Electronic Supplementary Material (ESI) for Chemical Communications

Discrimination of colon cancer stem cells using noncanonical amino acid

Xinrui Duan [a], Honglin Li [a], Hexin Chen [b] and Qian Wang*[a]

[a] Department of Chemistry and Biochemistry & Nanocenter
University of South Carolina, Columbia, SC 29208 (USA)
E-mail: WANG263@mailbox.sc.edu
[b] Department of Biology, University of South Carolina
Columbia, SC 29208 (USA)

Supplementary Information

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1. CSCs proportion in HCT-116

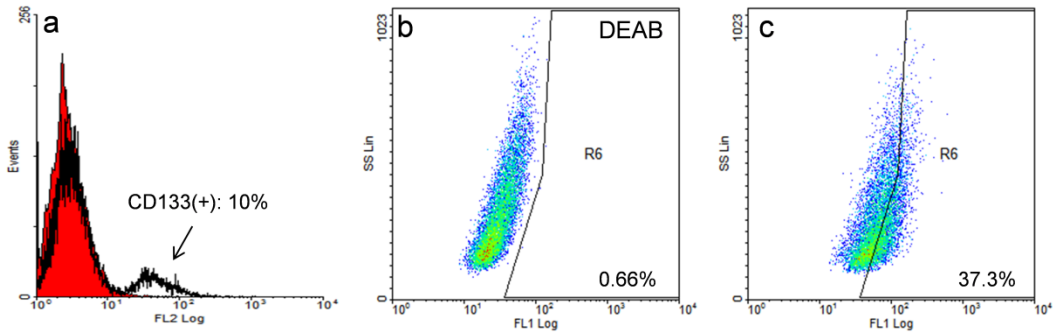

Figure S1 CSCs proportion in HCT-116 by using CD133 (a) and ALDEFLOUR assay (b, c).

2. Experimental section

Cell culture:

Colon cancer cell line HCT-116 was obtained from ATCC. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine in a 5% CO_2 humidified incubator at 37°C. All media components were obtained from Hyclone laboratories Inc.

HPG labeling:

Cells were seeded at estimated 50% coverage on 6-well plate the 12 hours before labeling. HPG Labeling is accounting to our previous reports. In brief, Methionine-free medium supplemented with 2 mM L-glutaMAX replaced the culture medium 30 min before adding 100 µM HPG. After HPG incubation, 100 µM Met was added to medium for chasing and incubated for 2 hours.

Fluorescence microscopy:

After HPG labeling, Cells were rinsed twice with PBS, fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 for 10 min. Cells were treated with blocking solution (1% BSA in PBS) at least 1 hour and rinsed twice with PBS. Cells were stained with 3-azido-7-hydroxycurmarin in the presence of 200 µM $CuSO_4$, 400µM TBTA, and 500 µM Sodium ascorbate in PBS(pH 7.4). The fluorescence was visualized under microscope (Olympus IX81) with DAPI filter set. Mercury lamp was used as a light source.

FACS and Flow cytometry:

FACS Cells were detached by Accutase (Invitrogen Inc). CD133 and ALDH1 enzyme activity was tested by using PE conjugated Anti-CD133 (Miltenyi Biotec) and ALDEFLOUR assay kit (STEMCELL Technologies, BC, Canada) according to manufacturer's manual. Each sample was filtered through a 40 µm Cell Strainers (Fisher Scientific Inc, GA) before sorting. Cells were sorted on BD FACSAria II cell sorter at 4 °C.

Flow cytometry

In brief, after HPG labelling cells were detached by Accutase (Invitrogen Inc). cells were rinsed with PBS and stain with primary anti-CD133 antibody and secondary anti-mouse FITC antibody. Then cells were fixed with 4% paraformaldehyde and were permeabilized with 0.1% Triton X-100 for 15 min each. Cells were treated with blocking solution (100 mM glycine in PBS) at least 1 hour. Cells were stained with 2 μM azido-Cy5 in the presence of 50 μM $CuSO_4$, 50 μM L-histidine, 500 μM Sodium ascorbate in PBS (pH 7.4) [2]. Then samples were washed with PBS with 0.5% tween 20 three times. Each sample was filtered through a 40 μm Cell Strainers (Fisher Scientific Inc, GA) before analysis on Beckman Coulter FC500 Flow Cytometers.

3. Reference

1. K. E. Beatty, J. C. Liu, F. Xie, D. C. Dieterich, E. M. Schuman, Q. Wang and D. A. Tirrell, *Angew. Chem. Int. Ed.*, 2006, 45, 7364-7367.
2. D. C. Kennedy, C. S. McKay, M. C. B. Legault, D. C. Danielson, J. A. Blake, A. F. Pegoraro, A. Stolow, Z. Mester, and J. P. Pezacki, *J. Am. Chem. Soc.* 2011, 133, 17993-18001.