

Ratiometric Fluorescent Biosensor for Visual Discrimination of Cancer Cells with Different Telomerase Expression Levels

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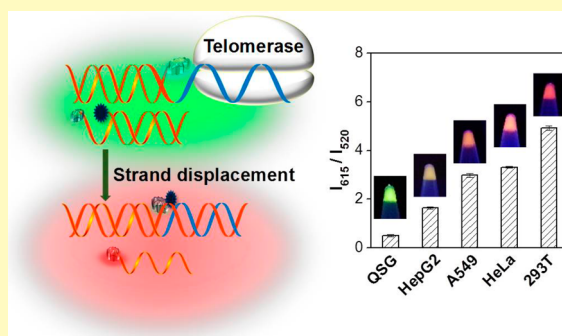
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S Supporting Information

ABSTRACT: Telomerase is inactive in normal somatic cells but highly activated in tumor cells to maintain their indefinite proliferation and immortal phenotype. As a specific marker for the generation and progress of almost all tumors, the detection of telomerase activity by classical PCR techniques has served in the biological research of tumors. However, the detection of in situ telomerase activity in cell extracts to evaluate the malignancy, progress, and metastasis of tumors remains a daunting challenge. Here, a precisely designed FRET-based ratiometric fluorescent oligonucleotide probe has achieved high-fidelity detection of telomerase activity for accurate discrimination of different cancer cells toward advanced diagnosis of tumors. Our method is superior to other methods in its capabilities to quantify telomerase activity in cell extracts and visualize various tumor cell extracts with different telomerase expression levels by the naked eye for clinical diagnosis. In particular, the ratiometric fluorescent probe used in the assay could exclude other experimental factors influence, and further avoid false positive signal generation. The method reported here could provide a reliable, accurate, and convenient way in medical diagnostics and therapeutic response assessment.

KEYWORDS: ratiometric fluorescent probe, telomerase activity detection, cancer cell discrimination, naked eye, FRET



Telomeres at the ends of chromosomes are a special structure to protect genome integrity from degradation, rearrangements, and end-end fusion.^{1–7} Progressive telomere shortening over the course of cell division leads to “replicative senescence” of somatic cells, which is counteracted in tumor cells by activating telomerase to compensate telomeric depletion.^{1,8} Telomerase is inactive in normal somatic cells, which would ultimately induce cell apoptosis.^{9–11} However, it is highly activated in almost all tumor cells to maintain their immortal phenotype or indefinite proliferation. Therefore, telomerase activity has become an important marker for malignant tumor generation and progression in the diagnosis and therapy of cancers.^{4,12,13}

Several strategies based on the telomeric repeat amplification protocol (TRAP)^{2,14} have been developed for detecting telomerase activity, such as the electrochemical method,^{15–17} surface-enhanced Raman scattering,^{18,19} and fluorescent method.^{20–24} These methods have significantly improved the sensitivity and feasibility of telomerase activity detection, but they still suffer from several drawbacks that include being time-consuming and requiring expensive instruments and sophisticated experimental steps. In contrast, the fluorescent method holds great promise for telomerase activity detection because of its high signal intensity, real-time detection, and easy use and

visualization. Despite these attractions, a number of notable challenges associated with this method still exist. For instance, most fluorescent methods are a one-color fluorophore-modified DNA probe that can be opened or closed by telomerase sequence through elongating a part of the DNA probe under the catalysis of telomerase,²⁵ leading to difficulty in distinguishing different types of cancer cells by the naked eye. In addition, many external factors will affect DNA unwinding during the telomerase catalysis process in cells, generating false positive signals.²⁶ Herein, we describe a ratiometric fluorescent probe to qualitatively and quantitatively detect telomerase activity based on two double strand DNA (dsDNA) labeled with two fluorophores and one quencher.²⁷ Upon the addition of telomerase, one dsDNA constructed by telomerase primer sequence (TS) and TS complementary sequence labeled with FAM (F1) was catalyzed and prolonged to form telomeric repeats, which would fully complement with Dabcyl-modified single-strand DNA (ssDNA). The previous FRET from Texas Red to Dabcyl of partially complementary dsDNA sequence (T1) was broken, and a new FRET from FAM and Dabcyl of

Received: January 17, 2018

Accepted: March 13, 2018

T1 was formed, which involves a color variation from green to red under UV irradiation by the naked eye. This novel assay offers not only high sensitivity and excellent specificity but also avoids false positive signals and distinguishes normal/cancer cells, which is favorable for the reliable and accurate detection of telomerase activity and easy discrimination of various tumor cell extracts with different telomerase expression levels by the naked eye. The proposed strategy for assaying telomerase activity of cell extracts was considered to play an important role in early diagnosis and therapy for cancer.

