of a 96-well plate. A 10-μL aliquot of concentrated telomerase stock solution was then added to the DNA solution to initiate telomerization. Incubation was performed at 37°C for 30 minutes. Afterwards, a 10 μL aliquot of 20μM lead stock solution was added to the DNA solution to initiate the cleavage reaction. The fluorescence intensity was recorded for 100 μL buffer containing 50 mM Tris-acetate (pH7.2), 100 mM KCl, 100nM TeloD9 and 1μM M9 solution for three sets of conditions: without telomerase, with telomerase, and with both telomerase and lead solution.). Bound telomerase was removed by detergent such as sodium dodecyl sulfate (SDS) solution. The excitation and emission wavelengths were set to 473 nm and 520 nm, respectively. Signal enhancement (S/B in Table 2) was calculated using the equation $(F_{\text{cleaved}} - F_{\text{buffer}})/(F_{\text{annealed}} - F_{\text{buffer}})$, where F_{cleaved} represents fluorescence signals from M9 after cleavage of the substrate fragment in the presence of Pb^{II} , F_{annealed} represents the fluorescence signals from the hairpin probes without cleavage, and F_{buffer} represents the fluorescence signals of buffer.

Gel-Based Activity Assay

Two-μL TeloD9 samples were annealed in 50 mM Tris-acetate buffer containing 100 mM NaCl (pH 7.2). After removing a 5 μL aliquot as a zero time point, Pb^{II} was added to the remaining solution with a final concentration of 50 nM. Aliquots were removed at 60-second intervals. The TeloD9-only sample was also loaded onto the gel to judge the effect without telomerase, without Pb^{II} , and without both telomerase and Pb^{II} . The cleaved and uncleaved substrates were separated by 20% denatured polyacrylamide gel electrofluorescence imaging, and the gel was analyzed by a fluorescence imager (FLA-3000G; Fuji, Tokyo, Japan) by exciting at 473 nm. Gels containing 20% polyacrylamide were run on a FB-VE10-1 electrophoresis unit (Fisher Biotech) at room temperature (200V, constant voltage) for 50 minutes in TAE/ Mg^{II} buffer.

Results and Discussion

Design and Optimization of Probe for Catalytic Reaction to Detect Telomerase

Several analytical procedures for the determination of telomerase activity have been developed, including TRAP (Figure 2), which involves intensive PCR amplification, or the functionalization of telomeres with fluorescent labels.^[27] In contrast, we have designed a new probe to monitor telomerase activity using a Pb^{II} -initiated DNAzyme catalytic reaction. Telomerase detection with this assay involves three basic steps (Figure 1). First, telomerase is extracted from cancer cells and mixed with substrate oligonucleotides, which are then annealed with 8-17 DNAzyme at the 3'-end. These oligonucleotide sequences are both telomerase-binding substrates and strands to partially help 8-17 DNAzyme form its catalytically active tertiary structure. Second, telomerase elongation is induced, and an elongation product is formed and hybridized to 8-17 DNAzyme, in which the 8-17 DNAzyme is forced to form its catalytically active structure. Specifically, the bound

telomerase can catalytically elongate the DNA strands in TTAGGG repeats in the presence of four deoxyribonucleotide triphosphate (dNTP) monomers (dATP, dCTP, dGTP, and dTTP). The 8-17 DNAzyme is formed into a catalytically active structure because the elongation product is able to recognize its complementary target, which is the flanking binding arm at the 5'-end of 8-17 DNAzyme. Third, in the presence of 10 μ M Pb^{II}, the telomere product is cleaved and dissociated, while the probe returns to its initial state and awaits the next round of elongation. Subsequently, the dissociated cleavage product then hybridizes with a complementary molecular beacon (M9), and the fluorescence signal enhancement of the opened molecular beacon is measured. To increase cleavage reaction efficiency, we constructed a hairpin-structured telomerase probe with the substrate in close proximity to the 8-17 DNAzyme, thereby ensuring total hybridization efficiency. With this design, the Pb^{II} binding pocket was well formed, thus increasing the apparent cleavage efficiency.

