Supporting Information for

Phenotype-Related Drug Sensitivity Analysis of Single CTCs for Medicine Evaluation

Haimeng Pei,[a] Mei Yu,[a] Defang Dong,[a] Yiguo Wang,[b] Qingling Li,[a] Lu Li*[a] and Bo Tang*[a]

[a]*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, Institute of Molecular and Nano Science, Shandong Normal University, Jinan, 250014, P.R. China. E-mail: tangb@sdnu.edu.cn, lilu5252@163.com.*
[b]*Shandong Provincial Qianfoshan Hospital, the First Hospital Affiliated with Shandong First Medical University, Jinan, 250014, P.R. China.*

E-mail: tangb@sdnu.edu.cn, lilu5252@163.com.

Table of Contents

1. Experimental Procedures

1.1 Materials and Instruments

TAMRA-labeled EpCAM aptamer probe (TAMRA-EpCAM) was artificially synthesized and purified by Sangon Biotech (Shanghai, China), and the base sequences are given as follows: 5′-TAMRA-CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG-3′. Alexa 647-labeled N-cadherin antibody probe (Alexa 647-N-cadherin) was purchased from Abcam (Cambridge, MA, USA). TGF-β was purchased from R&D Systems (Minneapolis, MN, USA). Annexin V-FITC/PI Apoptosis Detection Kit and Curcumin were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Enhanced CCK-8 Kit was purchased from Saint-Bio (Shanghai, China). Docetaxel (T1034) was purchased from TargetMol. Tirofiban and eptifibatide were purchased from MCE. Purified water was obtained from Wahaha (Hangzhou Wahaha Group, Co., Ltd.) All chemicals and solvents used were of analytical grade.

Fluorescence spectra measurements were carried out using an Edinburgh FLS 920 fluorescence spectrophotometer (Edinburgh, UK). The bright-field images were obtained using a Leica DFC300FX inverted fluorescence microscope (Oskar-Barnack-Straße, Germany) equipped with a high-speed camera pco.dimax cs1 (Kelheim, Germany). Confocal fluorescence images were obtained by confocal laser scanning microscopy (Leica TCS SP8, Germany). The syringe pump containing four channels was made by Baoding Longer Precision Pump Co., Ltd. (Baoding, China).

1.2 Cell lines and culture

MCF-7 and MDA-MB-231 cells were purchased from Procell (Wuhan, China). PANC-1 cells were purchased from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell culture consumables were purchased from Xinyou Biotech Co., Ltd. (Hangzhou, China). MCF-7 cells were grown in DMEM (Biological Industries, BI) with 10% fetal bovine serum (BI) and 1% penicillin-

streptomycin (BI). MDA-MB-231 and PANC-1 cells were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) (Hyclone) with 10% fetal bovine serum. All cells were maintained at 37 °C in a 5% CO_2 atmosphere.

1.3 Cell sample preparation

The cells were collected and centrifuged at 1000 rpm for 2 min in culture medium, followed by two washes with PBS. Then, Optiprep 16% (v/v) density gradient solution (Sigma, USA) was added to the cell suspension to disperse the cells. The cell density was determined using a hemocytometer. Cells were treated with TGF-β (0, 5, 20 ng/mL) 3 days to form three EMT gradients, and then co-culture with curcumin (0-50 μM) respectively. After 24 h, the cells were incubated with TAMRA-EpCAM, Alexa 647-N-cadherin and Annexin V-FITC for 1 hour for fluorescent labeling.

1.4 Fluorescence Measurements

After PBS and FITC infused into the two inlets of the DS-Chip through syringe pump, the fluorescence signal was measured at the end of the drug gradient generators by a Cary Eclipse fluorescence spectrophotometer. The fluorescence of FITC was collected between 510 and 600 nm with excitation wavelength at 490 nm. The fluorescent signal located at 520 nm was used for fluorescence analysis. All experiments were performed at least three times.

1.5 Confocal fluorescence imaging

After cells loaded into the cell traps of the DS-Chip, TAMRA-EpCAM, Alexa 647-N-cadherinand Annexin V-FITC were infused into the chip for fluorescence labeling. Fluorescence imaging of the cells was carried out using confocal laser scanning microscopy (CLSM) at three excitation wavelengths (488, 561 and 633 nm). The collecting range of corresponding emission wavelengths was 500-560, 570-620, and 650-710 nm, respectively.

