Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information Applications of hepatoma carcinoma cell aptamers *in vitro*

Fei Ding, [‡ [a]] *Chunhui Yuan,* [‡[ab]] *Fan Wu,* [‡ [a]] *Shuang Peng*[b], *Sufang Tian*[c], *Xiao-Lian Zhang*[b]* *and Xiang Zhou*[ab]*

Materials and Methods

SELEX library and primers. The DNA library contained a randomized sequence of 40nt at the center and flanked by two 19nt primer hybridization sites (5'-ACCGAC CGTGCTGGACTCT-N40-AGTATGAGCGAGCGTTGCG-3').[1] A FITC-labeled primer (5'-FITC- ACCGACCGTGCTGGACTCT -3') was used in the PCRs for the synthesis of single-stranded DNA strand. After performed asymmetric PCR, the FITC-conjugated sense ssDNA strand was separated and used for next-round selection. The selection process was monitored by flow cytometry and confocal image.

Cell lines and buffers. Huh7.5.1 cell (human liver carcinoma cells), GES-1 cell (Human gastric epithelial cells), Hs578Bst (human mammary epithelial cells) were obtained from American Type Culture Collection, and cultured in DMEM medium contained 15% FBS (GIBCO) and 100 units/ml penicillin–streptomycin (Beyotime). HepG2 cell (Human liver carcinoma cells), 97L cell (human liver carcinoma cells), LM9 cell (Human liver carcinoma cells), HT29 cell (Human colon adenocarcinoma cells) were obtained from American Type Culture Collection, and cultured in RPMI-1640 medium supplemented with 10% FBS (Hyclone) and 100 units/ml penicillin–streptomycin (Beyotime), HeLa cell (human cervical carcinoma cells)

were preserved in our laboratory, and cultured in MEM medium contained 10% FBS (Hyclone) and 100 units/ml penicillin–streptomycin (Beyotime). L-02 (human normal liver cells) were obtained from www.bgswkj.com, and cultured in RPMI-1640 medium supplemented with 15% FBS (GIBCO) and 100 units/ml penicillin–streptomycin (Beyotime). Washing buffer contained 4.5 g/L glucose and 5mM $MgCl_2$ in Dulbecco's PBS (Hyclone). Binding buffer was prepared by adding 1 mg⁄mL BSA, 0.1mg/ml yeast tRNA and 15% fetal bovine serum into the washing buffer to reduce background binding. All the DNA sequences used in this article were custom-designed and then synthesized by Takara Bio Inc. Trypsin and proteinase K were purchased from Fisher Biotech.

Tissues. Human gastric tumor tissues and human normal gastric tissues were obtained from Zhongnan Hospital of Wuhan University, School of Medicine.

Experimental Procedures

SELEX procedures. The ssDNA pool (300nmol) was dissolved in 200 μL binding buffer and then 95℃ heating for 5min, quickly cooled on ice for 10min until used. Then the ssDNA pool was incubated with 3×10^6 Huh7.5.1 cells (target cells) at 37˚C for 60min. After washing, the bound DNAs were eluted by heated at 95℃ for 10 min. The eluted DNAs were then incubated with 1×10^7 L-02 cells (negative cells) at 37˚C for counter selection for 1 h. After centrifugation, the supernatant was precipitated by ethanol and then amplified by PCR with FITC- primers (30 cycles of 45s at 95℃, 45s at 60℃, and 45s at 72℃, followed by 7 min at 72℃). The selected sense ssDNA was separated by rubber cutting and extracted by poly-gel extraction kit (Omega) For the

first-round of selection, the amount of initial ssDNA pool was 7nmol, dissolved in 500 μL of binding buffer, and the counter selection step was not progressed. To obtained high affinity and specificity aptamers, we gradually increased the amount of negative cells and its incubation time, meanwhile decreased the incubation time of target cells. The wash strength was enhanced gradually by increasing the number of washes (from one to five) and the volume of washing buffer (from 500 μL to 3000 μL). After 11 rounds of selection, the selected ssDNA pool was PCR-amplified by using unmodified primers and cloned into Escherichia coli by using the TA cloning kit (ComWin Biotech). Cloned sequences were determined by the Genome Sequencing Services (Invitrogen).

