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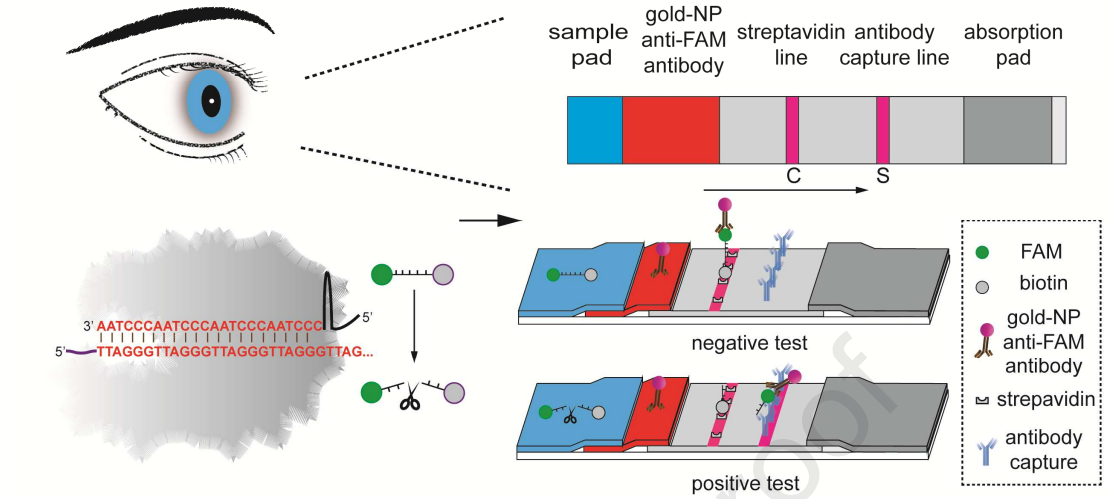
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Author statement outlining

Xiaolong Chen: Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing
Yuanyi Deng: Conceptualization, Methodology, Roles/Writing - original draft.
Gaihua Cao: Validation, Methodology and Formal analysis
Xinyi Liu: Methodology, Software
Tao Gu: Validation, Software
Ruoyang Feng: Methodology, Writing - Original Draft.
Danqun Huo: Supervision, Project administration
Faliang Xu: Project administration
Changjun Hou: Writing - Review & Editing, Project administration, Funding acquisition

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Graphical Abstract



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An ultrasensitive and point-of-care sensor for the telomerase activity detection

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Abstract: Telomerase owns great application potential in diagnosis, therapy, prognosis，and drug screening of cancers. Thus, the ultrasensitive and point-of-care detection of telomerase activity meets the clinical demands extremely. Here, a sensor based on telomerase extends activators to unlock the ssDNase activity of CRISPR/Cas12a was created for the first time to detect the telomerase activity. Based on the fluorescence or CRISPR/Cas12a-based lateral flow assay, we achieve the ultrasensitive and point-of-care detection of telomerase activity in MCF-7 cells low to 57 cells·mL^{-1} and 5.7×10^{2} cells·mL^{-1} in about one hour, respectively. Besides, the detection of telomerase activity in different subtype breast cancer cells indicates that the proposed sensor possesses potential in the classification of breast cancer cell subtypes.

Keywords: Telomerase; breast cancer cells; CRSPR/Cas12a; point-of-care testing

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1. Introduction

Cancer is one of the serious public health problems which seriously endanger people's physical and mental health worldwide [1]. Studies show that the cure rate of early cancer treatment is significantly higher than that of advanced cancer [2,3]. Therefore, the early diagnosis of cancer is of great significance to the prevention and treatment of cancer. In early cancer screening, cancer markers are important indicators for early cancer diagnosis. The cancer markers are specific substances produced by different types of cancer at different times [4]. Among which, telomerase has received much attention on account of its relationship with tumorigenesis [5-7]. Telomerase is a special reverse transcriptase composed of protein and RNA, and is directly related to the synthesis of telomere at the end of chromosomes in eukaryotic cells [8]. Telomere terminal $(TTAGGG)_n$ sequence can maintain the length and stability of the telomere during cell division [9]. The length of normal somatic cells telomere is gradually shortened with the division of the cell, while the telomerase activity is enhanced to maintain the length of the telomere without shortening, resulting that the cells permanently proliferate and become cancerous [10-12]. This indicates that the telomerase activity is closely related to cell immortality and cell carcinogenesis. Since the content of telomerase in normal cells is low and it is easily degraded in vitro, the ultrasensitive and point-of-care determination of telomerase activity owns great clinical significance.

PCR-based telomere repeats amplification protocol (TRAP) is an authoritative method for the evaluation of telomerase activity [13]. Meanwhile, its disadvantages

also are protruding, such as false results and long detection time. To overcome these defects, various methods have been established, including colorimetric [14-16], fluorescence [17-20], electrochemistry [21-23], and so on. However, the major disadvantages of those sensors are complicated pretreatment, the need of instruments and modification of nanoprobes, which are difficult to apply for point-of-care testing (POCT). Up to date, a few POCT have been developed to evaluate the telomerase activity. For example, Zhu used a handheld luminometer to detect telomerase activity, which achieved a detect limit low to 9 HeLa cells in a linear range from 10 to 1000 cells [24]. But the modification process between primer and magnetic beads is time-consuming and complicated. The whole inspection process is more than 2 hours. Wang reported a colorimetric approach through hydrogen peroxide test strip. This visual strategy realized a low detect limit of 10 cells·μL^{-1} [25]. Regrettably, this method has the disadvantages of time-consuming, narrow detection range, low detection sensitivity, and complicated operation process. Since telomerase is easily inactivated and low in content, it is still necessary to establish ultrasensitive and point-of-care method to detect telomerase activity.