1.6 Patient samples collection

Blood samples from cancer patients were received from Qianfoshan Hospital after obtaining informed patient consent. The experiments using patients' blood were agreed by the Shandong Provincial Qianfoshan Hospital Institutional Review Board. All blood samples were provided by Shandong Provincial Qianfoshan Hospital after obtaining informed signed consent from patients. For treatment with glycoprotein IIb/IIIa inhibitors, tirofiban and eptifibatide were added to fresh blood samples at final concentrations of 0.5 μg/mL and 20 μg/mL, respectively. CTCs were isolated from the blood samples based on an integrated microfluidic device that enables automated label-free isolation of CTCs and further purification and concentration based on the principle of deterministic lateral displacement (DLD)[1].

2. Supplementary Figure

Device Design and Fabrication

The PDMS microchip was designed by our group and made by Wenhao Chip Technology Co., Ltd. (Suzhou, China). The structure and size of the chip channels are shown in Fig S1. The DS-Chip included two structures: drug gradient generators and parallel cell traps. The microfluidic gradient generator is composed of three stages of microfluidic meander channels, with an incremental number from three to five in each stage. Each channel of the microfluidic mixer is about 250 μm wide and 14 μm deep. The cell trapping site array is linked to the liquid gradient channel with a width of 1 mm (the gap is 8 μm, with a width of 25 μm and a depth of 14 μm). The row shift distance was set to 50 μm. The cell trapping site gap is 70 μm.

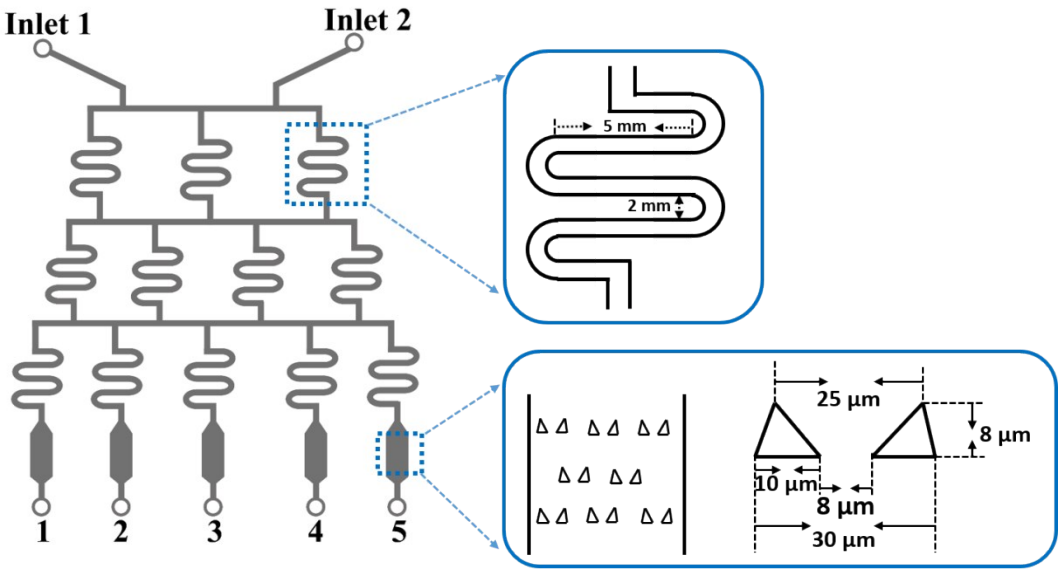

Figure S1. The schematic construction and dimension of the DS-Chip.

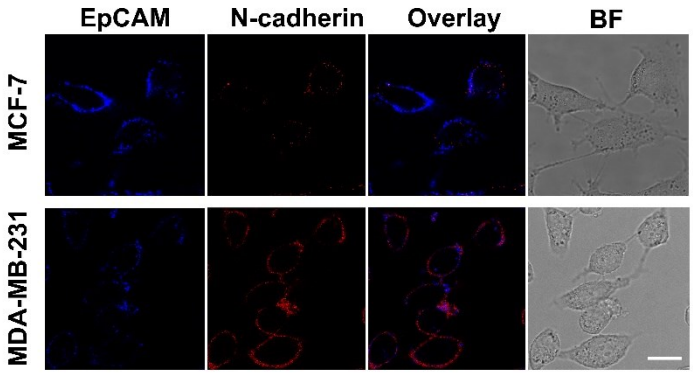

Figure S2. Confocal images of EpCAM and N-cadherin expression in MCF-7 and MDA-MB-231 cells, respectively. Scale bar is 20 μm.

