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Discrimination of colon cancer stem cells using noncanonical amino acid

Xinrui Duan^a, Honglin Li^a, Hexin Chen^b, and Qian Wang^a

Qian Wang: wang263@mailbox.sc.edu

^aDepartment of Chemistry and Biochemistry & Nanocenter, University of South Carolina, Columbia, SC 29208 (USA)

^bDepartment of Biology, University of South Carolina, Columbia, SC 29208 (USA)

Abstract

Cancer stem cells (CSCs) may responsible for tumor recurrence. Metabolic labelling of newly synthesized proteins with non-canonical amino acids allows us to discriminate CSCs in mixed population due to quiescent nature of these cells.

Cancer stem cells (CSCs) are rare population exist in primary tumor and cell lines. These cells can go to self-renewal or differentiation into all the cell types seen in bulk tumor.¹ CSCs are resistant to chemo and radio therapies thus might cause tumor recurrence. Better understanding of proteome of these cells might offer a promising way to identify marker and therapeutic target. At present, most of proteomic studies of CSCs focus on glioblastoma stem cells because they have a well-established *in vitro* adherence culture model,^{2–5} which not available in others cancer types.⁶ Otherwise, isolation of CSCs population via fluorescence activated cell sorting (FACS) from primary tumors or cell lines is necessary.^{7,8} Thus developing a labelling method for discrimination of CSCs in heterogeneous population meanwhile compatible with downstream proteomics analysis is highly demanded.

CSCs shares property of slow cycling with stem cells,⁹ which generally have an extended G0 (quiescent) phase, and rate of de novo synthesis of protein is lower than other phases.¹⁰ Because of the lower rate of cellular protein synthesis in CSCs, using pulse-labeling to label the newly synthesized proteins in mixed population, non-CSCs will be labelled faster than CSCs. Conventional pulse-labeling method uses radioactive ³⁵S-methionine.¹¹ Recently, a large number of noncanonical amino acids (ncAAs) have been incorporated into cells site-specifically or residue-specifically,^{12,13} and served as powerful tools for protein engineering and tagging.^{14–23} As one of the most widely used methods, methionine analogues with side chains containing azide and alkyne groups have been employed as substrates for the natural translational machinery of cells.^{24,25} Compare to radioactive ³⁵S-methionine, these analogues can undergo bioorthogonal ligation reaction, which allows further introduce of additional functionalities for downstream proteomics analysis of proteome.²² For example,

Correspondence to: Qian Wang, wang263@mailbox.sc.edu.

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we previously have shown that coumarin based fluorogenic dye and reactive methionine analogue homopropargylglycine (HPG) could be used to investigate the spatial and temporal features of protein synthesis in *E coli* and mammalian cells by cell imaging.^{21,23}

In this proof of concept study, as shown in Scheme 1, metabolic incorporation of HPG incorporation has been used to distinguish CSCs and non-CSCs in a heterogenous population by controlling incorporation time of HPG. Cell staining with fluorescent dye was used to monitor cell population changes via flow cytometry. By using 3-azido-7-hydroxycoumarin and azido cyanine5 to react with the alkyne group in HPG through copper (I) catalyzed alkyne-azide cycloaddition (CuAAC) reaction,^{26,27} the successful incorporation of HPG in model cell line HCT-116 was confirmed by fluorescent microscopy and flow cytometry. Non-CSCs show distinct high fluorescence intensity than CSCs, which is due to CSCs have lower rate of protein synthesis. Co-staining HPG with CSCs marker CD133 further confirmed the population with low HPG incorporation is CSCs population. For living cell applications, the potential cytotoxicity of copper is a major concern. The Cu(II)-bis-L-histidine complex used in our study has been shown low cytotoxicities.²⁸ In addition, we are seeking the possibility of new copper-free reaction conditions for living cells labelling.^{29,30}

First of all, we tested HPG incorporation in colon cancer cell line HCT-116 by fluorescence microscopy. It has been reported HCT-116 has CSCs subpopulations, which can be isolated by well-studied CSCs marker CD133 and aldehyde dehydrogenase (ALDH) enzyme activity.^{31,32} Incorporation of HPG in this model cell line was visualized by using 3-azido-7-hydroxycoumarin. The fluorescence of fluorophore in 3-azido-7-hydroxycoumarin is quenched by the azide group and will recover after the CuAAC reaction upon the formation of the triazole ring.^{26,33} This fluorogenic nature ensures low background and high signal/noise ratio of the detection.^{21,23,34–36} Compare to Met control, HPG treated cells emit bright fluorescence as we expected (Fig. 1). These images indicate HPG can be successfully metabolically incorporated in our model cell line HCT-116.