CRISPR/Cas12a has been demonstrated to own a non-specific ssDNA trans-cleavage after it specifically recognizes its target [26]. So when non-specific ssDNA is replaced by labelled ssDNA reporters, CRISPR/Cas12a system shows great potential in development of sensors. Up to now, CRISPR/Cas12a systems as novel biosensor platforms have become a research hotspot. Recently, Yin reported a DAMR (dynamic aqueous multiphase reaction) system. Based on 3D-printed microfluidic

device, this study realized multiplex determination of HPV16/18 within 1 h [27]. Dai proposed a CRISPR-Cas12a based electrochemical biosensor (E-CRISPR), which achieved picomolar sensitivity for viral nucleic acid detection [28]. Wang developed a microRNA sensing platform, which combined rolling circle transcription (RCT) and CRISPR-Cas12a. As reported, this method could detect microRNA in attomolar level [29]. Besides, other biomolecules, including bacteria [30], proteins [31,32], and chemicals [33], have already been detected extensively using the CRISPR-based system. All these reports show the CRISPR/Cas12a system owns huge potential in biosensors due to its high specificity and sensitivity. The telomerase mentioned above are closely related to the changes of nucleic acids in organisms. Furthermore, the final recognition unit of the CRISPR/Cas12a is also nucleic acid. This provides us a new idea for utilizing the CRISPR/Cas12a to detect the telomerase activity.

Here, we make full advantage of the telomerase property which can add the unit of *5'-TTAGGG-3'* to the 3' ends of DNA fragments repeatedly and the ssDNase activity of the CRISPR/Cas12a to establish an ultrasensitive and naked eye sensor. In the presence of telomerase, the primers are continuously extended to generate multiple activation regions which can initiate the ssDNase activity of the CRISPR/Cas12a. Sensor that relies on fluorescence and CRISPR/Cas12a-based lateral flow assay has achieved ideal detection results under optimized conditions. The sensor we proposed can simply achieve the ultrasensitive and point-of care detection of telomerase activity in breast cancer cells within one hour. We think it owns

practical significance in telomerase activity detection and is enlightening for other enzymes activity detection.

2. Materials and methods

2.1 Materials and instrumentations

All nucleic acid sequences adopted in this text were supplied by Sangon Biotech (Shanghai, China) and purified by HPLC. The detailed information is displayed in Table 1. The MiRcute miRNA Isolation Kit (DP501) bought from TIANGEN Biotechnology Co., Ltd (Beijing, China) was used to purify the synthetic crRNA. The mixture of deoxy-ribonucleoside triphosphate (dNTPs) was purchased from Sangon Biotech (Shanghai, China). The mixture of ribonucleoside triphosphate (rNTPs), T7 Mix, DNase I, RNase inhibitors, phi 29 DNA polymerase (phi 29), T4 Polynucleotide Kinase (T4 PNK), Uracil-DNA Glycocasylase (UDG) and LbCas12a were ordered by New England Biotechnology Co., Ltd (Beijing, China). CHAPS lysis buffer (1 mM $MgCl_2$, 10 mM Tris-HCl, 5 mM mercaptoethanol, 1 mM ethylenebis (oxyethylenenitrilo) tetraacetic acid, 0.5% 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate, 0.1 mM phenylmethanesulfonyl fluoride, 10% glycerol, pH=7.5) was prepared by Shanghai Qincheng Biotechnology Co., Ltd.. HCC38, MCF-7, SUM-159 and MDA-MB-231 cells were offered by Chongqing Cancer Institute and Hospital (Chongqing, China). 1×Telomerase reaction buffer (63 mM KCl, 1.5 mM $MgCl_2$, 20 mM Tris-HCl, 0.005% Tween-20, 0.1 mg/mL BSA, 1 mM ethylene glycol tetraacetic acid (EGTA), pH=8.3@25°C) were prepared in our laboratory. Test strip for CRISPR-Cas12a-based lateral flow assay was bought from HybriDetect. Human

Telomerase Reverse Transcriptase ELISA Kit was bought from Lanji Biotechnology Co., (Shanghai, China). LS-55 spectrophoto fluorometer (P. E. USA) was used to record the fluorescence spectrum of the system by stimulating the system at 490 nm from 400 nm to 700 nm and receiving the signal at 556 nm. During the measurement, the slit-widths of emission and excitation spectra were selected as 10 nm and 12 nm, respectively.

Table 1 Nucleic acid sequences adopted in this text.

Oligo names	Sequences (5'-3')
crRNA-template	TTA GGG TTA GGG TTA GGG TTA GGG ATC TAC ACT TAG TAG AAA TTA CCC TAT AGT GAG TCG TAT TAA TTT C
T7 promoter	GAA ATT AAT ACG ACT CAC TAT AGG G
Telomerase primer	GGA TCA ACT GC
ssDNA reporter	HEX-TAT TAT T-BHQ1
FAM/Bio reporter	6-FAM-TTA TTA TT- Biotin

2.2 Synthesis of the crRNA

First, 20 μL of mixture contained 16 μL RNase-free water, 10 μM T7 promoter, and 10 μM crRNA-template were mixed in 200 μL PCR tubule. Then the mixture was kept at 95°C for 5 minutes to anneal. After that, the mixture was cooled to 25°C slowly and maintained for 20 minutes to fully combine the T7 promoter with the crRNA-template. Subsequently, 1.5 μL of T7 polymerase and 10 μL of 100 mM rNTPs were added. The ultimate system was incubated at 37°C for overnight to obtain abundant crRNA. Then 3 μL of DNase I buffer and 5 μL of DNase I were added into the system to digest the ssDNA/dsDNA for 60 min at 37°C. The obtained crRNA were purified using miRcute miRNA Isolation Kit and stored at -20°C for later use. NanoDrop 2000C was used to measure the concentration of crRNA. The wide and

bright strip showed in Fig. S1 illustrates the successful synthesis of crRNA.