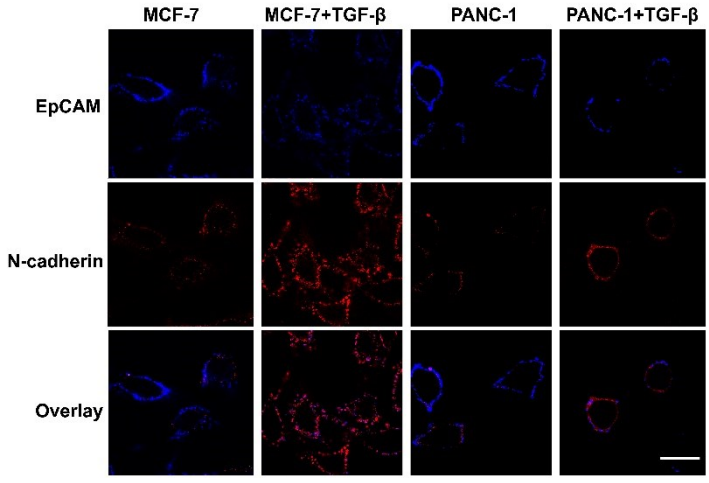

Figure S3. Confocal images of EpCAM and N-cadherin in MCF-7 and PANC-1 cells treated with or without TGF-β. Scale bars is 20 μm.

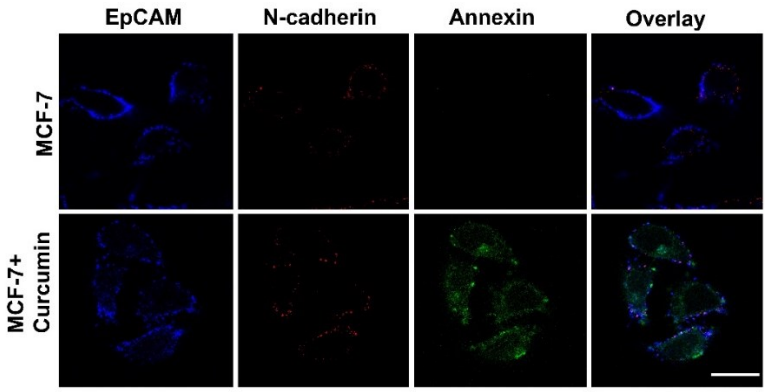

Figure S4. Confocal images of EpCAM, N-cadherin and Annexin V in MCF-7 cells. MCF-7 cells treated with or without curcumin. Scale bars is 20 μm.

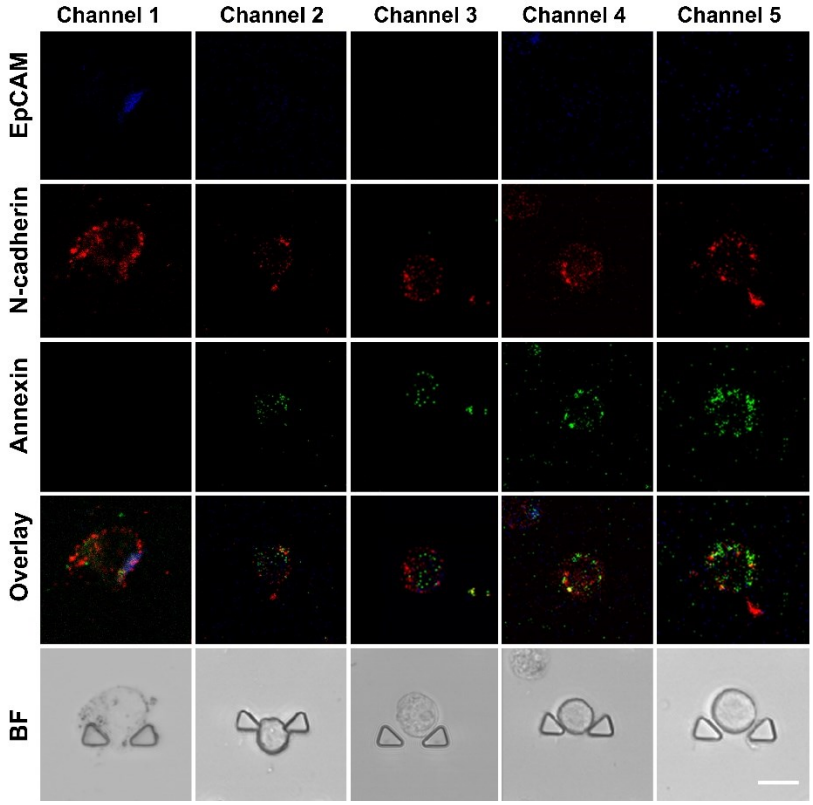

Figure S5. Confocal images of EpCAM, N-cadherin and Annexin V on single MCF-7 cells captured in DS-Chip. MCF-7 cells were pretreated with 10 ng/mL TGF-β to induce EMT, and were then treated with different concentrations of doxorubicin treatment. The scale bar represents 20 μm.

