Supplementary Information

A lateral flow assay strip for simultaneous detection of miRNA and exosome in liver cancer

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EXPERIMENTAL SECTION

Reagents and Materials

All the oligonucleotides in this work were purchased from Sangon Biotech Co., Ltd. Lateral flow assay strip was purchased from Beijing Biowinner Biotechnology Co., Ltd. Dulbecco's modified Eagle medium (DMEM) and phosphate buffer saline (PBS) were purchased from Vivacell, Shanghai, China. The human hepatoellular carcinomas（HepG2）cell line and normal liver cells (HL 7702) were purchased from AOLU Biological Technology Co., Ltd. Fetal bovine serum (FBS) in cell culture medium was purchased from Gibco. All aqueous solutions were prepared using sartorius ultrapure water (18.2 MΩ·cm^{-1}).

Instruments

Transmission electron microscopy (TEM) images were characterized on the HT7700 (Japan). Native polyacrylamide gel electrophoresis analysis was performed on gel electrophoresis apparatus (Bio-Rad, USA). The Zeta potential was recorded on a Malvern Zeta Sizer Nano (Malvern Instruments). Fluorescence spectra were carried out on a FLS-980 Edinburgh. The UV-Vis absorption spectra were obtained by UV-1700 (Shimadzu, Japan).

Preparation of FAM-EpCAM aptamer functionalized Au NPs (FAM-EApt-Au).

FAM-EpCAM aptamer (20 μM, 10 μL) was mixed with Au NPs (3 nM, 1mL) and slowly shaken at 180 rpm for 12 h. The SDS was added into the solution (final concentration of SDS is 0.01%) and then the NaCl solution (1 M) dissolved in PBS buffer (pH = 7.4) was added to the reaction system over 8 h period (final concentration of NaCl was 100 nM). The solution continued to react 12 h and then further aged for 48 h at room temperature. Finally, the FAM-EApt-Au probes were collected via centrifugation (13000 rpm, 20 min) and washed with water for subsequent use.

Fluorescence curves of FAM-EpCAM.

FAM-EpCAM solutions with a concentration gradient from 20-60 nM were prepared, and the fluorescence curves from 500-600 nm were recorded.

Isolation and characterization of exosomes.

The DMEM media was removed when the cell density reached above 80%, And then the cells were treated with new DMEM media without FBS containing 0.6% insulin-transferrin-selenium (ITS-G) for three days. Exosomes were obtained from the supernatant by gradient centrifugation. First, the

supernatant culture medium was collected and centrifuged at 500 g for 5 min, then centrifuged at 2000 g for 10 min and at 10000 g for 30 min orderly to remove the large vesicles and cell fragments. Next the obtained supernatant was treated by ultrahigh-speed centrifugation (Beckman, U.S.A., Optima XPN-100 Ultracentrifuge) at 120000 g for 1 h. Finally, the exosomes precipitation was resuspended in PBS and stored at -80 °C for subsequent use.

The native polyacrylamide gel electrophoresis (native PAGE) analysis of CHA.

The feasibility of CHA between EApt-H1 and Biotin-H2 was verified by native-PAGE. Loading buffer (6X, 2 μL) was added to DNA sample (PApt-H1 (2 μL, 2 μM), Biotin-H2 (2 μL, 2 μM), miRNA 223 (2 μL, 200 nm), PBS buffer (14 μL)) and the mixture was injected into the notch of gel (15%). Next, the gel was run in 1× TBE buffer under constant pressure 120 mV for 120 min. Finally, the gel was stained with the GelRed dye for 20 min and was analyzed by the JS-680D automatic digital gel imaging analysis system to obtain the electrophoresis result.

Cell culture.

HepG2 cells and HL 7702 (L02) cells were incubated in cell culture dishes with a diameter of 10 cm. The cells were incubated with DMEM media containing 10% (v/v) fetal bovine serum (FBS), 1% penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Detection of miRNA 223 and exosome in buffer.

PApt-H1 (2 μL, 2 μM) and Biotin-H2 (2 μL, 2 μM) were mixed with two kinds of targets (miRNA 223 (2 μL, 200 nm), HepG2-Exo (2 μL, 10^9 particles/μL)) at 25 °C for 10 minutes. Then FAM-EApt-Au probes (10 μL, 30 nM) were added and incubated at room temperature for 15 minutes. Finally, the LFA was inserted and ran for 15 min, corresponding results were read visually.

Detection of clinical sample.

The targets were replaced with the serum of the clinical sample. PApt-H1 (2 μL, 2 μM) and Biotin-H2 (2 μL, 2 μM) were mixed with clinical samples at 25 °C for 10 minutes. Then FAM-EApt-Au probes (10 μL, 30 nM) were added and incubated at room temperature for 15 minutes. Finally, the LFA was inserted and ran for 15 min, corresponding results were read visually.

Ethics approval and consent to participate.

This study was approved by the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University (No. 2021S128). Written informed consents were signed by all patients

before sample collection. Experiments of human serum samples were carried out in accordance with the approved guidelines of China.