EXPERIMENTAL SECTION

Materials. All DNA sequences used in the experiment were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The DNA stock solution (HB buffer) was prepared by mixing 1 mM KCl, 1 mM Tris-HCl, 0.5 mM MgCl₂ (pH 7.5). Each hybrid DNA probe was prepared by mixing two single-strand DNA (ssDNA) and heating to 94 °C for 3 min and then cooling to room temperature slowly. The RNase inhibitor, gel electrophoresis loading buffer, CHAPS lysis buffer, DNA marker, diethyl pyrocarbonate (DEPC), PBS (136.89 mM NaCl, 2.67 mM KCl, 8.24 mM Na₂HPO₄, and 1.76 mM KH₂PO₄, pH 7.4) and Tween 20 were all purchased from Sangon Biological Engineering Technology & Co., Ltd. (Shanghai, China).

Cell Cultures. HeLa (human cervical cancer cell line), KB (human oral epidermoid cancer cell line), HepG2 (human liver cancer cell line), and A549 (human lung cancer cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 10% fetal calf serum (FCS, Sigma) and 1% penicillin-streptomycin. QSG (human normal hepatic cell line) was cultured in RPMI-1640 (GIBCO) with 10% fetal calf serum and 1% penicillin-streptomycin. All of the cell lines were cultured in a humidified atmosphere containing 5% CO₂ at 37 °C in a culture dish.

Preparation of Cell Lysates. Cells were lysed by the NP-40 method according to a well-established protocol.²⁸ Briefly, cells were trypsinized from culture dishes, washed twice with PBS (pH 7.4), and resuspended in 1 mL of cooled lysis buffer (10 mM Tris-HCl, 1% NP-40, 150 mM NaCl, 10% glycerol, 0.25 mM sodium deoxycholate, 0.1 mM AEBSF, pH 8.0) at a concentration of 1.0×10^7 cells/mL. The cells were kept at 4 °C for 30 min under shaking, and the mixture was centrifuged at 11,000 rpm for 20 min at 4 °C to remove any cell debris. The lysates were flash frozen in liquid nitrogen and stored at -80 °C for further use.

Detection of Telomerase Activity. Different volumes of cell lysates were incubated with a mixture containing of 2 μ L of 50 μ M probe T1, 2 μ L of 50 μ M probe F1, 20 μ L of 10 $\times$ TRAP reaction buffer (200 mM Tris-HCl, pH 8.3, 15 mM MgCl₂, 630 mM KCl, 0.5% Tween 20, 10 mM EGTA), and 4 μ L of 10 mM dNTPs at 37 °C for 3 h (in all tests, the final volumes were adjusted to 200 μ L with DEPC-treated water). For control experiments, telomerase in the cell lysates was deactivated by heating at 90 °C for 30 min.

Gel Electrophoresis and PCR Amplification. The reaction for the elongation of telomerase primer with the telomeric repeat TTAGGG was performed using cell lysate. Two microliters of cell lysate was mixed with 3 μ L of telomerase primer (100 μ M), 16 μ L of PCR water, 2.5 μ L of 10 $\times$ TRAP reaction buffer, 0.5 μ L of 50 $\times$ dNTP mix (10 mM, 2.5 mM each of dATP, dTTP, dGTP, and dCTP in RNase-free H₂O), 0.5 μ L of TRAP primer mix (36 bp internal standard control (TSNT), 5'-AATCCGTCGAGCAGAGTTAAAG-GCCGAGAAGCGAT-3'; ACX primer, 5'-GCGCGGCTTACCCTT-ACCCTTACCCTAACC-3'; NT primer, 5'-ATCGCTTCTCGGCC-TTTT-3'), and 0.5 μ L of Taq polymerase (5 units/ μ L) in a centrifuge tube. The mixing solution was then incubated in a water bath at 37 °C for 30 min to elongate the TP primer. The elongated TP strands with (TTAGGG)_n were amplified in the PCR instrument through 32 circular steps, and each step included an incubation at 94 °C for 30 s, 62 °C for another 30 s, and 72 °C for 1 min. The amplification of hybridized duplex DNA chains was also performed according to identical steps.

The working principle of the probe was verified by analyzing gel electrophoresis. The samples were mixed with 2.0 μ L of loading buffer and then injected into polyacrylamide hydrogel. Electrophoresis was carried out in tris-borate EDTA (TBE) buffer at 110 V in TBE buffer for 65 min. The gel was stained for 10 min with 4S red plus and imaged using a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company).

RESULTS AND DISCUSSION

Principle of Assay. The principle of this reliable ratiometric probe to quantitatively detect telomerase activity based on the FRET mechanism²⁹ is illustrated in Scheme 1 and

Scheme 1. Schematic Illustration of the Ratiometric Fluorescent DNA Probe for Detecting Telomerase Activity in Cell Extracts