To determine the difference of metabolic incorporation of HPG in CSCs and non-CSCs population, these two populations were isolated by using combination of CD133 and ALDEFLUOR assay, which can detect ALDH enzyme activity in living cells.³⁷ In general, the ALDH⁺CD133⁺ cells are considered as the enriched CSCs population. As shown in Fig. 2, the ALDH⁺CD133⁺ (i.e. non-CSCs) population shows more fluorescent cells than the ALDH⁺CD133⁺ (i.e. CSCs) population. Based on the microscopy images, fluorescent cells have been counted and summed in both populations (Fig. 2g). Results indicate rate of metabolic incorporation of HPG in CSCs is much slower than non-CSCs.

Based on the above results, HPG positive population was determined using flow cytometry at different labeling time points. Cells growing at the log phase were used for the HPG labeling experiment in order to reduce the influence of the cell-cell contact and obtain appropriate amount of cells for down-stream studies. To achieve this, cells were seeded as single cell suspension 12 hours before labeling at a seeding density of estimated 50% coverage of culture surface. Fig. 3a shows an overlap between HPG (green line) and L-Met (red histogram) and HPG with protein synthesis inhibitor anisomycin¹⁶ (black line) treated cells, which indicate existence of HPG negative population (30%) in HCT-116. To confirm

this HPG negative population only related to protein synthesis rate of these cells, we doubled the HPG incubation time. As shown in Fig. 3b, HPG negative population decreased to less than 3%. According to our results (Fig. S1) and literature reports³¹, CSCs proportion in HCT-116 should be around 10% to 30%, which are evaluated by CD133 antibody staining or ALDEFLUOR assay. Our results indicate that by simply changing the incubation time, we can label whole population (4 hours incubation) or only non-CSCs (2 hours incubation). To further confirm HPG negative population (after 2 hours incubation) is the CSCs, we conducted the co-staining of HPG and the CSCs marker CD133. Fig. 4 shows CD133 positive population only overlaps with HPG negative population, suggesting the CSCs population only co-exists in the HPG negative population.

In conclusion, bioorthogonal CuAAC reaction (a typical click chemistry) and homopropargylglycine incorporation has been used to distinguish CSCs and non-CSCs in a heterogenous population by controlling incorporation time of HPG. Because the slower protein synthesis rate of CSCs, non-CSCs will be labeled at 2 hour incubation and CSCs need more than 4 hours. These results revealed the possibility of the rapid protein profiling of CSCs in heterogenous population. Since our method utilizes the universal property of CSCs with a lower rate of cellular protein synthesis, rather than surface proteins or other biomarkers to differentiate CSCs and non-CSCs, it has the potential to directly apply to any cancer cell model including primary cancer cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Sarkar B, Dosch J, Simeone DM. Chem Rev. 2009; 109:3200–3208. [PubMed: 19522504]
2. He H, Nilsson CL, Emmett MR, Marshall AG, Kroes RA, Moskal JR, Ji Y, Colman H, Priebe W, Lang FF, Conrad CA. J Proteome Res. 2010; 9:2098–2108. [PubMed: 20199106]
3. He J, Liu Y, Zhu TS, Xie X, Costello MA, Talsma CE, Flack CG, Crowley JG, DiMeco F, Vescovi AL, Fan X, Lubman DM. J Proteome Res. 2010; 10:330–338. [PubMed: 21110520]
4. He J, Liu Y, Xie X, Zhu T, Soules M, DiMeco F, Vescovi AL, Fan X, Lubman DM. J Proteome Res. 2010; 9:2565–2572. [PubMed: 20235609]
5. Nilsson CL, Dillon R, Devakumar A, Shi SDH, Greig M, Rogers JC, Krastins B, Rosenblatt M, Kilmer G, Major M, Kaboord BJ, Sarracino D, Rezai T, Prakash A, Lopez M, Ji Y, Priebe W, Lang FF, Colman H, Conrad CA. J Proteome Res. 2009; 9:430–443. [PubMed: 19899826]
6. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, Lander ES. Cell. 2009; 138:645–659. [PubMed: 19682730]
7. Dai L, Li C, Shedden KA, Lee CJ, Li C, Quoc H, Simeone DM, Lubman DM. J Proteome Res. 2010; 9:3394–3402. [PubMed: 20486718]
8. Zhu J, He J, Liu Y, Simeone DM, Lubman DM. J Proteome Res. 2012; 11:2272–2281. [PubMed: 22335271]
9. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, Bernard L, Viale G, Pelicci PG, Di Fiore PP. Cell. 2010; 140:62–73. [PubMed: 20074520]