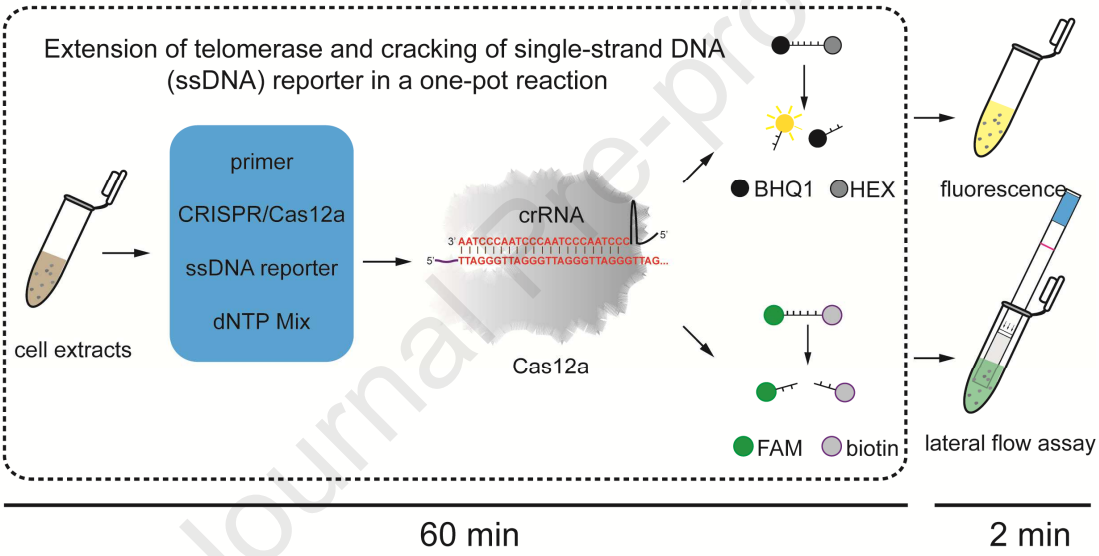
2.3 Cell culture and telomerase extraction

Dulbecco's Modified Eagle's medium (DMEM, Hyclone), RPMI-1640 medium (SIGMA), and Dulbecco's Modified Eagle's medium/Nutrient Mixture F-12 Ham (SIGMA) were utilized to culture MCF-7 cells, HCC38 cells, and SUM-159 and MDA-MB-231 cells, respectively. All mediums contained penicillin (100 $U \cdot mL^{-1}$), fetal bovine serum (10%), and streptomycin (100 $U \cdot mL^{-1}$). The cells were cultivated in a thermostat containing 5% CO_2 at 37°C. About 1 mL of exponential growth cells were transferred into a 1.5 mL EP tube and collected through centrifugation at 5000 rpm for 5 min at 4°C. Then, 1×PBS was used to wash cells twice at 5000 rpm for 5 min at 4°C. Next, the cells were mixed with 200 μL of ice-cold CHAPS lysis buffer. The process of lysing cells was reacted on ice bath for 30 min. Finally, the cell lysis products were centrifuged at 12000 rpm for 20 min at 4°C and the obtained supernatant was used for the subsequent telomerase extended reaction. The obtained cell extracts were frozen in -80°C for standby application.

2.4 Detection of telomerase activity

Extension of telomerase and cracking of single-strand DNA (ssDNA) reporter was conducted in a one-pot reaction. Fig. 1 illustrates the flow chart of this sensor. Specifically, 2 μL of cell extracts were incubated with 20 μL of mixture which contained 1×telomerase reaction buffer, 500 nM primer, 500 μM dNTP Mix, 100 nM Cas12a, 100 nM crRNA, 500 nM ssDNA reporter and 5 mM $MgSO_4$. Subsequently, the mixture was cultivated at 37°C for 1 h. Then the system was adjusted to 100 μL by

160 double distilled water (ddH_2O). The fluorescence signal was measured on a LS-55
161 spectrophoto fluorometer with an excitation wavelength of 490 nm. For the
162 CRISPR/Cas12a-based lateral flow assay, the ssDNA reporter was replaced by
163 single-stranded DNA modified with 6-carboxy-fluorescein (FAM) on its 3' tail and
164 biotin on its 5' tail. After reacting in the same step for 60 minutes, the sample pad of
165 the test strip was immersed into the mixture and incubated for 2 min at room
166 temperature. The final result is observed with the naked eye.



167 **Fig.1.** The flow chart of the sensor.