Supporting Figures and Tables

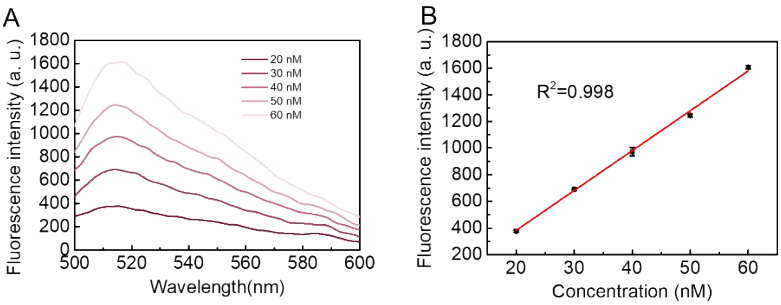

Figure S1 (A) The fluorescence spectra of FAM-EpCAM aptamer with different concentrations. (B) The fluorescence standard curves of FAM-EpCAM aptamer.

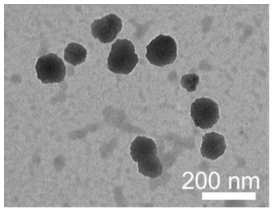

Figure S2 TEM image of L02-Exo.

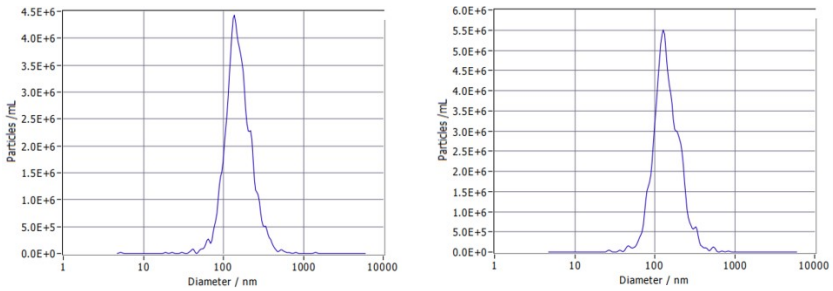

Figure S3 The diameters distribution of isolated exosomes analyzed by NTA.

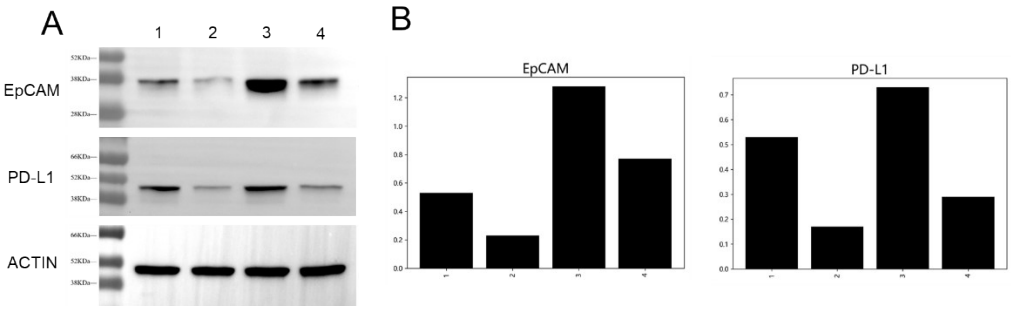

Figure S4 (A) Specific protein expression of different cells and exosomes analyzed by WB. (B) The relative gray value of proteins of different groups. (1: L02 cells, 2: L02-Exo, 3: HepG2 cells, 4: HepG2-Exo)

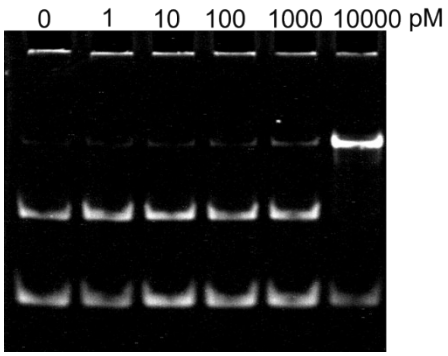

Figure S5 Native PAGE image of CHA triggered by different concentrations target.