10. Robinson JH. *Exp Cell Res*. 1985; 161:231–241. [PubMed: 4054233]
11. Hu VW, Heikka DS. *FASEB J*. 2000; 14:448–454. [PubMed: 10698959]
12. Ngo JT, Tirrell DA. *Acc Chem Res*. 2011; 44:677–685. [PubMed: 21815659]
13. Liu CC, Schultz PG. *Ann Rev Biochem*. 2010; 79:413–444. [PubMed: 20307192]
14. Liu W, Brock A, Chen S, Chen S, Schultz PG. *Nat Methods*. 2007; 4:239–244. [PubMed: 17322890]
15. Liu DR, Schultz PG. *Proc Natl Acad Sci U S A*. 1999; 96:4780–4785. [PubMed: 10220370]
16. Noren CJ, Anthony-Cahill SJ, Griffith MC, Schultz PG. *Science*. 1989; 244:182–188. [PubMed: 2649980]
17. Wang YS, Fang X, Wallace AL, Wu B, Liu WR. *J Am Chem Soc*. 2012; 134:2950–2953. [PubMed: 22289053]
18. Wan W, Huang Y, Wang Z, Russell WK, Pai PJ, Russell DH, Liu WR. *Angew Chem Int Ed*. 2010; 49:3211–3214.
19. Ngo JT, Champion JA, Mahdavi A, Tanrikulu IC, Beatty KE, Connor RE, Yoo TH, Dieterich DC, Schuman EM, Tirrell DA. *Nat Chem Biol*. 2009; 5:715–717. [PubMed: 19668194]
20. Dieterich DC, Hodas JLL, Gouzer G, Shadrin IY, Ngo JT, Triller A, Tirrell DA, Schuman EM. *Nat Neurosci*. 2010; 13:897–905. [PubMed: 20543841]
21. Beatty KE, Liu JC, Xie F, Dieterich DC, Schuman EM, Wang Q, Tirrell DA. *Angew Chem Int Ed*. 2006; 45:7364–7367.
22. Dieterich DC, Link AJ, Graumann J, Tirrell DA, Schuman EM. *Proc Natl Acad Sci U S A*. 2006; 103:9482–9487. [PubMed: 16769897]
23. Beatty KE, Xie F, Wang Q, Tirrell DA. *J Am Chem Soc*. 2005; 127:14150–14151. [PubMed: 16218586]
24. Kiick KL, Saxon E, Tirrell DA, Bertozzi CR. *Proc Natl Acad Sci U S A*. 2002; 99:19–24. [PubMed: 11752401]
25. van Hest JCM, Kiick KL, Tirrell DA. *J Am Chem Soc*. 2000; 122:1282–1288.
26. Wang Q, Chan TR, Hilgraf R, Fokin VV, Sharpless KB, Finn MG. *J Am Chem Soc*. 2003; 125:3192–3193. [PubMed: 12630856]
27. Tornøe CW, Christensen C, Meldal M. *J Org Chem*. 2002; 67:3057–3064. [PubMed: 11975567]
28. Kennedy DC, McKay CS, Legault MCB, Danielson DC, Blake JA, Pegoraro AF, Stolorow A, Mester Z, Pezacki JP. *J Am Chem Soc*. 2011; 133:17993–18001. [PubMed: 21970470]
29. Sletten EM, Bertozzi CR. *Acc Chem Res*. 2011; 44:666–676. [PubMed: 21838330]
30. Clorissa YC, Washington-Hughes L, Duan Xinrui, Cai Li, Lee L Andrew, Wang Qian. *Mol Pharm*. 2012 Submitted.
31. Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, Li C, Wang LP, Roby KF, Orsulic S, Connolly DC, Zhang Y, Montone K, Bützow R, Coukos G, Zhang L. *PLoS ONE*. 2010; 5:e10277. [PubMed: 20422001]
32. Cui X, Oonishi K, Tsujii H, Yasuda T, Matsumoto Y, Furusawa Y, Akashi M, Kamada T, Okayasu R. *Cancer Res*. 2011; 71:3676–3687. [PubMed: 21454414]
33. Sivakumar K, Xie F, Cash BM, Long S, Barnhill HN, Wang Q. *Org Lett*. 2004; 6:4603–4606. [PubMed: 15548086]
34. Le Droumaguet C, Wang C, Wang Q. *Chem Soc Rev*. 2010; 39:1233–1239. [PubMed: 20309483]
35. Li K, Lee LA, Lu X, Wang Q. *Biotechniques*. 2010; 49:525–527. [PubMed: 20615206]
36. Varazo K, Droumaguet CL, Fullard K, Wang Q. *Tetrahedron Lett*. 2009; 50:7032–7034.
37. Storms RW, Trujillo AP, Springer JB, Shah L, Colvin OM, Ludeman SM, Smith C. *Proc Natl Acad Sci U S A*. 1999; 96:9118–9123. [PubMed: 10430905]