168 **2.5 Gel electrophoresis**

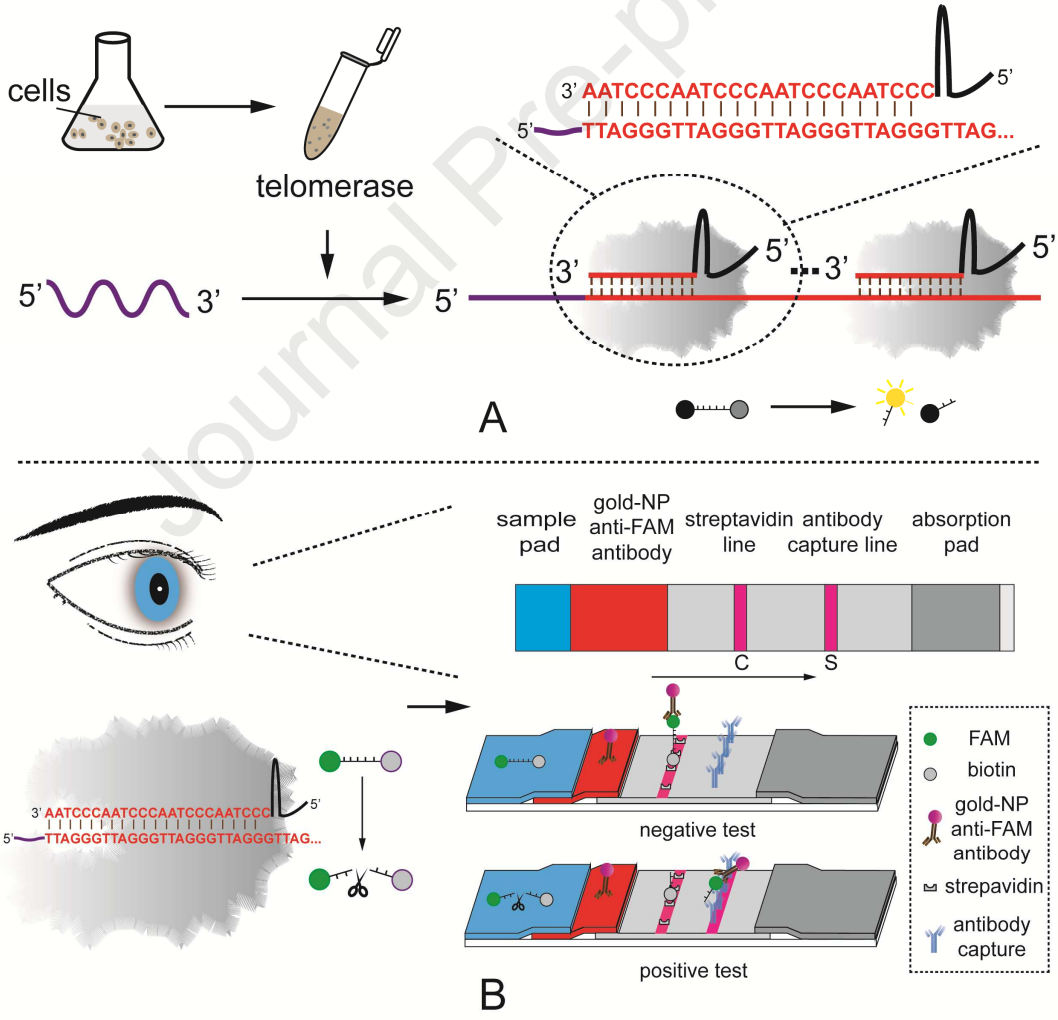
169 2.5% agarose gel was used to verify the synthesis of crRNA. It was implemented
170 in 1×TBE buffer at 130 V for 50 min. 15% of polyacrylamide gel electrophoresis
171 (PAGE) was used to observe the experimental response more intuitively. It was
172 executed in 1×TBE buffer at 130 V for 60 min. The gel was stained using SYBR
173 Green I and observed on An Azure Biosystems C150 (USA).

174 **3. Results and discussions**

3.1 Principle of the detection

The schematic diagram of the fluorescence strategy is shown in Scheme 1A. In the presence of telomerase, the sequence *5'-TTAGGG-3'* is added to the 3' ends of the DNA fragments continuously, generating DNA tails which contain multiple repeated sequences. Ingeniously, we designed the recognition region of crRNA to pair four *5'-TTAGGG-3'* sequences. Therefore, the generated repeated sequences can act as activators to recruit multiple Cas12a/crRNA system and activate their trans-cleavage activity, cleaving the ssDNA reporter indiscriminately and realizing signal amplification. Thus a rapid and efficient multiple amplification method is realized to ultra-sensitively test the telomerase activity. Moreover, to achieve the point-of-care and naked eye detection of the telomerase activity, we established a CRISPR/Cas12a-based lateral flow assay under optimized conditions. The principle of detection is illustrated in Scheme 1B. In the absence of a target, the ssDNase activity of CRISPR/Cas12a will not be activated. Therefore, the FAM/Bio reporter modified with FAM and biotin at both ends will not be cleaved. The control line and sample line of the test strip are fixed with streptavidin and antibody capture, respectively. When complete FAM/Bio reporter flows through the gold-NP anti-FAM antibody region with the buffer, the FAM on the FAM/Bio reporter can be integrated by gold-NP anti-FAM antibody, forming the conjugate of FAM/Bio reporter and gold-NP anti-FAM antibody. But when this conjugate continues to flow through the control line with the buffer, the streptavidin on the control line can specifically bind to the biotin at the other end of the FAM/Bio reporter, causing it cannot flow through the

sample line on the test strip. The final result displayed is that only a red band appears at control line on the test strip. But when there is a target, the ssDNase activity of the CRISPR/Cas12a system can be activated, leading to the FAM/Bio reporters are cut. The cleaved FAM/Bio reporters will produce two ssDNA with FAM and biotin respectively. The ssDNA with FAM then combine with gold-NP anti-FAM antibody and swim to the sample line and captured by the antibody capture here. The final result displayed is that two red band appears at the control line and sample line on the test strip.



