Supporting Information

Cyclic chain displacement amplification-based dual-miRNA detection: a triple-line lateral flow strip for the diagnosis of lung cancer

Ping Zhou, Fei Lu, Wei Pan, Jiaqi Yin, Na Li,* and Bo Tang*

College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Institute of Molecular and Nano Science, Shandong Normal University, Jinan 250014, P. R. China.

*E-mail: lina@sdnu.edu.cn, tangb@sdnu.edu.cn.

Table of Contents

Experimental Section……S-3

Reagents and Materials. ……S-3

Methods ……S-3-6

Ethics approval and Consent to Participate.……S-6

Supporting Figures and Tables ……S-7

Table S1. ……S-7

Figure S1. ……S-7

Figure S2. ……S-7

Figure S3. ……S-8

Experimental Section

Reagents and Materials.

The hairpin DNA 1, 2, 3 and 4 were purchased from Sangon Biotech Co., Ltd. The triple-line nucleic acid test strip was purchased from Beijing Biowinner Biotechnology Co., Ltd. Phosphate buffer saline (PBS) was purchased from Biological Industries, Israel. Other aqueous solutions used in experiments were prepared using deionized water (18.2 MΩ·cm).

Methods

The native polyacrylamide gel electrophoresis (native-PAGE).

To verify the feasibility of target miRNA-triggered cyclic chain displacement reaction (CCDR), 15% polyacrylamide gel containing with polyacrylamide, ammonium persulfate, N,N,N',N'-Tetramethylethylenediamine (TEMED), and TBE, was prepared.

15% polyacrylamide gel (15 mL): 5.625 mL Acryl/Bis 40% solution (19:1), 1 mL 10 x TBE buffer, 7.725 mL ddH_2O, 135 μL 10% Ammonium persulfate solution (APS) and 10 μL TEMED.

The DNA1, DNA2, DNA3 and DNA4 were pretreated by heating into 95 ℃ for 5 min, and cooling into 0 ℃ rapidly and maintained for 10 min. Then DNA1, DNA2 and DNA3, DNA4 was mixed with miRNA-223 (100 nM) and miRNA-200b (100 nM), respectively. The mixture were reacted at room temperature for 10 min. Then four testing groups, one without miRNAs, one only with miRNA-223 or miRNA-200b and one with two target miRNAs were separately mixed with loading buffer. The gel was run under constant pressure120 mV for 90 min. After that, the gel was dyed by GelRed for 20 min and photographed by peiqing JS-680D automatic digital gel imaging analysis system.

The time optimization for miRNA-triggered CCDR.

The detailed principle of the miRNA-mediated CCDR is displayed in Figure S1. In the presence of miRNA-223, the stem-ring structure of the hairpin DNA1 was opened from 5’ end with the generation of a single-stranded fragments in 3’ end. The fragments

can invade and hybridize with the hairpin DNA2. Then hybridization chains of DNA1 and DNA2 was generated and the miRNA-223 will be substituted out from DNA1-miRNA-223 hybrid complex. Furthermore, the released-miRNA-223 acts as a cyclic progenitor that can attack the next hairpin DNA1 and result in continuous hybridization chain of hairpin DNA1 and DNA2. The principle of miRNA-200b-triggered CCDR with DNA3 and DNA4 is similar with that of miRNA-223. In order to efficiently suppress the nonspecific background between the hairpin DNAs in the CCDR, four hairpin DNAs were dissolved in PBS buffer containing with 50-150 mM NaCl and heated to 95 ℃ and maintained for 10 min, and cooled to 0℃ for 10 min before using. After that, the DNA solution was store at 4 ℃ until further application.

In order to explore the optimal reaction time of the miRNA-triggered CCDR, 15% polyacrylamide gel was chosen and prepared. At first, the DNA1 was mixed with DNA2, and DNA3 was mixed with DNA4 in PBS buffer, respectively. Then the corresponding miRNA was added into the mixture and incubated for different times (1, 5, 10, 20, 30, 60 min) at room temperature. Subsequently, the reaction solution was run by the 15% native-PAGE under 120 mV. With the staining of GelRed for 20 min, the gels were scanned by peiqing JS-680D automatic digital gel imaging analysis system.
The feasibility verification experiment. To explore the feasibility of this dual-miRNA detection strip, DNA1, DNA2, DNA3 and DNA4 were pretreated with heating at 95 ℃ for 5 min and cooling at 0 ℃ for 10 min. Then DNA1, DNA2, DNA3 and DNA4 were added into PBS buffer and mixed with each other in a 1.5 mL centrifuge tube. The mixture was divided into four group: group one without miRNA was the control group; group two was incubated with the target miRNA-223; group three was incubated with the target miRNA-200b; and group four was incubated with the two miRNAs. After the reaction for 10 min, the triple-line test strips were inserted into the four reaction solutions for data readout.