Fig. 1.

Fluorogenic labelling of protein in colon cancer cell line HCT-116 with 3-azido-7-hydroxycoumarin. Imaging of HCT-116 cells pulse-labelled in media containing 100 μ M HPG (top) or 100 μ M Met (bottom). Blue represents coumarin and red represents propidium iodide (PI) which stains cell nuclei. Scale bar is 50 μ m.

Fig. 2.

Fluorogenic labelling of ALDH⁺CD133⁺ (CSCs) and ALDH⁻CD133⁻ (non-CSCs) subpopulations in HCT-116 with 3-azido-7-hydroxycoumarin. CSCs population is isolated based on high ALDH enzyme activity and surface marker CD133. Double positive ALDH⁺CD133⁺ and negative ALDH⁻CD133⁻ are considered as CSCs and non-CSCs, respectively. The top panels show fluorescence images of CSCs (a–c) and non-CSCs (d–f). Proportion of HPG positive cells in two populations represents at bottom (g). Total more than 300 cells have been counted in both populations. Cells were treated with 100 μ M HPG for 4 hours and L-methionine treated cells were used as control. Scale bar is 100 μ m.

Fig. 3.

Flow cytometry histogram of HPG labelling in HCT-116 Cells. Cells were treated with 100 μ M HPG (green line) for (a) 2 or (b) 4 hours. L-Met (red histogram) and HPG with protein synthesis inhibitor (black line) treated cells were used as control. Azide-Cy5 was used to label HPG.

Fig. 4.

Flow cytometry diagram of co-staining of HPG labelling (x-axis, FL4) and CD133 (y-axis, FL1) in HCT-116 Cells. Cells were treated with 100 μ M HPG for 2 hours. HPG positive population is gated by using L-Met and HPG with protein synthesis inhibitor as control. Azide-Cy5 was used to label HPG.

Scheme 1.

a) Schematic representation of discrimination of colon cancer stem cells using noncanonical amino acid. **b)** Cu(I) catalysed alkyne-azide cycloaddition (CuAAC) reaction between HPG with 3-azido-7-hydroxycoumarin and azido cyanine5 (Cy5-Azide).