Scheme 1. Flow chart of telomerase activity detection.

3.2 Feasibility of the sensor

To verify the feasibility of the proposed sensor, the cell extracts was added to the positive group and the negative group was not added. To prove that the experimental effect was caused by enzymes, we did an essay in inactivated positive group which the cell extracts was inactivated at 95°C for 5 minutes. As displayed in Fig. 2A, the fluorescence strength of the positive group is obviously higher than negative group and inactivated positive group, proving that the ssDNA reporter only is sheared in the presence of telomerase. The system is almost interference-free in theory due to the activation sequence do not exist originally. To intuitively reveal that the primer indeed extends the repeat *5'-TTAGGG-3'* sequence in the presence of cell extracts, a 15% of PAGE was done. As illustrated in Fig. 2B, the control group only appears the band of primer (lane 3), while the experimental group appears the bands represented larger molecular weight DNA bands (lane 2). All these results indicate that it is feasible to use this strategy to detect the telomerase activity.

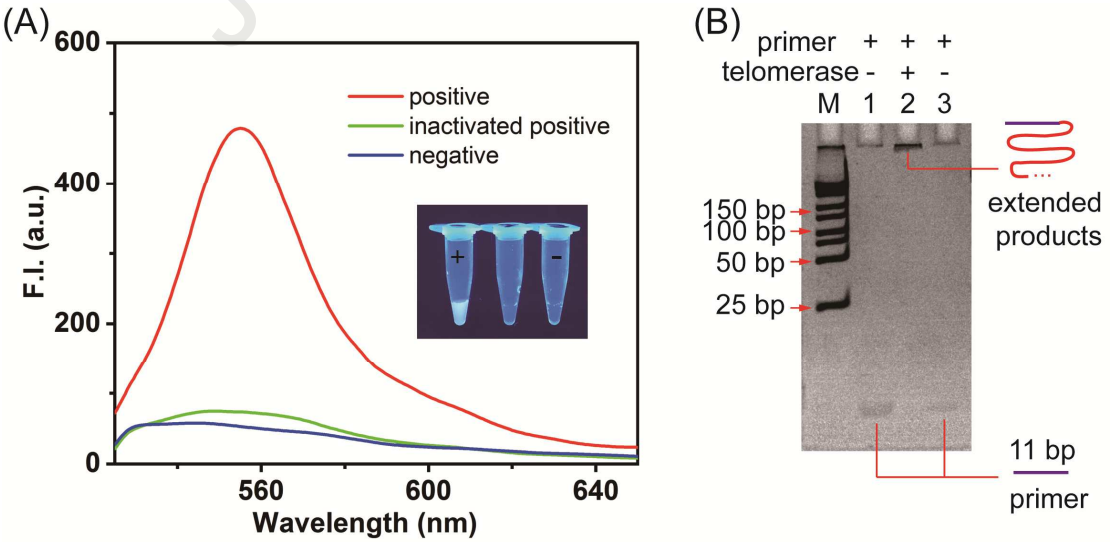


Fig.2. Feasibility of the proposed sensor. (A) the fluorescence spectrum of positive,

inactivated positive, and negative group (the amount of cell extracts is 5.7×10^4 cells/mL); (B) the electropherogram of 15% PAGE (M: Marker; lane 1: primer; lane 2: experimental group; lane 3: control group).

3.3 Analysis of the telomerase activity

Before detecting telomerase activity in different concentrations of cell extracts, the amplification time which affects the system mainly was optimized. As seen in Fig.S2, the net difference of the fluorescence intensity (ΔF) between the positive and negative samples increases with the amplification time increases. However, the amplitude of the increase is significantly reduced after it is greater than 60 min, indicating that the activity of telomerase begins to decrease, and some ssDNA reporter cracked non-specifically and generate interference signals from then on. To further study the analytical performance of the proposed sensor in telomerase activity detection, the different concentrations of cell extracts was investigated via CRISPR/Cas12a based fluorescence assay. As illustrated in Fig.3A, the fluorescence strength of the system at 556 nm reduces as the concentration of MCF-7 cells decreases. The more telomerase existed, the stronger recorded fluorescence intensity. It is shown that the ΔF owns a good linear relationship with the number of MCF-7 cells from 57 to 5.7×10^5 cells·mL^{-1}. The linear regression equation between the ΔF and the logarithmic value of the concentrations of MCF-7 cells is $Y=128.43\lg X-199.52$ ($R^2=0.98041$), where Y represents the net difference of the fluorescence intensity (ΔF) between the positive and negative samples under a certain amount of MCF-7 cells, X represents the number of MCF-7 cells (Fig.3B). The actual

limit of detection (LOD) is estimated at about 57 cells·mL^{-1}. These results indicate that the CRISPR/Cas12a based approach have an outstanding sensitivity and very little fluorescent background interference. Compared with other telomerase activity sensors, our method shows a wider detection range and ideal actual LOD in a short time (Table 2). Besides, we integrate the extension of telomerase and the fluorescence assay based on the ssDNase of the CRISPR/Cas12a in one pot, which simplifies the procedure and shortens the testing time. With this method, we can detect the activity of telomerase in MCF-7 cells as low as 57 cells·mL^{-1} in one hour.

