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Impacts of CD44 knockdown in cancer cells on tumor and host metabolic systems revealed by quantitative imaging mass spectrometry

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ABSTRACT

CD44 expressed in cancer cells was shown to stabilize cystine transporter (xCT) that uptakes cystine and excretes glutamate to supply cysteine as a substrate for reduced glutathione (GSH) for survival. While targeting CD44 serves as a potentially therapeutic stratagem to attack cancer growth and chemoresistance, the impact of CD44 targeting in cancer cells on metabolic systems of tumors and host tissues *in vivo* remains to be fully determined. This study aimed to reveal effects of CD44 silencing on alterations in energy metabolism and sulfur-containing metabolites *in vitro* and *in vivo* using capillary electrophoresis-mass spectrometry and quantitative imaging mass spectrometry (Q-IMS), respectively. In an experimental model of xenograft transplantation of human colon cancer HCT116 cells in superimmunodeficient NOG mice, snap-frozen liver tissues containing metastatic tumors were examined by Q-IMS. As reported previously, short hairpin CD44 RNA interference (shCD44) in cancer cells caused significant regression of tumor growth in the host liver. Under these circumstances, the CD44 knockdown suppressed polyamines, GSH and energy charges not only in metastatic tumors but also in the host liver. In culture, HCT116 cells treated with shCD44 decreased total amounts of methionine-pool metabolites including spermidine and spermine, and reactive cysteine persulfides, suggesting roles of these metabolites for cancer growth. Collectively, these results suggest that CD44 expressed in cancer accounts for a key regulator of metabolic interplay between tumor and the host tissue.

1. Introduction

Methionine is an essential amino acid provided by nutrition that starts remethylation cycle to synthesize S-adenosylmethionine (SAM), a donor metabolite necessary for transfer of methyl group to DNA and proteins, playing a critical role for epigenetic modification. SAM is also decarboxylated to yield polyamines including spermidine and spermine through methionine salvage pathway which plays a crucial role for regulation of cancer proliferation [1,2]. After donating methyl group, SAM is converted to S-adenosylhomocysteine (SAH) and then to homocysteine (Hcy) and methionine; these metabolites collectively form remethylation cycle, contributing to epigenetic regulation. A portion of Hcy that does not recycle to generate methionine is catalyzed by cystathionine β-synthase (CBS), the rate-limiting enzyme of transsulfuration pathway that generates cystathionine. This pathway plays a crucial role in ameliorating xenobiotic toxicity for cancer chemoresistance. Cystathionine γ-lyase (CSE), the 2nd enzyme of the pathway, catalyzes cystathionine to generate cysteine (Cys), providing a substrate for glutathione synthesis. Operation of methionine cycle and glutathione synthesis requires ATP.

Besides remethylation and transsulfuration pathways, the extracellular pathway dependent on CD44/xCT complex serves as an alternative mechanism to supply Cys to cells [3,4]. Through this mechanism in cancer cells, extracellular cystine (Cys-Cys) has been thought to enter cells to provide Cys for glutathione synthesis, so far as CD44 knockdown breaks down xCT stabilization and suppresses the entry of cystine and reduces GSH contents in cancer cells [4]. Previous studies revealed that Cys-Cys serves as a substrate for

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Table 1
Lists of metabolites with their theoretical and actual *m/z* values and MS^2 ion species in positive and negative ion mode.