In order to maximize probe performance, several design features must be considered. First, once the primer is elongated, the linker length must be such that the product will reach a range that allows rapid hybridization with the DNAzyme, subsequently leading to cleavage and release. Second, the design is initialized by telomerase recognition of the primer; therefore, the telomerase primer, which is located at the 3'-end of TeloD9, must have higher binding affinity with telomerase than with the 8-17 DNAzyme binding arm. This allows the primer sequence to be quickly recognized in the presence of telomerase and then easily dissociated from the intramolecular DNAzyme binding arm, thereby allowing elongation to resume. In order to achieve suitable optimization toward these requirements, we investigated the probe sequences by measuring the background fluorescence and melting temperatures of the hairpin structures (Table-2) to compare efficient structure formation (Figure 3). Third, optimization of the length of the 5'-end binding arm of DNAzyme depends on the nature of the telomere, which is a known sequence (TTAGGG, human telomere repeating unit).

To implement this plan, we designed probes termed TeloD6, TeloD9 and TeloD12 containing 6, 9, and 12 nucleotides (nt) in telomerase primer sequence fragments, respectively. No probe was designed containing a longer substrate because dissociation with a base pair number in excess of 12 would, in all likelihood, have been unfavorable due to the strong binding effect,^[28] resulting in low efficiency of elongation. As shown in Figure 3, the fluorescence background using TeloD9 probes without Pb^{II} was very low. However, in the presence of Pb^{II}, the elongated duplex structures bind the ions, thereby activating the DNAzyme to catalyze the cleavage reaction. Figure 3 also shows that TeloD9, with a moderate length of telomeric primer fragment, possessed maximum efficiency in the catalytic amplification reaction, giving an approximately 15-fold signal enhancement when the Pb^{II} was added. This outstanding signal enhancement in comparison to other candidates can be explained by enhanced telomerization efficiency based on the competition between two binding events: 1) the intramolecular hybridization between the primer and DNAzyme, and 2) the intermolecular binding between primer and the elongation template within the telomerase protein. Duplex stability (binding event 1) increases while the primer is elongated by telomerase enzyme, and the elongated product recognizes its complementary strand in the 5'-end binding arm of the DNAzyme fragment. In the presence of Pb^{II} ions, the elongation product is correctly positioned for cleavage by the DNAzyme and dissociation from the 5'-end binding arm.

At room temperature, stable duplex formation of the primer with the complementary region of the DNAzyme probe will not be achieved unless the two DNA segments are linked by the poly-T in a single probe unit. Comparing the melting temperature of TeloD9 (57.3°C) to unlinked DNAzyme and its substrate (36°C), the enhanced stability is quite significant. This enhanced duplex stability also results in improved cleavage efficiency.

The effect of primer length is demonstrated in Figure 3. Unlike TeloD9, TeloD6 showed low fluorescence enhancement because of low telomerization efficiency, indicating that a 6-nucleotide primer may be too short to efficiently produce enough telomerase elongation product. Lack of elongation product would produce incomplete hybridization (no formation of structure II in Figure 1), thereby resulting in an inefficient cleavage reaction.^[29] On the other hand, no noticeable cleavage was observed for TeloD12, because the telomerase could not bind to the primer. With 12 base pairs, the hybridization between telomeric primer fragment and 8-17 DNAzyme binding arm is so strong that the telomerase protein cannot compete, and elongation does not occur. In contrast, TeloD9, in the absence of Pb^{II} , showed a low fluorescence background signal, but upon the addition of Pb^{II} (10 μM), fluorescence enhancement was observed within 5 minutes. This experiment shows that the increased fluorescence was indeed a result of DNAzyme-catalyzed cleavage with Pb^{II} as a cofactor.

Because Mg^{II} , Cu^{II} , and Zn^{II} are common divalent metal ions present in biological samples, we also checked for possible cross-reactivity for a series of metal ions. According to the results in Figure 4, TeloD9 presented significant response to Pb^{II} over other divalent metal ions.

Characterization of the Probe with Analytical Parameters

To further obtain the full profile of the performance of TeloD9, we determined its dose response and enzymatic reaction kinetics. Figure 5 shows the profile of lead ion dependent fluorescence signal enhancement for the three cell types studied. A linear response is observed at low lead concentrations. Above the linear region, fluorescence enhancement continues to rise for the cancer cell lines (LH86 and M7617), with much less enhancement for the normal cells (HBE135). However, when the $[Pb^{II}]$ was higher than 10 μM , no improvement was observed, and 10 μM was chosen as the optimum concentration.

