Supporting Information

Nondestructive Capture, Release, and Detection of Circulating Tumor Cells with Cystamine Mediated Folic Acid Decorated Magnetic Nanospheres

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1. Methods

1.1 Cell culture

Hela cell, the human cervical cancer cell line, and HEK293T cell, human renal epithelial cell line, were grown in regular commercially available DMEM supplemented with 10% FBS and penicillin, streptomycin and L-glutamine and grown in a T75 flask at humidified atmosphere with 5% CO$_2$ at 37°C in a cell culture incubator. The culture medium was replaced once every three days with fresh medium.

The Hela cells at about 70% confluences were replaced fresh medium with no FA for serval hours and harvested using trypsinization with 1 mL of trypsin ethylene diamine tetraacetic acid (EDTA) solution at 37°C for 1-2 min to detach the cells from the flask. The trypsin was neutralized by adding 5 mL of fresh supplemented 10% FBS medium, and the harvested cells in the medium suspension were transferred into a 15 mL centrifuge tube and centrifuged at 1200 rpm for 2 min. Then harvested cells were stained with 10 μL Hoechst.33342 dye (1 mg/mL) per mL and incubated for 20 min, followed by PBS wash (three times) and eventual resuspension in RPMI1640 medium with no FA and serum.

1.2 Proteomics sample preparation of cultured CTCs

Cultured model CTCs were washed with cold PBS and collected by mechanical scrape. The cells were collected in a 1.5 ml eppendorf vials and lysis with 8 M urea in 100 mM Tris-HCl containing 1 mM sodium orthovanadate, phosphoSTOP (Roche) and complete mini EDTA free (Roche) in 4°C for 15 min with constant shaking. Then,
lysates were disrupted and homogenized by sonication for 5 min (5 s on, 25 s off) to
dissolve proteins and shear DNA. Cell debris were removed by ultracentrifugation
(12,000 g for 10 min at 4°C), and the supernatant was collected in ultrafiltration
centrifuge filters with cut-offs of 10 KD to depletion of detrimental low-molecular-
weight components. Then, adding Tris(2-carboxyethyl) phosphine (TCEP) to the filters
and incubated for 15 min at room temperature with final concentration at 20 mM to
reduce disulfide bonds. Followed by alkylation of free cysteines through adding 2-
Iodoacetamide (IAA) with final concentration of 50 mM and incubated at dark for 30
min. Finally, samples were washed according to the filter aided proteome preparation
(FASP) protocol [1] and digested with trypsin with the ratio of 1:50 (trypsin: protein)
in 50 mM ammonium bicarbonate buffer (pH 8.0). Digestion was performed overnight
at 37°C. After desalting process with Sep-Pak tC18 cartridges (Waters) [2], digested
sample was analyzed by LC-MS/MS Fusion Lumos.

1.3 LC-MS/MS analysis

MS experiments were performed on a nanoscale EASY-nLC 1200UHPLC system
(Thermo Fisher Scientific) connected to an Orbitrap Fusion Lumos equipped with a
nanoelectrospray source (Thermo Fisher Scientific). Mobile phase A contained 0.1%
formic acid (v/v) in water; mobile phase B contained 0.1% formic acid in 80%
acetonitrile (ACN). The peptides were dissolved in 0.1% formic acid (FA) and
separated on a RP-HPLC analytical column (75 um×25 cm) packed with 2 μm C18
beads (Thermo Fisher Scientific) using a linear gradient ranging from 7% to 28% ACN
in 90 min and followed by a linear increase to 44% B in 20 min at a flow rate of 300
nL/min. The Orbitrap Fusion Lumos acquired data in a data-dependent manner alternating between full-scan MS and MS2 scans. The spray voltage was set at 2.2 kV and the temperature of ion transfer capillary was 300°C. The MS spectra (350–1500 m/z) were collected with 120,000 resolution, AGC of $4 \times 10^5$, and 50 ms maximal injection time. Selected ions were sequentially fragmented in a 3 s cycle by HCD with 30% normalized collision energy, specified isolated windows 1.6m/z, 15,000 resolution. AGC of $5 \times 10^4$ and 30 ms maximal injection time were used. Dynamic exclusion was set to 40 s. Unassigned ions or those with a charge of 1+ and >7+ were rejected for MS/MS.
2. Results

Figure S1. (A) HRTEM (lattice fringe image) characterization of magnetic nanospheres; (B) Selected-area electron diffraction (SEAD) characterization of magnetic nanospheres.

Figure S2. MNs@Cys@PEG$_{2k}$-FA nanoprobes were collected with small magnet for 0 min and 2 min, respectively. MNs@Cys@PEG$_{2k}$-FA were recovered within 2 min.
Figure S3. Energy-dispersive spectroscopy (EDS) characterization of MNs@Cys.

Figure S4. (A) Hydrodynamic diameter distribution of MNs@Cys@PEG\(_{2k}\)-FA conjugates in PBS. (B) Diameter distribution of MNs@Cys@PEG\(_{2k}\)-FA conjugates which were stored over 6 months in PBS.
**Figure S5.** Image of CTCs capture with MNs@Cys@PEG\(_{2k}\)-FA (Top) and magnetic captured CTCs after DTT treatment (bottom).

**Figure S6.** MTT assay. The cells were incubated with MNs@Cys@PEG\(_{2k}\)-FA (0.1 mg/mL) for 15 min, DTT (50 mM) for 30 min, and MNs@Cys@PEG\(_{2k}\)-FA (0.1
mg/mL) for 15 min followed by DTT (50 mM), respectively.

**Figure S7.** Released CTCs were cultured *in vitro* for 24 h, 48 h and 72 h, respectively.
Figure S8. FRs level (*FOLR1* transcriptional level) in cholangio carcinoma (CHOL), glioblastoma multiforme (GBM), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), testicular germ cell tumors (TGCT), uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD) and thyroid carcinoma (THCA). Red color represents the tumor tissues (T) and the prey color represent the corresponding normal tissues (N). The data was obtained from visual database GEPIA.
References