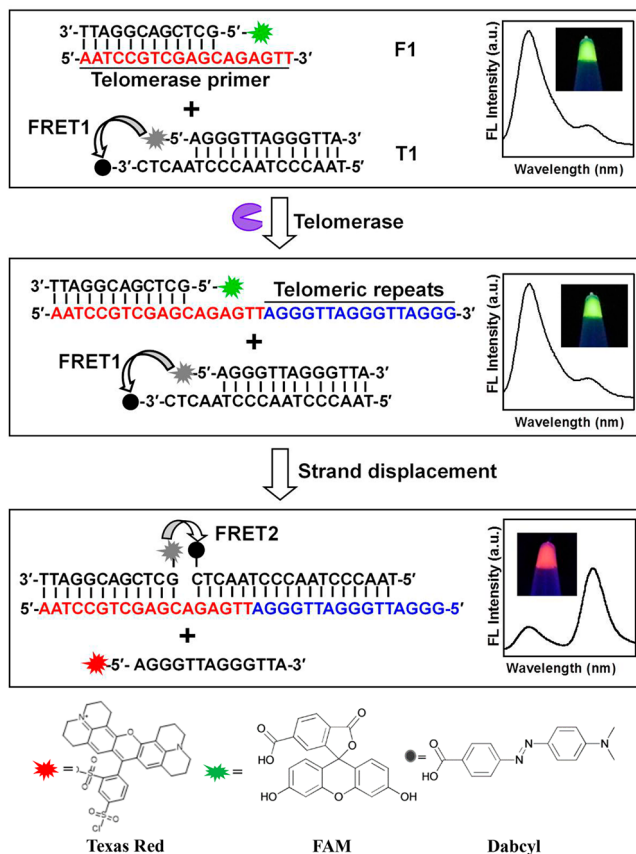


Table S1. The ratiometric fluorescent oligonucleotide probe is constructed using a quencher- and fluorophore-labeled duplex oligonucleotide (T1) as well as another fluorophore-labeled duplex oligonucleotide (F1). T1 is designed as a partly complementary dsDNA modified with Texas Red at the 5'-end as a donor of one ssDNA and Dabcyl at the 3'-end as a quencher of another ssDNA. T1 exhibits no color under an excitation wavelength of 365 nm due to the FRET1 effect from Texas Red to Dabcyl through the formation of partly complementary dsDNA structure. F1 is labeled with FAM at the 5'-end of ssDNA, which is partly complementary with telomerase primer sequence that could be catalyzed and prolonged by telomerase. Consequently, the F1 and T1 mixture display green fluorescence under the excitation of a UV lamp ($\lambda_{\text{ex}} = 365$ nm). In the presence of the target telomerase, F1 was catalyzed and prolonged in a dNTP mixture

to form telomeric repeats at the 3'-end of the telomerase primer sequence. The prolonged F1 dsDNA sequence would completely complement with Dabcyl-modified ssDNA of T1 and subsequently induce the breaking of FRET1 between Texas Red and Dabcyl and formation of new FRET2 between FAM and Dabcyl, which involves a color variation from green to red. Compared to conventional single-dye-labeled DNA to detect telomerase activity,³⁰ this ratiometric fluorescent probe based on the FRET mechanism was expected to possess the excellent capability of avoiding false positive signals, which improves the reliability and accuracy of telomerase activity detection in cancer cell extracts.

Telomerase-Responsive Fluorescence Variation. For the telomerase-responsive fluorescence variation of the ratiometric probe to be evaluated, the fluorescent spectra of the ratiometric fluorescent probe (2 μ L of 50 μ M probe T1, 2 μ L of 50 μ M probe F1) in the presence of dNTPs (4 μ L of 10 mM) was observed after incubation for different amounts of time in HeLa cell extracts. Over time from 0 to 320 min, the fluorescence intensity of FAM centered at 520 nm gradually decreases, whereas the red fluorescence of Texas Red centered at 615 nm steadily increases (Figure 1A), demonstrating the

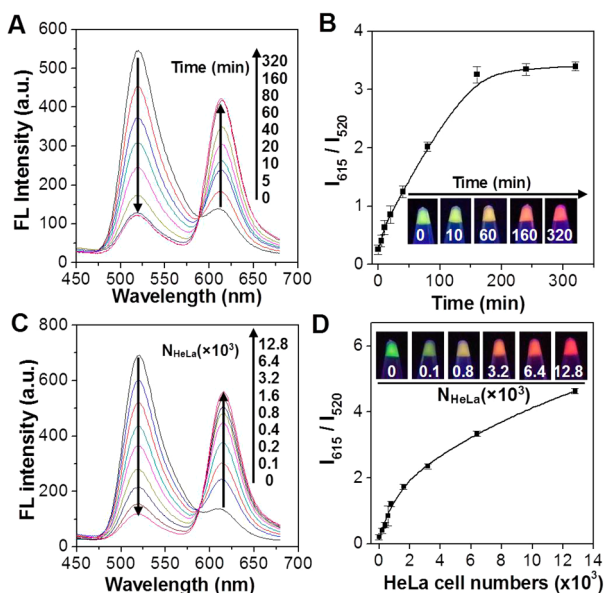


Figure 1. (A) Fluorescence spectra of 50 nM ratiometric fluorescence probe in 6.4×10^3 HeLa cell extracts in response to increasing reaction time. (B) Plot of the fluorescence intensity ratio (I_{615}/I_{520}) vs reaction time. The insets show the corresponding fluorescence colors at different times under a 365 nm UV lamp. (C) Fluorescence spectra of 50 nM ratiometric fluorescence probe response to cell extracts of different cell numbers for 3 h. (D) Plot of fluorescence intensity ratio (I_{615}/I_{520}) vs different cell numbers in HeLa cell extracts. The insets show the corresponding fluorescence colors with different HeLa cell numbers under a 365 nm UV lamp.