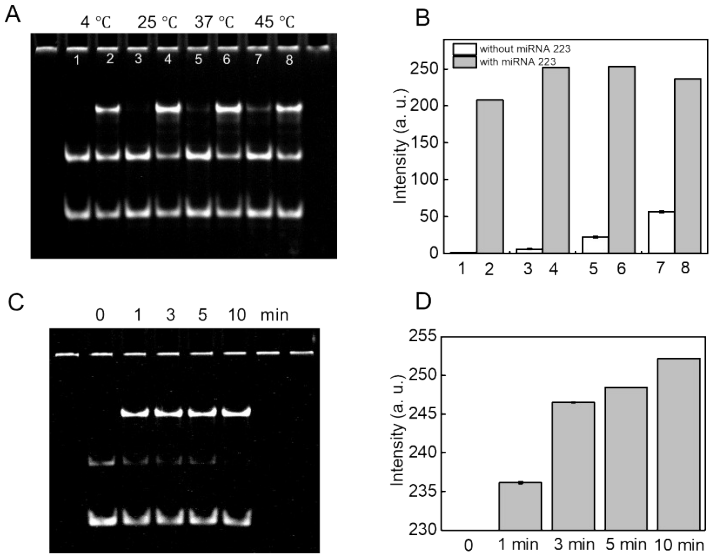

Figure S6 (A) Native PAGE image of CHA amplification at different reaction temperatures. Lines 1, 3, 5, and 7 are PApt-H1+ Biotin-H2 group; lines 2, 4, 6, 8 are PApt-H1+ Biotin-H2+miRNA 223 group. (C) The optimization of the CHA reaction time. (B) and (D) are the quantization of T line in A and C, respectively.

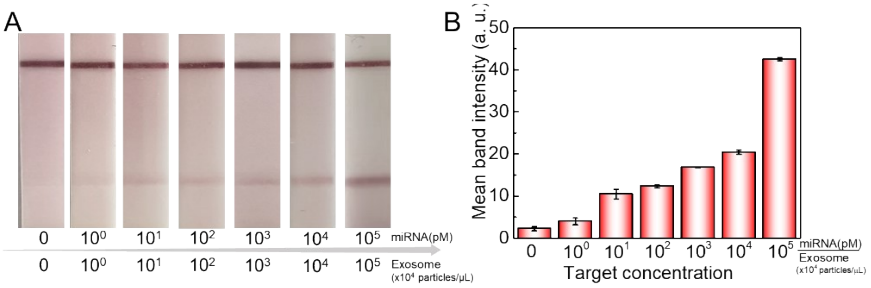

Figure S7 (A) The change of T line signal with the increasing concentration of dual targets. (B) Image J grayscale analysis results of (A).

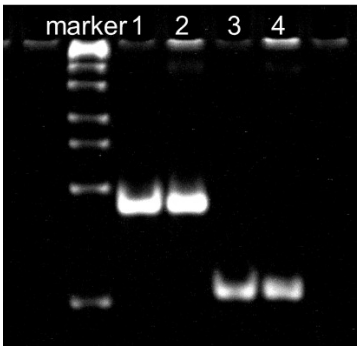

Figure S8 The stability experiment of the PApt-H1+ Biotin-H2 in serum solution. PBS: 1, 3; Serum: 2, 4.

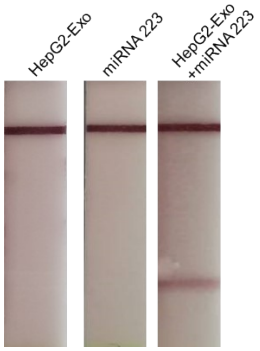

Figure S9 The detection feasibility of LFA in serum solution.

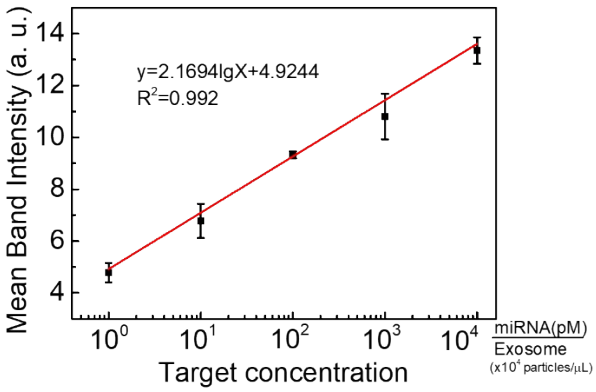

Figure S10 The linear relationship between T line intensity and logarithmic values of target concentrations.

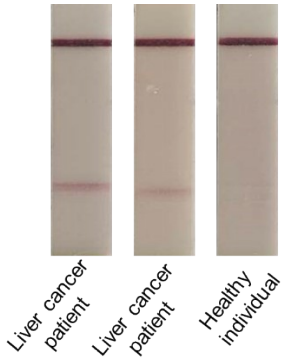

Figure S11 The detection results of LFA on the clinical sample after the reagent stored for a week at 4 °C.

Table S1. Sequences of DNA/RNA employed in this work.

Oligonucleotide	Sequence (5'-3')
PApt-H1	TACAGGTTCTGGGGGGTGGGTGGGGAACCTGTTAAAAAAAACTCAGCT T GTCAAATATGGTGTGAGTTATTTGACAAG
Biotin-H2	Biotin-AAAAATGTCAAATAACTCACACCATATTTGACAAGTGGTGTGAGTT
EpCAM Aptamer	TTTCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCC T G
miRNA 223	CGUGUAUUUGACAAGCUGAGUU
miRNA 122	UGGAGUGUGACAAUGGUGUUUG
miRNA 21	GCUUAUCAGACUGAUGUUGA
miRNA 210	AGCCCCUGCCCACCGCACACUG
miRNA 155	UUAAUGCUAAUCGUGAUAGGGGU