The specificity verification experiment.

At first, miRNA-155, miRNA-21 and miRNA-205 were chosen as the non-target miRNA to verify the specificity of this dual-miRNA detection system. Herein, all of

the miRNA concentration was 50 nM, and the incubation time was 10 min at room temperature. After reacting, the triple-line test strips were used to read out the result. The picture was obtained by a digital camera.

The detection of miRNA in serum.

The serum samples were obtained from clinical healthy individuals and lung cancer patients. For detecting, 50 μL serum was mixed with 150 μL PBS buffer that containing DNA1, DNA2, DNA3 and DNA4 sequences. After running by the triple-line strip, the chromogenic signals were obtained and recorded by a digital camera. This experiment was repeated at least three times.

The quantification of detection results by Image J.

The test line and control line in the strip were quantified with Image J. We measured the mean intensities of detection line and background, respectively. Next, we treated the mean intensities of the test line and subtracted the intensities of the background. This experiments were repeated at least three times and the error bars were obtained.

The stability experiment.

The hairpin DNAs and triple-line lateral flow strips were stored at 4 ℃ and room temperature, respectively. After more 3 months, the feasibility experiments of the dual-miRNA detection system were conducted to verify the stability of this detection system. The detection result of target miRNAs was as shown in Figure S4.

Quantitative Real-time polymerase chain reaction (qRT-PCR).

Total miRNA was extracted from 200 μL serum based on miRNeasy extraction Micro Kit (TianGen, DP501) according to the manufacturer' protocol. The concentration and the quality of the RNA were analyzed by nanodrop. The cDNA is synthesized by program containing poly A tailing modification and reverse transcription reaction using miRcute Plus miRNA First-Strand cDNA Kit (TIANGEN, KR211). According to the manufacturer's protocol, 7 μL total miRNA as the template was added to the reaction solution. Then the mixture was reacted under 42 °C for 60 min and then 95 °C for 3 min. Polymerase chain reaction (PCR) instrument was

purchased from Bioer Technology Co., Ltd. (Hangzhou, China, LineGene 9620). The concentration and the quality of the cDNA were analyzed by nanodrop. The qRT-PCR assay was performed with miRcute Plus miRNA qPCR Kit (SYBR Green, TIANGEN, FP411). According to the manufacturer's protocol, 2 μL cDNA as the template was added to the SYBR Green mix. The specific PCR primers of miRNA-223, miRNA-200b and RNU-6 were purchased by TIANGEN BIOTECH (Beijing) CO., LTD. The data were normalized with RNU-6 and the relative expression of miRNA-223 and miRNA-200b were determined by the 2-△△Ct method.

The calculation of limit of detection.

The calibration curves of strip-based detection could be formed according to the quantitative value of test strips. As shown in Figure 3B and 3C, the standard curve of miRNA-223 was fitted into the following regression equation: y=11.98462*logx+10.91199, R2=0.992. The limit of detection (LOD) of the miRNA-223 was determined as the value of x at y=0. Thence, the LOD of miRNA-223 was calculated to be 0.123 pM. Similarly, the standard curve of miRNA-200b was fitted into the regression equation: y=14.01305*logx+5.34631, R2=0.9868. And the LOD of miRNA-200b was calculated to be 0.415 pM. All experiments were repeated at least three times.

Ethics approval and Consent to Participate.

This study was approved by the Ethics Committee of Shandong Normal University and written informed consents were signed by all patients. Experiments of human serum samples were carried out in accordance with the approved guidelines of China.

Table S1. The sequence of DNAs and RNAs employed in this work.

Sequence (5'-3')
miRNA-200b: 5'-UCUUACUGGGCAGCAUUGGA-3'
miRNA-223: 5'-UGUAU UUGAC AAGCU GAGUU-3'
miRNA-205: 5'-UCCUUCAUUCCACCGGAGUCU-3'
miRNA-21: 5'-GCUUAUCAGACUGAUGUUGA-3'
DNA1: 5'-Biotin-AAAAAAAACTCAGCTTGTCAAATATGGTGTGAGTTATTTGACAAG-3'
DNA2: 5'-TAMRA-AAAAATGTCAAATAACTCACACCATATTTGACAAGTGGTGTGAGTT-3'
DNA3: 5'-Digoxin-AAAAAATCCAATGCTGCCCAGTAATGGTGTGAGTTTACTGGGCAG-3'
DNA4: 5'-FAM-AAAAAACCCCAGTA AACTCACACCATTACTGGGCAGTGGTGTGAGTT-3'