occurrence of the FRET2 process between FAM and Dabcyl and the destruction of FRET1 between Texas Red and Dabcyl. The fluorescence ratio (I_{615}/I_{520}) gradually increased with increasing incubation time from 0 to 160 min and reached a plateau after 160 min (Figure 1B), which resulted in a fantastic color change from green to yellow green to yellow to orange red to red. In addition, with the addition of the ratiometric fluorescent DNA probe to different numbers (different concentrations of telomerase) of HeLa cell extracts, the

green fluorescence of FAM was dramatically quenched while the red fluorescence of Texas Red was gradually enhanced, similar to the trend of the fluorescent probe incubated in 6.4×10^3 HeLa cell extracts with prolonged incubation time. Importantly, the fluorescence intensity ratio (I_{615}/I_{520}) progressively increased from the initial value of 0.2:1 to 4.6:1 at 12.8×10^3 HeLa cell number, suggesting its high sensitivity to the HeLa cell number. When the number of HeLa cells was up to 12.8×10^3 , the fluorescence intensity of FAM became much weaker than that of Texas Red, leading to the remarkable color change from green to red under a UV lamp (inset of Figure 1B). The fluorescence intensity ratio increased proportionately with a good linearity ranging from 0 to 0.8×10^3 HeLa cell number by the correlation coefficient of 0.9666 in the equation $I_{615}/I_{520} = 1.18c + 0.16$ (c being the HeLa cell number, Figure S2). The detection limit in this method is defined as 3-times the standard deviation of background (3σ) divided by the slope of the standard curve, and it was calculated to be 102 HeLa cells, which is comparatively lower than the previous reported method of telomerase activity detection (Table S2).^{31–34}

Verification of the Oligonucleotide Reaction. To verify the mechanism we proposed in Scheme 1, gel electrophoresis was used to monitor the interactions among the oligonucleotides according to its ability to separate oligonucleotides based on their charge and molecular weight.³⁵ First, we investigated whether the TS sequence could amplify the repeats of TTAGGG in the dNTPs mixture in the presence of telomerase. The bands of lanes a and b in Figure 2A represent TS

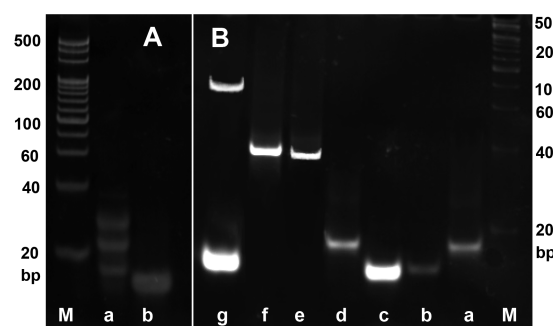


Figure 2. (A) Gel electrophoresis images of TS sequence (1) without telomerase and (2) with telomerase (cell extracts). (B) Gel electrophoresis images of (a) TS sequence, (b) FAM-ssDNA, (c) Texas Red-ssDNA, (d) Dabcyl-ssDNA, (e) F1, (f) T1, (g) incubation product of T1 and F1 at 37 °C for 3 h, and ladder DNA. M means DNA marker.

sequences in the absence and presence of telomerase, respectively. It can be seen that the 18bp TS sequence in lane b disappeared, and a series of amplified bands at ~ 28 bp was formed, confirming that the TS sequence could be elongated by telomerase-amplified function. Second, the response of the ratiometric fluorescent probe to telomerase activity was also investigated. As shown in Figure 2B, only one obvious low molecular weight band in lanes a–d is observed, which indicates the strong stability of the TS sequence, FAM-labeled ssDNA, Texas Red-labeled ssDNA, and Dabcyl-labeled ssDNA. The length of lane e is approximately equal to the sum of the length of the TS sequence and the FAM-ssDNA, and both F1 and FAM-ssDNA exhibit strong fluorescence, demonstrating that F1 is hybridized by the TS sequence and FAM-ssDNA. Meanwhile, the length of lane f is also found

equal to the sum of the length of the Texas Red-ssDNA and the Dabcyl-ssDNA, and only Texas Red-ssDNA displays fluorescence, further revealing that the fluorescence of T1 is quenched by the hybridization of fluorophore labeled-ssDNA and quencher labeled-ssDNA. Once the telomerase was added to the ratiometric fluorescent probe, one distinct fluorescence band could be seen in line g, indicating the telomerase triggered the unwinding of T1 to form a new FRET process from FAM to Dabcyl. The gel electrophoresis results further confirm the principle we proposed in Scheme 1.