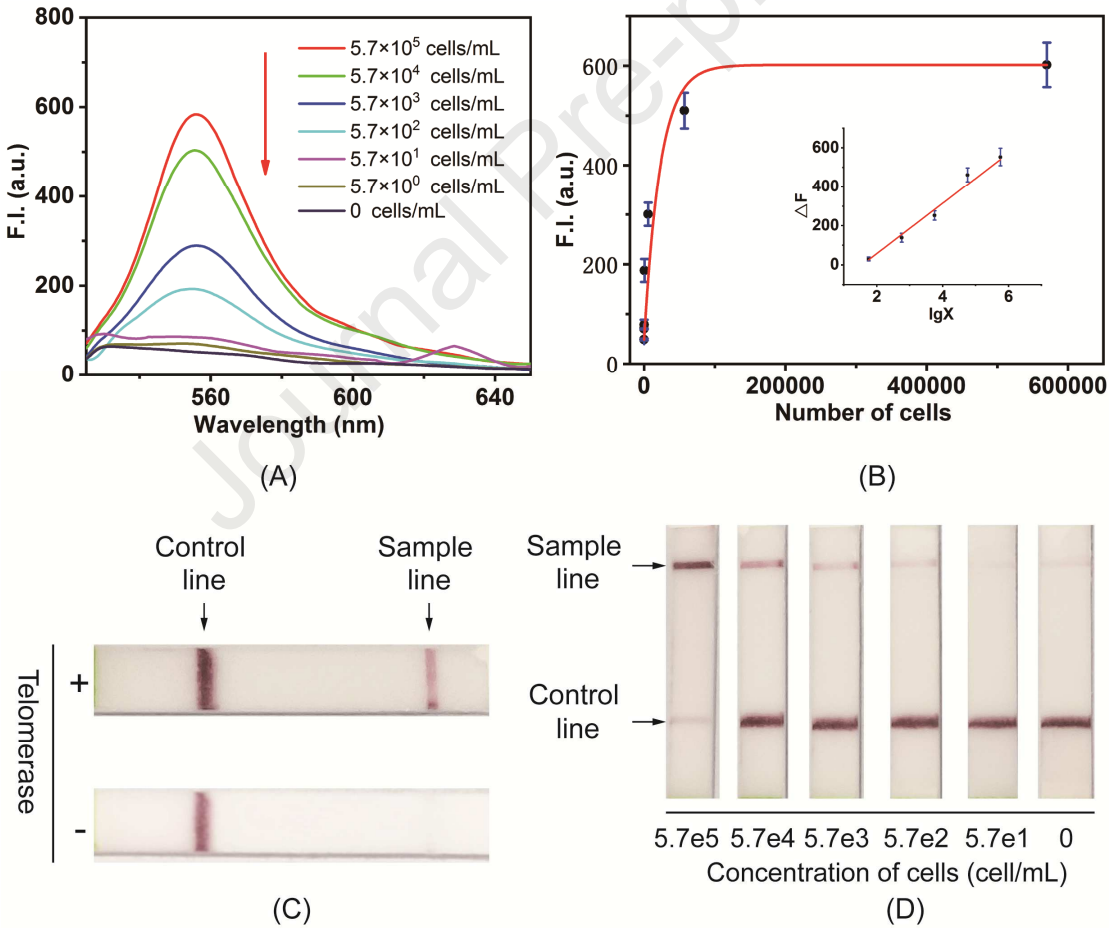


Fig.3. Analysis for telomerase activity in various amounts of MCF-7 cells. (A) is the corresponding fluorescence spectrum at various amounts of MCF-7 cells; (B) is the

fluorescence intensity of the system at 556 nm in various amounts of MCF-7 cells and related data simulation (illustration); (C) is the assay of the CRISPR/Cas12a-based lateral flow assay; (D) is the corresponding CRISPR/Cas12a-based lateral flow assay under different concentrations of MCF-7 cells.

Table 2 Comparison of this sensor with other methods in detecting telomerase activity.

Methods	Detection range	Actual detection limit	Time	Ref.
Electrochemistry	5×10^2-1.62×10^7 cells·mL^{-1}	5×10^2 cells·mL^{-1}	>1.5 h	[34]
	2×10^2-2×10^6 cells·mL^{-1}	2×10^2 cells·mL^{-1}	>3 h	[35]
	1×10^2-1.04×10^7 cells·mL^{-1}	1×10^2 cells·mL^{-1}	1 h	[36]
Colorimetry	1-50 cells·μL^{-1}	1 cells·μL^{-1}	-	[37]
	20-500 cells	20 cells	70 min	[15]
	100-10^6 cells·mL^{-1}	100 cells·mL^{-1}	80 min	[38]
	10-500 cells·μL^{-1}	10 cells·μL^{-1}	>3 h	[39]
Fluorescence	5-1000 cells	5 cells	>3 h	[40]
	6-2000 cells	6 cells	>4 h	[41]
	3-400 cells	3 cells	1 h	[42]
	1-100 cells	1 cells	>3 h	[43]
	10-1200 cells	10 cells	150 min	[44]
	100-5×10^4 cells	100 cells	3 h	[45]
	100-2000 cells·mL^{-1}	100 cells·mL^{-1}	>4 h	[46]
	2-4000 cells·mL^{-1}	13 cells·mL^{-1}	>1 h	[47]
	57-5.7×10^5 cells·mL^{-1}	57 cells·mL^{-1}	1 h	**This work**

