

RESEARCH ARTICLE

HIF-1 α and GLUT1 Gene Expression is Associated with Chemoresistance of Acute Myeloid Leukemia

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Abstract

Aims: Much evidence suggests that increased glucose metabolism in tumor cells might contribute to the development of acquired chemoresistance. However, the molecular mechanisms are not fully clear. Therefore, we investigated a possible correlation of mRNA expression of HIF-1 α and GLUT1 with chemoresistance in acute myeloid leukemia (AML). **Methods:** Bone marrow samples were obtained from newly diagnosed and relapsed AML (M3 exclusion) cases. RNA interference with short hairpin RNA (shRNA) was used to stably silence GLUT1 or HIF-1 α gene expression in an AML cell line and HIF-1 α and GLUT1 mRNA expression was measured by real-time quantitative polymerase chain reaction assay (qPCR). **Results:** High levels of HIF-1 α and GLUT1 were associated with poor responsiveness to chemotherapy in AML. Down-regulation of the expression of GLUT1 by RNA interference obviously sensitized drug-resistant HL-60/ADR cells to adriamycin (ADR) *in vitro*, comparable with RNA interference for the HIF-1 α gene. **Conclusions:** Our data revealed that over-expression of HIF-1 α and GLUT1 might play a role in the chemoresistance of AML. GLUT1 might be a potential target to reverse such drug resistance.

Keywords: GLUT1 - HIF-1 α - glucose metabolism - chemoresistance

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Introduction

The majority of tumors are known to have an altered metabolism compared their origin tissue, with high rates of glucose uptake and glycolysis, which meets the energy need for unlimited proliferation of tumor cells (Bayley et al., 2012; Wynn et al., 2012). Tumor cells increase glucose uptake and expression of glucose transporters to markedly enhance glycolysis (aerobic glycolysis; the Warburg effect) (Nakajima et al., 2013). The enhanced glucose metabolism is one of the essential phenotypes of malignant tumors, and participate in tumor progression, metastasis, and relapse (Chen, 2012). In the last decade, a growing body of evidence suggested that tumor increasing glucose metabolism might contribute to the development of acquired chemoresistance (Lu et al., 2008; Suh et al., 2011; Zhou et al., 2012; Tamada et al., 2012).

Hypoxia inducible factor 1 α (HIF-1 α) is a transcriptional factor that up-regulates the expression of specific isoforms of glucose transporter (GLUT), hexokinase (HK), phosphofructokinase (PFK)-1, PFK-2, aldolase (ALD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGAM), enolase (ENO), pyruvate kinase (PYK), and lactate dehydrogenase (LDH) (Robey et al., 2005). In consequence, the elevated expressions of these genes

increase glycolytic flux and glucose utility. HIF-1 α is commonly over-expressed in many types of tumors (Comito et al., 2011; Huang et al., 2012). High levels of constitutive HIF-1 α expression in primary cancer samples have been associated with chemotherapy resistance in multiple solid tumor types (Patiar et al., 2006). Recently, it was shown that leukemic blasts in the bone marrow of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) also over-expressed HIF-1 α (Hulleman et al., 2009; Takubo et al., 2010; Deeb et al., 2011; Matsunaga et al., 2012).

GLUT is one of the main controlling steps of the glycolytic flux in cells (Moreno-Sánchez et al., 2007). Changes in the isoform pattern of GLUT expression occur in several tumor cells compared with normal cells. GLUT1, an over-expressed isoform in several solid tumor cells, is associated with tumor progression and chemoresistance (Amann et al., 2009; Chung et al., 2009). Moreover, the higher expression of GLUT1 were demonstrated in ALL cells (Manel et al., 2005), which produce the high levels of lactate and are sensitive to the glycolysis inhibitor 2-deoxy-D-glucose (2DG) (Hulleman et al., 2009). This provides an empirical evidence that acute leukemic cells might have a similar metabolic profile with solid tumors.

Drug resistance has been associated with the high levels

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of glycolysis in solid tumor. Resistance to glucocorticoids resulted in treatment failure in ALL was associated with increased glucose consumption and inhibition of glycolysis sensitized prednisolone-resistant ALL cell lines to glucocorticoids (Hulleman et al., 2009; Buentke et al., 2011). High level of glycolysis was also associated with resistance to apoptosis induction by a combination of ATRA and ATO in AML blasts (Herst et al., 2011). Several mechanisms for the enhanced glycolysis in tumor cells have been proposed. Among these mechanisms, HIF-1 α and GLUT1 play the important roles in genetic regulation of glycolysis and altered glucose metabolism in tumor cells. However, the exact mechanisms are not fully clear. In this study, we investigated a possible correlation of the expressions of HIF-1 α and GLUT1 with chemoresistance in AML cells.

Materials and Methods

Patient samples

Bone marrow (BM) were obtained from newly diagnosed (n=90) and relapsed (n=18) patients with AML (M3 exclusion), after informed consent, in our hospitals between October 2010 and November 2011. All samples contained at least 80% leukemic cells. Within 24 hours after sampling, mononuclear cells from bone marrow samples were obtained after red blood cell lysis (Boster Ltd, Wuhan, China) of the lower fraction after density gradient centrifugation. Isolated mononuclear cells were washed twice by phosphate-buffered saline (PBS), and stored in liquid nitrogen until further use. The study was performed in accordance with the modified Helsinki Declaration, and the protocol was approved by our ethical review boards before study initiation. Informed consent was obtained from the patients and healthy volunteers.