Reliability of the Ratiometric Fluorescence Probe

Method. To further ensure the reliability of this method, we conducted several control experiments. First, a comparison of normal HeLa cell extracts and heat-inactivated HeLa cell extracts at 95 °C was examined (Figure S1), and negligible fluorescence enhancement of heat-inactivated HeLa cell extracts was observed due to the high temperature inducing enzyme inactivation, further demonstrating that using the ratiometric probe to detect telomerase activity is reliable and accurate. Second, the introduction of 3'-azido-3'-deoxythymidine (AZT), a widely used telomerase enzymatic activity inhibitor, was also used to investigate the feasibility of the ratiometric fluorescent probe method. Different concentrations of AZT were added to the reaction solution during the telomerase elongating process. The cell extracts were extracted from 2000 HeLa cells and the concentrations of AZT were chosen from 0 to 5 nM. As shown in Figure 3A, with the increasing concentration of AZT, the

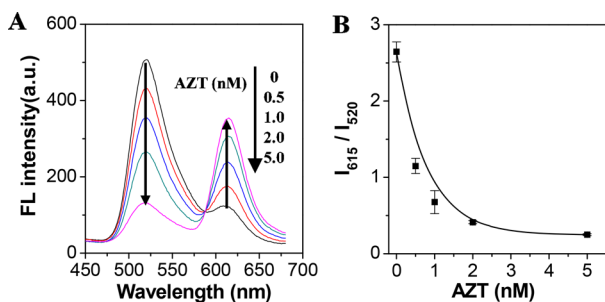


Figure 3. (A) Fluorescence spectra of 50 nM ratiometric fluorescence probe in 2×10^3 HeLa cells extracts in response to different concentrations of the telomerase inhibitor (AZT). (B) Plot of the fluorescence intensity ratio (I_{615}/I_{520}) vs different concentrations of AZT.

fluorescence intensity at 620 nm corresponding to Texas Red gradually decreased, whereas the fluorescence intensity of FAM at 515 nm steadily increased. The relationship between telomerase activity (fluorescence intensity ratio) and AZT concentration is shown in Figure 3B, demonstrating that the AZT could effectively inhibit the activity of telomerase. The AZT inhibitor experiment also proves that the ratiometric fluorescence probe method is reliable.

Detection of Telomerase in Different Cell Extracts. To evaluate the potential of this ratiometric fluorescent method for clinical diagnosis, we examined different human tumor cells (HepG2, A549, 293T) and QSG as one of the normal human somatic cells as experimental samples to investigate telomerase activities. It is well-known that telomerase in tumor cells has higher expression levels than those in normal cells.^{36,37} As shown in Figure 4A, the fluorescence intensity ratio (I_{615}/I_{520}) of HeLa cells was much higher than that of QSG with the increase in cell number, whereas the telomerase activity of QSG

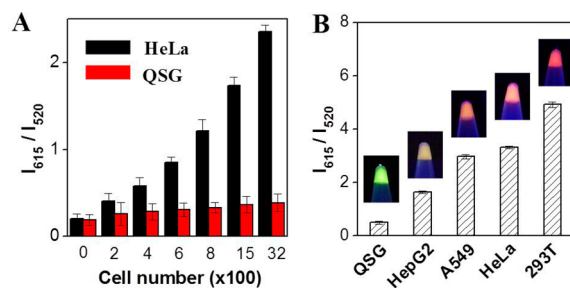


Figure 4. (A) Telomerase activity in HeLa (cancer) and QSG (normal) cells with increasing cell number. (B) Comparison of the telomerase activity for the ratiometric fluorescence probe treated with the normal cell line (QSG) and different cancer cell lines (HepG2, A549, HeLa, and 293T). The error bar is the standard deviation calculated from three replicated experimental results.

cells was almost the same at different cell numbers, illustrating that the ratiometric fluorescent probe exhibits a stable and specific response to the telomerase in HeLa tumor cell extracts rather than that in QSG normal cell extracts, which is a complex environment, without the interference of nucleic acids and enzymes. Figure 4B displays telomerase activity for the ratiometric fluorescence probe (2 μ L of 50 μ M probe T1, 2 μ L of 50 μ M probe F1) treated with QSG, HepG2, A549, HeLa, and 293T cell extracts with a cell number of 1000. As expected, HepG2, A549, HeLa, and 293T cancer cells all exhibit positive telomerase activity, which is consistent with the overexpression of telomerase in most known human tumors. In contrast, the QSG cells generate negligible improved fluorescence signal due to the lack of telomerase activity in normal cells. Interestingly, different cells, including QSG, HepG2, A549, HeLa, 293T, can be easily distinguished by the different fluorescence intensity ratios of the ratiometric fluorescent probe, which induce different color changes by the naked eye under UV irradiation from yellow green to orange to orange red to red (inset of Figure 4B). These results clearly demonstrate the feasibility of the proposed method for the detection of telomerase activity in clinical samples using a UV lamp.