To achieve the point-of-care and naked eye detection of the telomerase activity in MCF-7 cells, we established a CRISPR/Cas12a-based lateral flow assay under the optimized conditions. The cell extracts of MCF-7 was diluted to different concentrations (5.7×10^5, 5.7×10^4, 5.7×10^3, 5.7×10^2, 5.7×10 cells·mL^{-1}). The following procedure is similar to the above, except that the radical group of ssDNA reporter is replaced by FAM and biotin at both ends. After the reaction, the system was diluted to 100 μL with attached buffer. Then the strip was inserted directly into

the solution and incubated for two minutes. As shown in Fig. 3C, the reporter is clipped in the presence of positive sample and an obvious band appears at the sample line. However, the same phenomenon is not observed in negative samples. The strip assay of cell extracts of different concentrations of MCF-7 is shown in Fig. 3D. We can see that the naked eye determination of the telomerase activity in MCF-7 cells can reach to 5.7×10^2 cells·mL^{-1}. At present, other point-of-care and naked eye strategies to detect telomerase require 2 hours at least (table 3). But our sensor can detect the telomerase activity in MCF-7 cells low to 5.7×10^2 cells·mL^{-1} in about one hour. This scheme provides a simpler and more direct detection method for the telomerase activity determination in breast cancer cells.

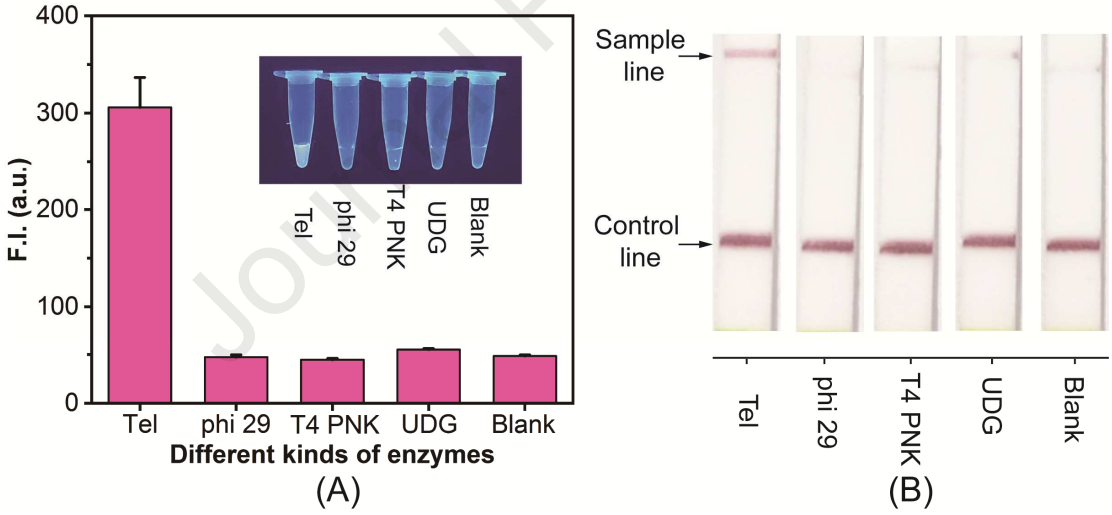
Table 3 Comparison of this sensor with other POCT in detecting telomerase activity.

Readout	Detection range	Actual detection limit	Time	Ref.
ATP meter	25-2500 cells	25 cells	1 h	[48]
paper	10-500 cells	10 cells	>4 h	[49]
luminometer	10-1000 cells	10 cells	>4 h	[24]
H_2O_2 test strip	200-5000 cells	10 cells·μL^{-1}	>3 h	[25]
pressure meter	1-1000 cells	1 cell	>1.5 h	[50]
glucometer	10-10000 cells	10 cells	5 h	[51]
lateral flow assay	5.7×10^2-5.7×10^5 cells·mL^{-1}	5.7×10^2 cells·mL^{-1}	1 h	**This work**

3.4 Selectivity of the sensor

To further evaluate the selectivity of this sensor, phi29 (0.5 U·μL^{-1}), T4 PNK (0.5 U·μL^{-1}), and UDG (0.5 U·μL^{-1}) was used (Fig.4A). The fluorescence intensity of cell extracts (5.7×10^3 cells·mL^{-1}) is significantly stronger than that of other groups. In addition, compared with blank, the fluorescence intensity of phi29, T4 PNK, and UDG are not significant. This phenomenon indicates that the background interference

283 of this sensor is very weak and has excellent selectivity. The corresponding
284 CRISPR/Cas12a-based lateral flow assay also shows only in the presence of cell
285 extracts has a band appear on the sample line on the test strip (Fig.4B). The
286 CRISPR/Cas12a system inherently possesses excellent specificity due to its
287 trans-cleavage activity only be activated significantly when the recognition region of
288 the target single-stranded DNA reaches more than 15 bp or the double-stranded DNA
289 contains PAM sites and recognition regions [26]. Here, the recognition sequence for
290 the crRNA we designed does not exist in the system initially. So the ssDNase of the
291 CRISPR/Cas12a system can't be activated. Thus, the added ssDNA reporter will not
292 be incised.



293 **Fig.4**. Selectivity of the sensor. (A) is the fluorescence-based experiments (Illustration:
294 the image of the target and interferences under 302 nm fluorescence irradiation); (B)
295 is the lateral flow-based experiments.