One significant feature of the DNAzyme-based telomerase sensor is its excellent selectivity, which derives from unique secondary configurations enabling the probes to discriminate between target (cancer cell extracts which presented telomerase) and nontarget (normal cell extracts without telomerase). With this capability, TeloD9 possesses the selectivity needed to discriminate between healthy normal cells (trace levels of telomerase) and cancer cells (abundant telomerase). Thus, to demonstrate the excellent selectivity of TeloD9 for cancer cells against normal cells, the fluorescence signal changes of three types of cell lysates at different concentrations were obtained (Figure 6). As expected, the normal cell lysate induced little fluorescence change, indicating that the intramolecular engineering of TeloD9 accomplished the goal of *in vitro* early cancer diagnosis. We suspected that the false positive signal towards normal cell lysate may be attributed to the low percentage of M9 binding to the 3' end of TeloD9 itself, even without telomerase, since they are partially complementary to each other. To determine telomerase dependency, we also validated the fluorescence signal by adding different amounts of cell extract protein to the initial reaction mixture. As shown in Figure 6, TeloD9 can provide up to 16-fold signal enhancement in the presence of 1 μg cancer cell protein extract, compared to only 3-fold enhancement for normal cells. This large signal enhancement allows us to detect telomerase over a large dynamic range with a low detection limit of telomerase in approximately 0.1 μg total protein from cell extracts.

In addition to the excellent sensitivity and selectivity of TeloD9 towards telomerase, this probe also shows rapid cleavage kinetics. As shown in Figure 7, the signal reaches a plateau within 20 minutes. The time to reach this plateau was more than 30 minutes (data not shown) when 8-17 DNAzyme and its substrate (starting with telomere primer) were added separately, instead of as a single probe molecule.

The use of DNAzymes for amplified analysis of telomerase provides several advantages. First, the high turnover of telomerase induced elongation reaction assures formation of a large number of well-formed lead binding pockets. Second, the catalytic cleavage reaction allows a single probe to generate many product segments for detection by the molecular beacon design. Third, the molecular beacon fluorescence signal is detected within 5 minutes through fluorescence measurement, much faster than the total time for current PCR based assays, which take several hours from sample preparation to assay generation. Therefore, this new assay rivals the sensitivity and convenience of the conventional PCR-based method of telomerase detection.

Characterization of Probe Activity with PAGE-Based Assay

A PAGE-based assay was used to assess the multiple turnover kinetics of the elongation/cleavage reaction of TeloD9 in the presence of telomerase. For these experiments, 20 μM TeloD9 was incubated with 2 μg protein in pH 7.2 Tris-acetate buffer at room temperature for a total 20-minute *in vitro* reaction. Aliquots were periodically removed, and the reaction was terminated by the addition of formamide (90%, v/v) containing 15 mM EDTA.^[30] The cleaved DNA fragments from TeloD9 were separated by polyacrylamide gel electrophoresis, and the extent of conversion was quantified using a UV imager. K_m and k_{cat} values were obtained by fitting velocity of cleavage versus substrate concentration using the equation $V = k_{cat}[S]/(K_m + [S])$. All reactions were terminated and processed as described in the previous section.

The multiple turnover results shown in Figure 8 demonstrate that our probe has the advantage of enzymatic signal amplification according to Michaelis-Menten kinetics, ensuring its ability to detect low levels of telomerase, thus promising application in early cancer diagnosis. Under the conditions used in these experiments, $V_{max} = 0.97 \mu\text{M}/\text{min}$, $K_m = 0.71 \mu\text{M}$, and $k_{cat} = 0.017 \text{ s}^{-1}$. Specifically, in the presence of 2 μg protein, 20 μM TeloD9 was cleaved, resulting in over 700 catalytic turnovers of the DNAzyme used in the designed structure. In this experiment, prehybridization of the TeloD9 probe structure is not required. Thus, there is no need to heat or cool the TeloD9 before hybridization and subsequent cleavage by the introduction of Pb^{II} .

Conclusion

In conclusion, we demonstrated a PCR-free, yet highly sensitive and selective, DNAzyme-based telomerase sensor. Duplex formation between the Pb^{II} -specific DNAzyme fragment and telomere primer results in efficient and specific cleavage of telomere target after telomerase elongation. Our single-molecule probe design allows a quantifiable detection range from 0.1 μg -1 μg protein from cancer cell lysate. Not only is this measurement tool for telomerase detection highly sensitive and selective, it is simple, cost effective, and can be used in cancer cells. Thus, this novel probe can be applied to both cancer studies and cancer biomarker discovery. Given this degree of sensitivity and selectivity, our molecular engineering design may prove useful in the future development of other nucleic acid-based probes for clinical toxicology and cancer therapeutics.