Evaluation of Avoiding False Positive Signals. It is well-known that the general ratiometric fluorescence probe has the advantage of avoiding false positive signal.^{38,39} To prove that our probe could also avoid false positive signals, the ratiometric fluorescent probe to one typical enzyme DNase I was examined by coincubating them at 37 °C and then detecting the fluorescence of mixture. As shown in Figure 5, the fluorescent peak of Texas Red centered at 620 nm gradually

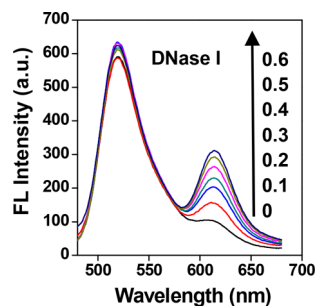


Figure 5. Effect of different concentrations of DNase I on the fluorescence intensity of the ratiometric fluorescence probe.

increased with the increase in DNase I concentration from 0 to 0.6 under the excitation wavelength of 365 nm, whereas the peak of FAM centered at 520 nm remained unchanged. One possible reason for this could be that the mixed dsDNA F1 and T1 were broken into several fragments under the cleavage of DNase I, forming several small single-stranded fragments. Thereby, the Dabcyl group on the 3'-end of T1 could not fully quench the red fluorescence of the Texas Red group on the same strand of T1, and telomerase could not extend the TS sequence in F1 with the increasing amount of DNase I. On the other hand, the results of the fluorescence spectra were clearly different in the absence of DNase I. F1 was catalyzed under the function of telomerase and prolonged in the dNTP mixture to form telomeric repeats at the 3'-end of the telomerase primer sequence, which could completely complement Dabcyl-modified ssDNA of T1 and subsequently induce the breaking of FRET between Texas Red and Dabcyl, leading to the decrease in the FAM fluorescent peak at 520 nm and increase in the Texas Red fluorescent peak at 620 nm. In other words, this method successfully excluded the influence of other experimental factors by comparing the fluorescence spectra of the reaction; only telomerase can stimulate the ratiometric fluorescent probe and induce a significant change in the fluorescence spectra (decrease in the FAM peak and increase in the Texas Red peak), which can avoid false positive results.

CONCLUSIONS

In summary, we have developed a general platform to discriminate various cancer cells with different telomerase expression levels by the naked eye based on FRET principles, which was achieved by designing a dual-colored dye-modified ratiometric fluorescence oligonucleotide probe for qualitative and quantitative detection of telomerase activity in cell extracts. This novel probe was constructed by a quencher- and fluorophore-labeled duplex oligonucleotide as well as another fluorophore-labeled duplex oligonucleotide. Once the target telomerase was added, dye-labeled dsDNA was catalyzed and prolonged to form telomeric repeats, which could fully complement Dabcyl-modified ssDNA, subsequently inducing the breaking of FRET and formation of a new FRET. In contrast to the traditional methods, the realization of a telomerase assay using two dye-modified ratiometric fluorescent oligonucleotide probes and FRET principles is rarely reported. By using this ratiometric fluorescence probe, telomerase activity in cell extracts could be precisely detected with a detection limit of 102 HeLa cells, which is comparatively lower than seen in other methods. This study is expected to exhibit exciting opportunities in medical diagnostics and therapeutic response assessment.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.8b00059.

DNA sequences used in the experiment, comparison of the pros and cons of various reported methods for telomerase activity detection, fluorescence spectra and intensity ratios of the ratiometric probe in normal and heat-inactivated HeLa cell extracts, linear correlation of the fluorescence intensity ratio vs different cell numbers in

HeLa cell extracts, and stability of the ratiometric fluorescence probe (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is supported by National Natural Science Foundation of China (Nos. 21335006, 21475135, 21375131, 21703255, 61705239) and Natural Science Foundation of Anhui Province (1708085MB35, 1608085QB32).