Flow cytometric analysis of pool enrichment. To monitor the enrichment of aptamer candidates during selection, the FITC-labeled ssDNA pool was incubated with 3×10^5 Huh7.5.1cells or L-02 cells in 200 μL of binding buffer at 37˚C for 30min. Cells were washed twice with 500 μL of washing buffer (containing 15% FBS, 0.1%NaN_3) and suspended in 200 μL of steriled PBS. The fluorescence was determined with a FACScan cytometer (BD Immunocytometry Systems) by counting 20,000 events. The FITC-labeled unselected ssDNA library was used as a negative control. The binding affinity of aptamers was determined by incubating Huh7.5.1 cells (3×10^5) at 37˚Cfor 30min with varying concentrations of FITC-labeled aptamers in a 200 μL volume of binding buffer. The FITC-labeled unselected ssDNA library was used as a negative control to determine the nonspecific binding. All of the experiments for binding assay were repeated three times. The mean fluorescence

intensity of target cells labeled by aptamers was used to calculate for specific binding by subtracting the mean fluorescence intensity of nonspecific binding from unselected library DNAs.[2] The equilibrium dissociation constants (Kd) of the aptamer–cell interaction were obtained by fitting the dependence of fluorescence intensity of specific binding on the concentration of the aptamers to the equation Y =B max X/ (Kd+X), using SigmaPlot (Jandel, San Rafael, CA).

Confocal imaging of cell bound with aptamer. For confocal image, the selected ssDNA pools or aptamers were labeled with FITC and incubated with cell monolayer in a 35-mm glass bottom culture dish in 500μL of binding buffer containing 15% FBS at 37˚C for 30min. After washing twice, the dishes with cells in 500μL of steriled PBS were placed above a 40× objective on the confocal microscope. The fluorescent images of cells were observed under excitation at 488 nm using confocal laser scanning microscopy (Nikon C1-si TE2000, Japan).

Confocal imaging of human tissues stained with aptamer. Human liver tumor tissues and normal tissues were formalin-fixed and embedded in paraffin. Five micrometer sections were cut from each paraffin block for microscopic examination. The 5-μm sections were first dewaxed, and then incubated with FAM labeled-aptamers (250 nM) in binding buffer containing 15% FBS at 37 ℃ for 20min. After washing, the slides were examined under excitation at 630 nm by confocal imaging (20× objective) (Nikon C1-si TE2000, Japan).

Flow cytometry assays with fluorescent aptamer probes. Generally, fluorescent aptamer probes were incubated with 3×10^5cells in 200μL binding buffer at 37℃ for

15 min in the dark and then immediately injected and determined with a FACScan cytometer (BD Biosciences) by counting 30,000 events. Especially for the detection sensitivity assay, different amounts of Huh7.5.1cells were stained by 250 nM activatable fluorescent aptamer probe in 200 μL binding buffer at 37 ℃ in the dark. After incubation for 15 min, the samples were immediately detected with flow cytometer by counting the aptamer probes-labeled events appearing in the upright (UR) region.

References:

1. Zhang, H. Q., Fang, N and Zhang, K. H. SELEX technology. *China Biotechnology.* 2008, **28,** 113.
2. Medley, C. D., Smith, J. E., Tang, Z., Wu, Y., Bamrungsap, S and Tan, W. Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells *Anal Chem***.** 2008. **4**,1067.

Figure S1 Generation of aptamer candidates by cell-SELEX to target cancer cells. Briefly, the ssDNA pool was incubated with Huh7.5.1 cells (target cells). After washing, the bound DNAs were eluted by heating to 95℃. The eluted DNAs were then incubated with L-02 cells (negative cells) for counterselection. After centrifugation, the supernatant was collected and the selected DNA was amplified by PCR. The PCR products were separated into ssDNA for next-round selection. When the selected pool was enriched enough, the PCR product of the evolved pool was cloned and sequenced for aptamer identification.