296 **3.5 Determination of the telomerase activity in subtype of breast cancer cells and**
297 **normal human serum**

Telomerase activity is closely related to cell immortality and canceration. Studies have shown that telomerase is overexpressed in breast cancer, but has no expression or low activity in benign lesions and normal tissues [52,53]. Yashima showed that the expression rates of telomerase activity in fibroadenoma, preinvasive carcinoma and invasive breast cancer were 67%, 92%, and 94%, respectively. Telomerase has different activities in different degrees of differentiation and pathological types [54]. Thus, the determination of telomerase activity in subtype of breast cancer cells can promote the understanding of the growth, division and metastatic ability of breast cancer. Simultaneously, the different telomerase activity may be used as one of the index for breast cancer cell typing. To extend the application of the proposed method, telomerase activity of subtype of breast carcinoma cells (HCC38, MDA-MB-231, SUM-159, and MCF-7) were detected. Each subtype breast cancer cells was diluted to the same concentration. As illustrated in Fig. 5A, different subtype breast cancer cells own different telomerase activity but all significantly different from the blank group. The activity of telomerase in subtype is ranked as: MCF-7>MDA-MB-231>SUM-159>HCC38. We can see that the telomerase activity of MCF-7 is significantly different from the HCC38 and SUM-159. The telomerase activity of MDA-MB-231 is also significantly different from the HCC38 and SUM-159. And the telomerase activity of HCC38 and SUM-159 is obvious difference. To verify our conclusions, we used Human Telomerase Reverse Transcriptase ELISA Kit on the market to detect the telomerase activity in different subtypes of breast cancer cells at the same concentration. The experiment was performed according to

the operating instructions attached to the kit. The result is shown in Fig. 5B. The results are consistent with the results of our proposed sensor. Those results indicate that the established way may own a potential in the classification of breast cancer cell subtypes. To further verify that the sensor can be used for the detection of telomerase activity in complex substrates, we did spiked experiments in normal human serum. The results are shown in Table 4. The recovery rates of the spiked experiments are between 94.7% and 109.2%, indicating that the sensor can be used to detect telomerase activity in complex substrates.

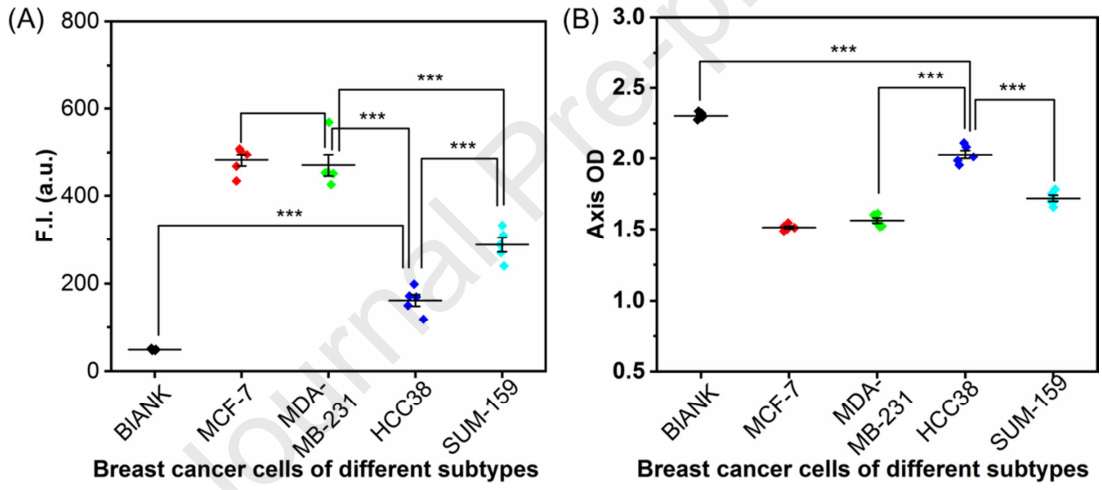


Fig.5. Detection of telomerase activity in subtype of breast cancer cells by proposed sensor (A) and Human Telomerase Reverse Transcriptase ELISA Kit (B).

Table 4 The detection of added telomerase activity in normal human serum using the proposed sensor

Target added (number of MCF-7 cells)	Detected results (number of MCF-7 cells)	Recovery (%)
0	--	--
100	109.2	109.2
400	378.6	94.7
4000	4327.2	108.2

4 Conclusions

In summary, this work established a sensor to ultra-sensitively and visually detect

telomerase activity. In the presence of telomerase, the primer can be extended to generate a tail containing unlimited *5'-TTAGGG-3'* units in a short time. While the continuous repeating *5'-TTAGGG-3'* sequence can play the role of multiple activators to switch on the ssDNase activity of the CRISPR/Cas12a system. Due to the extremely efficiency of the enzymes, the primers can be extended multiple activation regions to achieve the first rapid amplification of the signal. In addition, the activated CRISPR/Cas12a system essentially with signal amplification since it can quickly cut the single-stranded cleavage reporters indiscriminately. Thus the two-step signal amplification is realized. Besides, we integrated the extension of telomerase and the activation of the ssDNase of the CRISPR/Cas12a in one pot, which simplifies the procedure and shortens the testing time. Based on the fluorescence and CRISPR/Cas12a-based lateral flow assay, we achieve the ultra-sensitive and point-of-care detection of telomerase activity in MCF-7 cells low to 57 cells·mL^{-1} and 5.7×10^{2} cells·mL^{-1}, respectively. Compared with other methods for telomerase activity detection, our method owns the advantages of time-saving, high sensitivity and simplicity. Finally, we used this sensor to detect telomerase activity in subtype of breast carcinoma cells. The results indicate that the proposed method may possess great potential in the classification of breast carcinoma cells.

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☒ √ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
None

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