REFERENCES

- (1) Blasco, M. A.; Lee, H.-W.; Hande, M. P.; Samper, E.; Lansdor, P. M.; DePinho, R. A.; Greider, C. W. Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA. *Cell* **1997**, *91*, 25–34.
- (2) Cawthon, R. M. Telomere Measurement by Quantitative PCR. *Nucleic Acids Res.* **2002**, *30*, 47–47.
- (3) Jankovic, G. M.; Colovic, M. D.; Petrovic, M. D. Telomere Loss and Cancer. *Nature* **1991**, *350*, 197.
- (4) Blackburn, E. H. Structure and Function of Telomeres. *Nature* **1991**, *350*, 569–573.
- (5) Brown, W. R.; MacKinnon, P. J.; Villasante, A.; Spurr, N.; Buckle, V. J.; Dobson, M. J. Structure and Polymorphism of Human Telomere-Associated DNA. *Cell* **1990**, *63*, 119–132.
- (6) Morin, G. B. The Human Telomere Terminal Transferase Enzyme Is a Ribonucleoprotein That Synthesizes TTAGGG Repeats. *Cell* **1989**, *59*, 521–529.
- (7) Wang, L. J.; Ma, F.; Tang, B.; Zhang, C. Y. Sensing Telomerase: From *in Vitro* Detection to *in Vivo* Imaging. *Chem. Sci.* **2017**, *8*, 2495–2502.
- (8) Yu, T.; Zhao, W.; Xu, J. J.; Chen, H. Y. A PCR-Free Colorimetric Strategy for Visualized Assay of Telomerase Activity. *Talanta* **2018**, *178*, 594–599.
- (9) Schmidt, J. C.; Cech, T. R. Human Telomerase: Biogenesis, Trafficking, Recruitment, and Activation. *Genes Dev.* **2015**, *29*, 1095–1105.
- (10) Greider, C. W.; Blackburn, E. H. Identification of a Specific Telomere Terminal Transferase-Activity in Tetrahymena Extracts. *Cell* **1985**, *43*, 405–413.
- (11) Blackburn, E. H. Telomeres and Their Synthesis. *Science* **1990**, *249*, 489–490.
- (12) Sharma, H. W.; Sokoloski, J. A.; Perez, J. R.; Maltese, J. Y.; Sartorelli, A. C.; Stein, C. A.; Nichols, G.; Khaled, Z.; Telang, N. T.; Narayanan, R. Differentiation of Immortal Cells Inhibits Telomerase Activity. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 12343–12346.
- (13) Sommerfeld, H. J.; Meeker, A. K.; Piatyszek, M. A.; Bova, G. S.; Shay, J. W.; Coffey, D. S. Telomerase activity: a Prevalent Marker of Malignant Human Prostate tissue. *Cancer Res.* **1996**, *56*, 218–222.
- (14) Xiao, Y.; Dane, K. Y.; Uzawa, T.; Csordas, A.; Qian, J.; Soh, H. T.; Daugherty, P. S.; Lagally, E. T.; Heeger, A. J.; Plaxco, K. W. Detection of Telomerase Activity in High Concentration of Cell Lysates Using Primer-Modified Gold Nanoparticles. *J. Am. Chem. Soc.* **2010**, *132*, 15299–15307.
- (15) Aghaei, R.; Mazloum-Ardakani, M.; Abdollahi-Alibeik, M.; Moshtaghion, S. M.; Rezaei-poor-Anari, A.; Haghighijoo, Z.; Zamani,