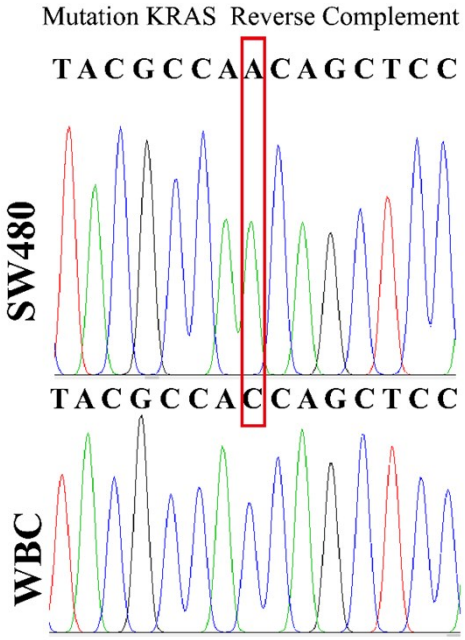

Figure S6. Sanger sequencing of the artificial blood samples. We spiked SW480 cells into blood samples as artificial patient sample, and we isolated tumor cells and performed genetic analysis of the frequently mutated KRAS gene. The artificial patient sample is positive for the 35G > T defect in codon 12, while the KRAS mutation was not detected in WBCs from the same blood sample.

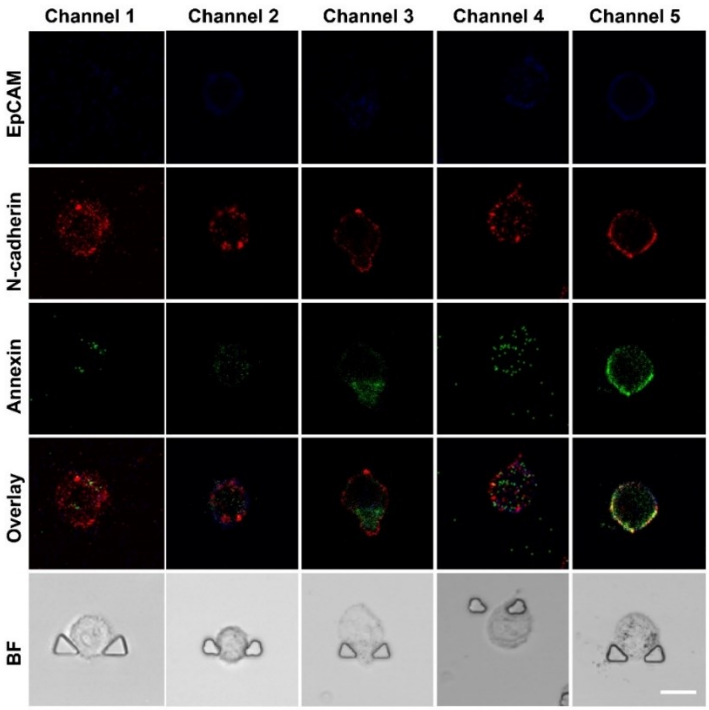

Figure S7. Confocal images of EpCAM, N-cadherin and Annexin V on single CTCs captured in DS-Chip. The images were obtained after the CTCs were stimulated on chip by different concentrations of curcumin.

3. Supplementary Table

Table S1. Clinical Characteristics of colorectal cancer patients

Patient ID	Age	Sex	Diagnosis	Stage
1	45	M	gastric cancer	III
2	49	F	gastric cancer	II
3	58	F	colon cancer	IV
4	63	M	colon cancer	IV
5	66	F	liver cancer	III

F, female; M, male; CRC, colorectal cancer.

4. References

1. H. Pei, L. Li, Y. Wang, R. Sheng, Y. Wang, S. Xie, L. Shui, H. Si and B. Tang, Anal. Chem., 2019, 91, 11078-11084.