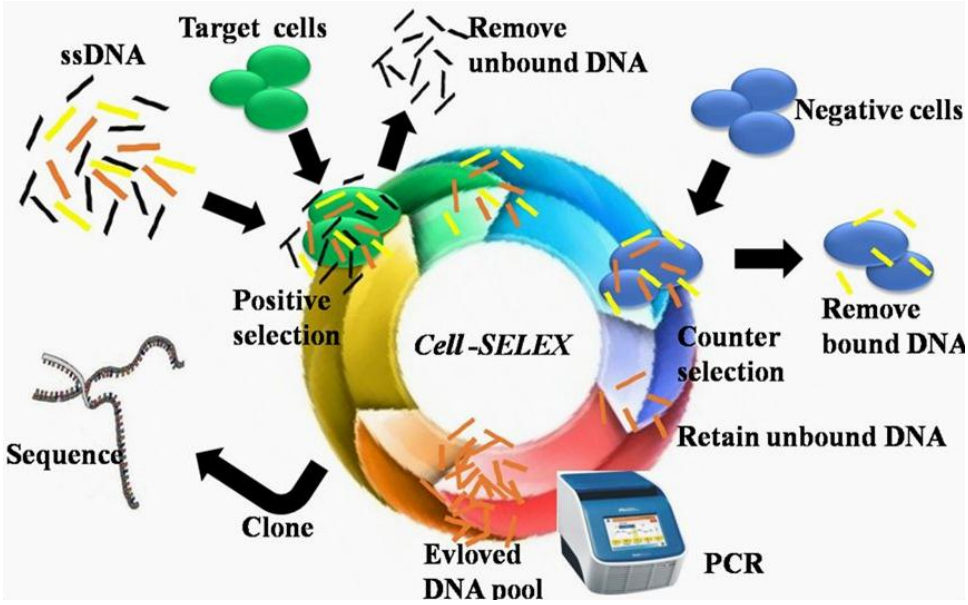
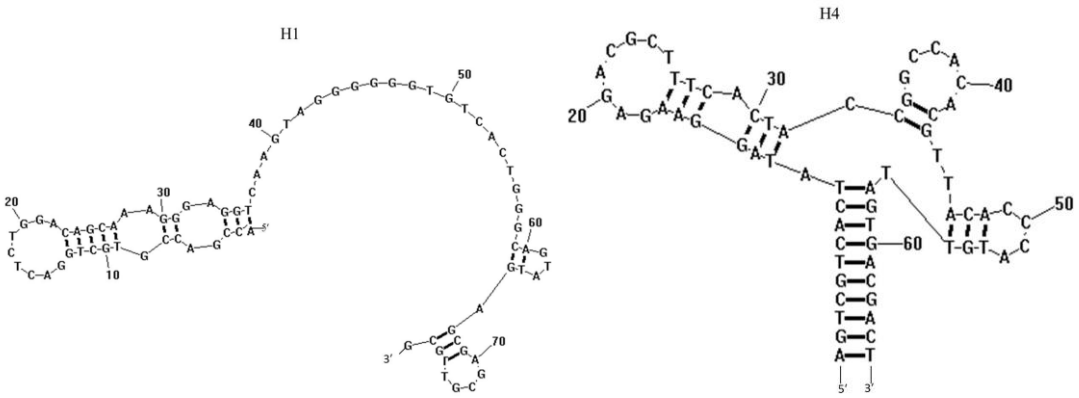


Figure S2 The secondary structures of aptamers and its truncated sequences by DNAMAN analysis.