- L. A New Electrochemical Biosensor Based on Telomeric G-Quadruplex DNA: In Silico and Experimental Study of Dihydropyridine Derivatives Potential Effect on Telomerase Inhibition. *J. Electroanal. Chem.* **2017**, 796, 24–32.
- (16) Alizadeh-Ghods, M.; Zavari-Nematabad, A.; Hamishehkar, H.; Akbarzadeh, A.; Mahmoudi-Badiki, T.; Zarghami, F.; Moghaddam, M. P.; Alipour, E.; Zarghami, N. Design and Development of PCR-free Highly Sensitive Electrochemical Assay for Detection of Telomerase Activity Using Nano-Based (Liposomal) Signal Amplification Platform. *Biosens. Bioelectron.* **2016**, 80, 426–432.
- (17) Lou, J.; Wang, Z. Y.; Wang, X.; Bao, J. C.; Tu, W. W.; Dai, Z. H. Highly Sensitive "Signal-on" Electrochemiluminescent Biosensor for the Detection of DNA Based on Dual Quenching and Strand Displacement Reaction. *Chem. Commun.* **2015**, 51, 14578–14581.
- (18) Zheng, T.; Feng, E.; Wang, Z.; Gong, X.; Tian, Y. Mechanism of Surface-Enhanced Raman Scattering Based on 3D Graphene-TiO₂ Nanocomposites and Application to Real-Time Monitoring of Telomerase Activity in Differentiation of Stem Cells. *ACS Appl. Mater. Interfaces* **2017**, 9, 36596–36605.
- (19) Shi, M.; Zheng, J.; Liu, C.; Tan, G.; Qing, Z.; Yang, S.; Yang, J.; Tan, Y.; Yang, R. SERS Assay of Telomerase Activity at Single-Cell Level and Colon Cancer Tissues via Quadratic Signal Amplification. *Biosens. Bioelectron.* **2016**, 77, 673–680.
- (20) Su, X.; Li, Z. H.; Yan, X. Z.; Wang, L.; Zhou, X.; Wei, L.; Xiao, L. H.; Yu, C. Y. Telomerase Activity Detection with Amplification-Free Single Molecule Stochastic Binding Assay. *Anal. Chem.* **2017**, 89, 3576–3582.
- (21) Li, X. Q.; Wang, W.; Chen, Y. Y.; Ding, C. F. Fluorescence Detection of Telomerase Activity in High Concentration of Cell Lysates Based on Strand-Displacement Mediated Recycling. *Analyst* **2016**, 141, 2388–2391.
- (22) Ma, F.; Wei, S. H.; Leng, J.; Tang, B.; Zhang, C. Y. A Simple "Mix-and-Detection" Method for the Sensitive Detection of Telomerase From Cancer Cells Under Absolutely Isothermal Conditions. *Chem. Commun.* **2018**, 54, 2483.
- (23) Zhu, G.; Yang, K.; Zhang, C. Y. A Single Quantum Dot-Based Biosensor for Telomerase Assay. *Chem. Commun.* **2015**, 51, 6808–6811.
- (24) Ma, F.; Li, Y.; Tang, B.; Zhang, C. Y. Fluorescent Biosensors Based on Single-Molecule Counting. *Acc. Chem. Res.* **2016**, 49, 1722–1730.
- (25) Qian, R.; Ding, L.; Yan, L.; Lin, M.; Ju, H. Smart Vesicle Kit for In Situ Monitoring of Intracellular Telomerase Activity Using a Telomerase-Responsive Probe. *Anal. Chem.* **2014**, 86, 8642–8648.
- (26) Yang, Y. J.; Huang, J.; Yang, X. H.; Quan, K.; Wang, H.; Ying, L.; Xie, N. L.; Ou, M.; Wang, K. M. FRET Nanoflares for Intracellular mRNA Detection: Avoiding False Positive Signals and Minimizing Effects of System Fluctuations. *J. Am. Chem. Soc.* **2015**, 137, 8340–8343.
- (27) Ueyama, H.; Takagi, M.; Takenaka, S. A Novel Potassium Sensing in Aqueous Media with a Synthetic Oligonucleotide Derivative. Fluorescence Resonance Energy Transfer Associated with Guanine Quartet-Potassium Ion Complex Formation. *J. Am. Chem. Soc.* **2002**, 124, 14286–14287.
- (28) Counter, C. M.; Avilion, A. A.; Lefevre, C. E.; Stewart, N. G.; Greider, C. W.; Harley, C. B.; Bacchetti, S. Telomere Shortening Associated with Chromosome Instability Is Arrested in Immortal Cells Which Express Telomerase Activity. *Embo. J.* **1992**, 11, 1921–1929.
- (29) Roy, R.; Hohng, S.; Ha, T. A Practical Guide to Single-Molecule FRET. *Nat. Methods* **2008**, 5, 507–516.
- (30) Ding, C. F.; Li, X. L.; Ge, Y.; Zhang, S. S. Fluorescence Detection of Telomerase Activity in Cancer Cells Based on Isothermal Circular Strand-Displacement Polymerization Reaction. *Anal. Chem.* **2010**, 82, 2850–2855.
- (31) Patolsky, F.; Gill, R.; Weizmann, Y.; Mokari, T.; Banin, U.; Willner, I. Lighting-Up the Dynamics of Telomerization and DNA Replication by CdSe-ZnS Quantum Dots. *J. Am. Chem. Soc.* **2003**, 125, 13918–13919.
- (32) Zhang, Z. X.; Sharon, E.; Freeman, R.; Liu, X. Q.; Willner, I. Fluorescence Detection of DNA, Adenosine-5'-Triphosphate (ATP), and Telomerase Activity by Zinc(II)-Protoporphyrin IX/G-Quadruplex Labels. *Anal. Chem.* **2012**, 84, 4789–4797.
- (33) Yi, Z.; Wang, H. B.; Chen, K.; Gao, Q.; Tang, H.; Yu, R. Q.; Chu, X. A Novel Electrochemical Biosensor for Sensitive Detection of Telomerase Activity Based on Structure-Switching DNA. *Biosens. Bioelectron.* **2014**, 53, 310–315.
- (34) Yang, W.; Zhu, X.; Liu, Q.; Lin, Z.; Qiu, B.; Chen, G. Label-Free Detection of Telomerase Activity in HeLa Cells Using Electrochemical Impedance Spectroscopy. *Chem. Commun.* **2011**, 47, 3129–3131.
- (35) Qian, R.; Ding, L.; Ju, H. Switchable Fluorescent Imaging of Intracellular Telomerase Activity Using Telomerase-Responsive Mesoporous Silica Nanoparticle. *J. Am. Chem. Soc.* **2013**, 135, 13282–13285.
- (36) Lehner, R.; Enomoto, T.; McGregor, J. A.; Shroyer, A. L.; Haugen, B. R.; Pugazhenth, U.; Shroyer, K. R. Quantitative Analysis of Telomerase hTERT mRNA and Telomerase Activity in Endometrioid Adenocarcinoma and in Normal Endometrium. *Gynecol. Oncol.* **2002**, 84, 120–125.
- (37) Wang, X.; Sun, L.; Wang, X.; Kang, H.; Ma, X.; Wang, M.; Lin, S.; Liu, M.; Dai, C.; Dai, Z. Acidified Bile Acids Enhance Tumor Progression and Telomerase Activity of Gastric Cancer in Mice Dependent on c-Myc Expression. *Cancer Med.* **2017**, 6, 788–797.
- (38) Xu, H.; Aylott, J. W.; Kopelman, R.; Miller, T. J.; Philbert, M. A. A Real-Time Ratiometric Method for the Determination of Molecular Oxygen Inside Living Cells Using Sol-Gel-Based Spherical Optical Nanosensors with Applications to Rat C6 Glioma. *Anal. Chem.* **2001**, 73, 4124–4133.
- (39) Zhang, K.; Zhou, H. B.; Mei, Q. S.; Wang, S. H.; Guan, G. J.; Liu, R. Y.; Zhang, J.; Zhang, Z. P. Instant Visual Detection of Trinitrotoluene Particulates on Various Surfaces by Ratiometric Fluorescence of Dual-Emission Quantum Dots Hybrid. *J. Am. Chem. Soc.* **2011**, 133, 8424–8427.