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Figure 1.

Scheme of the hairpin-structured, DNase-based telomerase sensor and its working principle. I: The TeloD9 DNase/substrate hybrid strand, which is composed of DNase (8-17 DNase, bottom) and telomere primer, upper, hybridize together in Tris-acetate buffer, containing 60mM K^+ . II: After telomerase-induced elongation, an elongation product is formed and hybridizes to 8-17 DNase, in which the 8-17 DNase is forced to form its catalytically active structure. III: In the presence of $10 \mu M Pb^{II}$, the elongation product is cleaved and dissociated. This dissociated ssDNA is then detected by complementary a molecular beacon, which reports the telomerase activity by fluorescence enhancement.

Figure 2.

Gel Image for TRAP assays of extracts from cancer samples (M7617 and LH76), and one normal sample (HBE135), which served as negative control. PCR products were separated by electrophoresis on a non-denaturing 12.5% polyacrylamide gel, stained with ethidium bromide, and visualized on a UV transilluminator.

Figure 3.

Optimization of synthesized probes using fluorescence signal comparison. Experiments were run in the presence of 2 μg protein after 10 min incubation with TeloD9, 6, and 12, respectively. Measurements were acquired after addition of Pb^{II} to a final concentration of 10 μM .

Figure 4.

Selectivity of the TeloD9 probe sensor. Sensor responses to all competing metal ions at the eight concentrations tested. The TeloD9 concentration was 200 nM, while M9 was 20 μM, and the buffer contained 50 mM Tris-acetate (pH 7.2) and 100 mM NaCl. The reaction time was 10 min. The values indicate means from three experiments, and the error bars represent standard derivations.

Figure 5.

Fluorescence increase over background fluorescence at varying Pb^{II} concentrations for three cell types. The TeloD9 concentration was 200 nM, while M9 was 20 μM , and the buffer contained 50 mM Tris-acetate (pH 7.2) and 100 mM NaCl. The values indicate means from three experiments, and the error bars represent standard derivations.

Figure 6.

Monitoring of dose-dependent telomerase elongation in presence of $10 \mu\text{M Pb}^{\text{II}}$ for different concentrations of total protein. The TeloD9 concentration was 200 nM , while M9 was $20 \mu\text{M}$, and the buffer contained $50 \text{ mM Tris-acetate (pH 7.2)}$ and 100 mM NaCl . The background-corrected intensity in each cell type has been normalized relative to $0 \mu\text{g}$ of protein. The values indicate the means from three experiments, and the error bars represent standard derivations.

Figure 7.

Analysis of DNase cleavage reaction for different Pb^{II} incubation times. M7617 and LH86 are cancer cells, and HBE135 is a normal cell line. The TeloD9 concentration was 200 nM, while M9 was 20 μM , and the buffer contained 50 mM Tris-acetate (pH 7.2) and 100 mM NaCl. The values indicate means from three experiments, and the error bars represent standard derivations.

Figure 8.

Catalytic activity of TeloD9 elongation/cleavage. The red line represents a nonlinear fit to the data using the Michaelis–Menten expression with $K_m = 0.71 \mu\text{M}$, and $k_{\text{cat}} = 0.017 \text{ s}^{-1}$. (Inset) The upper band represents uncleaved TeloD9, and the lower band represents the cleaved fragment.

Table 1
Names and sequences of DNA used in the paper. rA is the adenosine 5'-phosphate monomer. All syntheses were performed with an ABI synthesizer. The DNA/RNA chimers were purified by HPLC

Name	Sequences
TeloD12	5'-CTAACCCTAACCTCCGAGCCGGACGAAACCCTAACCCTTTTTTTTTTGGGTTAGGGTTrA-3'
TeloD9	5'-ACCCTAACCTCCGAGCCGGACGAAACCCTAATTTTTTTTTTTAGGGTTrA-3'
TeloD6	5'-TAACCTCCGAGCCGGACGAAACCCTTTTTTTTTTGGGTTTrA-3'
M9	5'-TTAGGGTTAACCCTAACCCTAACCCTAA-3'
D9	5'-ACCCTAACCTCCGAGCCGGTCGAAAACCCTAA
DS9	5'-TTAGGGTTA-3'

Table 2
Comparison of melting temperature and fluorescence enhancement among different design sequences

Hairpin sequence	T _m (°C)	S/B ratio
TeloD6	53.2	5
TeloD9	57.3	14
TeloD12	59.1	8
D9+DS9	36.2	2