Positive ion mode (Matrix: DHB)				
Compound name	Chemical formula	Monoisotopic molecular weight		Fragments observed in MS^2
		Theoretical	Actual	
		m/z	*m/z*	
SAM	$C_{15}H_{23}N_6O_5S$	399.145	399.141	250.093, 298.096, 136.064
SAH	$C_{14}H_{20}N_6O_5S$	385.129	385.126	250.087, 136.063, 134.027
Spermidine	$C_7H_{19}N_3$	146.166	146.155	129.027, 72.058, 112.088
Spermine	$C_{10}H_{26}N_4$	203.224	203.212	129.139,112.104, 84.082
Adenosine	$C_{10}H_{13}N_5O_4$	268.105	268.086	136.059
Negative ion mode (Matrix: 9AA)				
Compound name	Chemical formula	Monoisotopic molecular weight		Fragments observed in MS^2
		Theoretical	Actual	
		m/z	*m/z*	
UDP-HexNAc	$C_{17}H_{27}N_3O_{17}P_2$	606.074	606.068	385.031, 403.030, 282.039, 272.954, 323.046, 305.018, 362.055
GSH	$C_{10}H_{17}N_3O_6S$	306.076	306.067	254.087, 179,050, 272.072, 143,048, 159,932, 210.092, 128.030, 171.032
GSSG	$C_{20}H_{32}N_6O_{12}S_2$	611.144	611.137	306.071, 272.086, 338.058, 254.079, 482.103, 304.058, 288.058, 593.121
GSO_3^-	$C_{10}H_{17}N_3O_9S$	354.061	354.038	179.036, 336.099, 225.006, 210.075, 135.050, 254.062, 193.063, 143.031, 261.002
$GSSO_3^-$	$C_{10}H_{17}N_3O_9S_2$	386.033	386.008	306.058, 179.036, 254.062, 272.071, 288.049, 368.000, 160.000, 143.040, 210.076, 194.050
ATP	$C_{10}H_{16}N_5O_{13}P_3$	505.988	505.986	408.046, 272.955, 158.924, 176.934
ADP	$C_{10}H_{15}N_5O_{10}P_2$	426.022	426.032	328.070, 134.048
AMP	$C_{10}H_{14}N_5O_7P$	346.055	346.094	210.998, 149.988, 192.991, 134.041
Gln	$C_5H_{10}N_2O_3$	145.061	145.060	127.053, 109.042
Glu	$C_5H_9NO_4$	146.045	146.047	128.022, 102.046
Taurine	$C_2H_7NO_3S$	124.007	124.010	79.958, 106.978
Asp	$C_4H_7NO_4$	132.030	132.031	88.038, 115.013
Malate	$C_4H_6O_5$	133.014	133.015	115.006, 71.018

SAM: S-Adenosyl-L-methionine, SAH: S-Adenolsyl-L-homocysteine, UDP-HexNAc: UDP-N-acetyl hexosamine, GSH: Glutathione, reduced form, GSSG: Glutathione oxidized form, GSO_3^-: Glutathione sulfonate, $GSSO_3^-$: Glutathione S-sulfonate.

CBS and CSE to generate cysteine hydropersulfide (Cys-SSH) as a primary product of these enzymes. This reactive persulfide can react with reduced glutathione (GSH) to form glutathione hydropersulfide (GSSH) and other derivatives including cysteine-glutathione disulfide (Cys-SSG) and hydrogen sulfide anion (HS^-) and other polysulfide derivatives of thiol-containing peptides and proteins [5]. Compared with glutathione and hydrogen sulfide, Cys-SSH derivatives were superior nucleophiles and reductants and capable of detoxifying nucleophiles [5], benefiting amelioration of oxidative stress in cancer cells.

Since methionine and cysteine might be derived from host tissues during cancer development, it is not unreasonable to hypothesize that CD44 targeting by RNA interference or small molecular reagents does not only contribute to cancer regression but also alter metabolic systems of the host tissues. We have recently developed quantitative imaging mass spectrometry (Q-IMS) as a novel method to collect quantitative information of many metabolites in tumor-bearing tissues. Using this technique combined with super-immunodeficient mice for xenograft transplantation of human-derived cancer cells, the current study aimed to examine influence of CD44 knockdown in human-derived colon cancer HCT116 cells on metabolic systems in metastatic tumors and the liver as a host tissue. The results showed that the CD44 knockdown suppressed polyamines, GSH and energy charges not only in metastatic tumors but also in the host liver, suggesting that CD44 in cancer cells support the metabolic interplay between tumors and the host tissue, benefiting cancer proliferation and survival.

2. Materials and methods

2.1. Stable CD44 interference with shRNA

Using expression vectors encoding a shRNA specific for human CD44 mRNA or a scrambled shRNA obtained from Origene Technologies (Rockville, MD), we introduced it into HCT 116 cells by transfection with Lipofectamine 2000 [4]. The sequence of shRNA used for CD44 knockdown (shCD44) throughout all studies was 5'-GCTGACCTCTGCAAGGCTTTCAATAGCAC-3'. The control non-targeting shRNA sequence was 5'-GCACTACCAGAGCTAACTCAGATAGTACT-3', designated as control shRNA unless otherwise mentioned.

2.2. Human-derived cancer xenografts in livers of superimmunodeficient mice

All animal experiments were carried out in accordance with the guidelines of Experimental Animal Committee of Keio University School of Medicine. Human colon cancer (HCT116) cells were transfected stably with non-target control shRNA or with shCD44. The cells were injected into the spleen of superimmunodeficient NOG (NOD/SCID/IL-2Rγ^{null}) mice as described previously [6–8]. In brief, HCT116 cells were injected into the spleen of male NOG mice aged at 11~14 weeks at 1×10^6 cells/mouse. Two weeks after transplantation, liver lobules of the mice fasted for 17 hours were excised under sevoflurane anesthesia and snap-frozen with liquid nitrogen. To examine tumor growth *in vivo*, 5-μm thickness cryosections were stained with hematoxylin and eosin (H&E), serving as

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