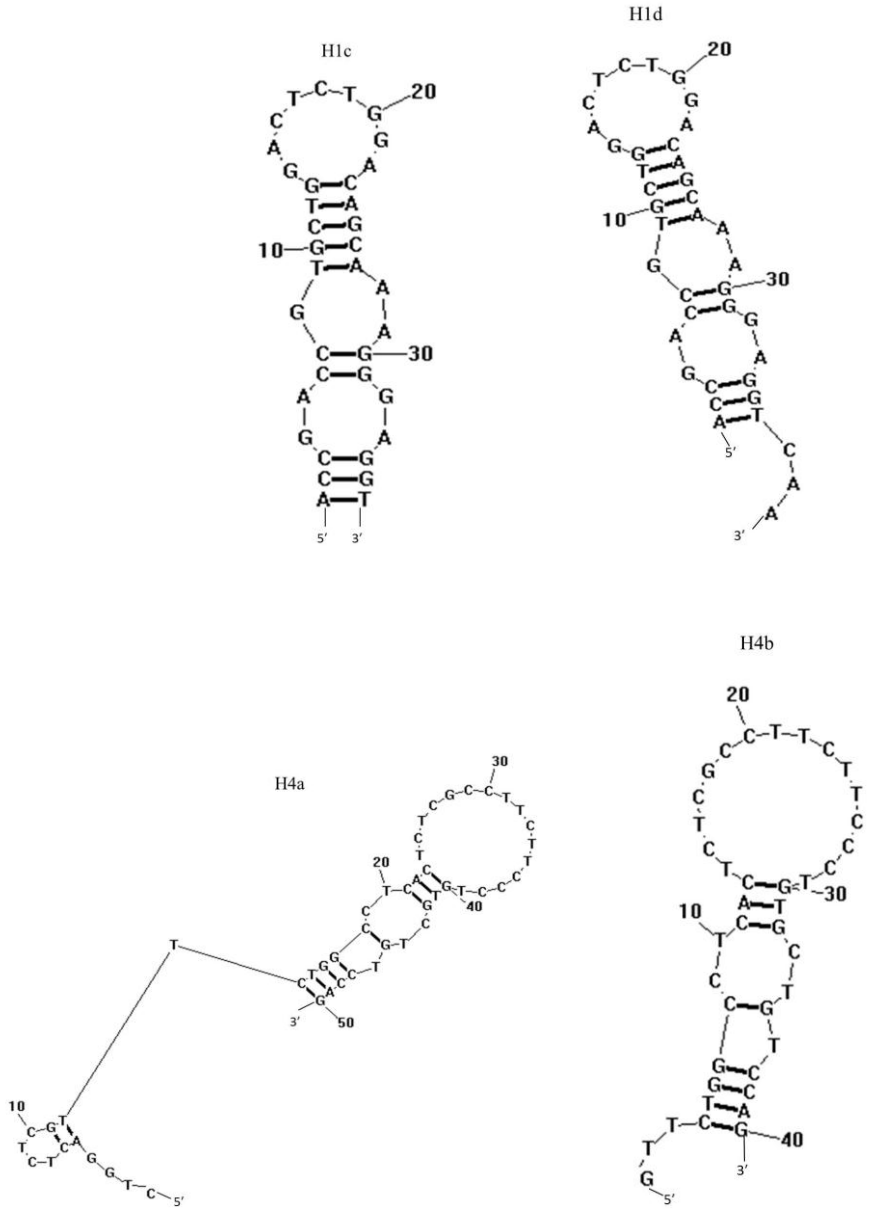


Table S1 Aptamer candidates selected with original sequences.

Aptamer candidated	Sequence
H1	ACCGACCGTGCTGGACTCTGGACAGCAAAGGGAGGTCAAGTAGGGGGGTGTCACTGGGCAGTATGAGCGAGCGTTGCG
H4	ACCGACCGTGCTGGACTCTCGTTCTGGCCTCACTCTCGCCTTCTTCCCTGTGCTGTCCAGAGTATGAGCGAGCGTTGCG

Black nuclotides are PCR binding region, red nucleotides are random sequences.