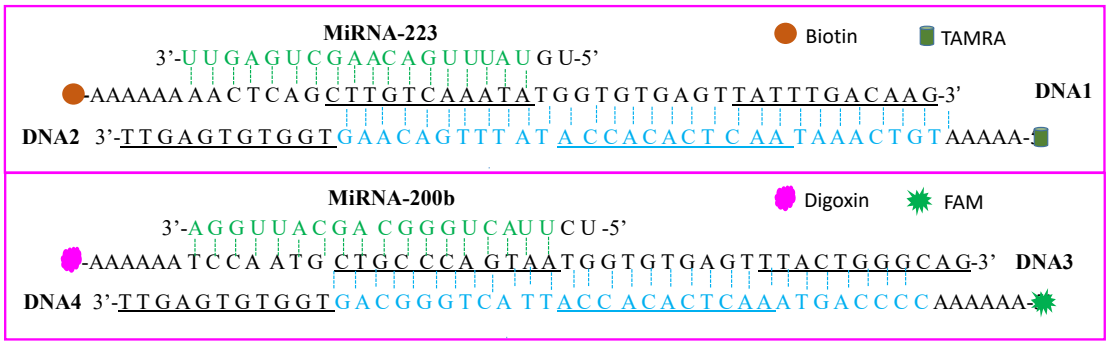

Figure S1. The schematic diagram of miRNA-223 and miRNA-200b-triggered chain hybridization displacement reaction.

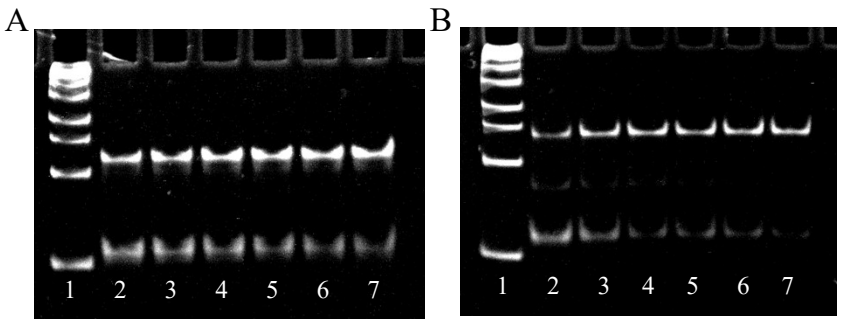

Figure S2. The native-PAGE images of the two pairs of hairpin DNA, DNA1/DNA2 (A), DNA3/DNA4 (B) incubated with miRNA-223 and miRNA-200b for different time (1, 5, 10, 20, 30, 60 min, from line 2-7), respectively.

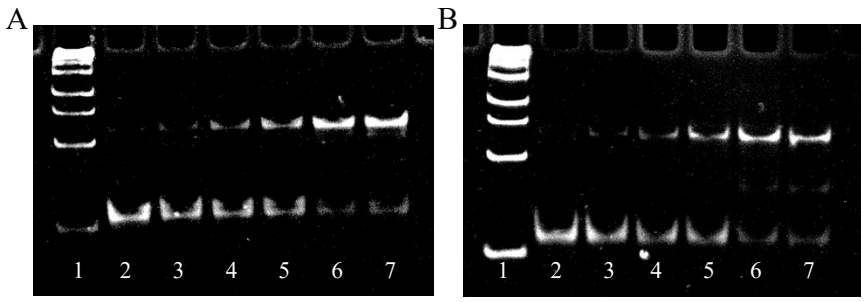

Figure S3. The native-PAGE images of the two pairs of hairpin DNA, DNA1/DNA2 (A), DNA3 /DNA4 (B) incubated with miRNA-223 and miRNA-200b for different concentrations (0, 1, 5, 10, 50, 100 nM, from line 2-line 7), respectively.

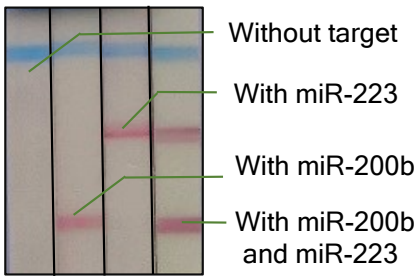

Figure S4. The detection image of target miRNAs using the dual-miRNA detection system after the DNAs and strips were stored at 4 ℃ and room temperature for 3 months.