Cell lines and cell culture

Human myeloid leukemia cell line HL-60 and its adriamycin (ADR)-resistant subline HL-60/ADR were obtained from laboratory of hematology, Nanfang Hospital of South medical University. Human Lenti-X 293T cell line and fibrosarcoma HT-1080 cell line were obtained from central laboratory, Nanfang Hospital of South medical University. These cell lines were cultured at 37°C in a 5% humidified atmosphere in Dulbecco modified Eagle medium (DMEM; Lenti-X 293T and HT-1080) or RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco BRL, MD, USA), penicillin (100 units/mL), streptomycin (100 μ g/mL) and 2mM glutamine. Before each experiment, HL-60/ADR cells were treated with 1 μ mol/ml ADR at least for 1 week and then cultured for 10 days without ADR exposure (Chen et al., 2005).

RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

Total RNA from cell lines and primary mononuclear cell samples were extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. The qPCR was performed using a SYBR Green reaction (TaKaRa, Tokyo, Japan) kit and a PRISM 7500 real time PCR detection system (ABI,

USA). The expression levels of HIF-1 α and GLUT1 were analyzed relative to the levels of β -actin gene transcript. Sequences of primers were as follows: HIF-1 α : forward primers, 5'- ACAGCCTCACCAAACAGAGCAGG-3' and reverse primers, 5'- CGCTTTCTCTGAGCATTCTG CAAAGC-3'; GLUT1: forward primers, 5'- GCGGGTT GTGCCATACTCATGACC-3' and reverse primers, 5'- AGGCCACAAAGCCAAAGATGGCC-3'; β -actin: forward primers, 5'- TGGCACCCAGCACAATGAA-3' and reverse primers 5'- CTAAGTCATAGTCCGCCTAG AAGCA-3'. The reaction mixture for each direct qPCR was performed using 10 μ l of SYBR Premix Ex, 0.8 μ l (0.4 μ M) of each primer and 2 μ l cDNA, and in a total reaction volume of 20 μ l. qPCR reaction condition included pre-denaturation at 95 °C for 30 sec, followed by 40 cycles of denaturation at 95 °C for 3 sec and annealing at 60 °C for 30 sec. Three independent experiments were performed on the cells in independent cultures at 3 different times.

Western blot analysis

Cells (2 \times 10⁶) were washed twice in ice-cold PBS and then incubated with 300 μ l of lysis buffer [1% Triton X-100, 0.1% SDS, 50mM Tris (pH 8.0), 150mM NaCl, 1mM phenylmethylsulfonyl fluoride, 0.1mM NaVO₄, 0.1mM benzamidine, 5 μ g/ml leupeptin, and 5 μ g/ml aprotinin] for 10 min on ice. Whole cell lysates were clarified by centrifugation at 15,000 rpm for 15 min at 4°C. The protein concentration was determined by using the Bradford protein assay (Bio-Rad Laboratory, Richmond, CA, USA) with bovine serum albumin as a standard protein. Thirty micrograms of protein was separated by electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel (10% gel). After electrophoresis, proteins were transferred to nitrocellulose membrane. After 1 hour incubation in a blocking solution (5% non-fat dry milk in PBS-0.5% Tween-20), the membrane was blotted with the anti-HIF-1 α monoclonal antibody (1:200; Santa Cruz, California, CA, USA), anti-GLUT1 monoclonal antibody (1:200; Sigma, St. Louis, MO, USA) and anti- β -actin monoclonal antibody (1:1,000; Sigma, St. Louis, MO, USA) overnight at 4°C. After washed, the blot was incubated with the secondary antibodies (1:1000 dilution) for 1 hour at room temperature. The immunoreactive bands were visualized with the enhanced chemiluminescence (ECL) reagent according to the manufacturer's instructions. All the tests were repeated three times.

Plasmid construction and production of lentiviral vectors

For knock-down of GLUT1 or HIF-1 α genes by RNA interference (RNAi), three pairs of short hairpin RNA (shRNA) sequences targeting HIF-1 α mRNA (NM_001530) or GLUT1 mRNA (NM_006516) were designed according to the Tuschl principles. BLAST research was performed to ensure that the shRNAs did not have significant sequence homology with other genes. We then transfected it into HL-60/ADR cells by the method of electroporation, and screened the optimal shRNA sequence by the efficiency of RNAi which was monitored at RNA level by qPCR and protein level by western blot, respectively. The

Table 1. The Clinical Features of AML Patients with Different Sensitivities to Chemotherapy

Characteristics	CR		PR		NR		p
	Mean	N (%)	Mean	N (%)	Mean	N (%)	
Total		64 (59.3)		16 (14.8)		28 (25.9)	
Age	43.2		44.6		36.3		0.42
<19		2 (3.1)		0		2 (7.1)	
19~60		50 (78.1)		14 (87.5)		24 (85.7)	
>60		12 (18.8)		2 (12.5)		2 (7.1)	
Sex							0.95
Male		18 (56.3)		10 (62.5)		16 (57.1)	
Femal		14 (43.8)		6 (37.5)		12 (42.9)	
WBC ($\times 10^9/L$)	58.2		47		108.8		0.017
Hb (g/L)	74.1		80.4		69.6		0.459
PLT ($\times 10^9/L$)	67.9		63.1		108.5		0.056
Blasts (%)	86.9		87		89.5		0.359
Disease status							0.083
Newly diagnosed		58 (90.6)		14 (87.5)		18 (64.3)	
Relapsed		6 (9.4)		2 (12.5)		10 (35.7)	
Subtypes							0.573
M2		42 (65.6)		14 (87.5)		14 (50.0)	
M4		6 (9.4)		2 (12.5)		4 (14.3)	
M5		14 (21.9)		0		10 (35.7)	
M7		2 (3.1)		0		0	

selected sequences for HIF-1 α shRNAs, GLUT1 shRNAs and control siRNAs were: HIF-1 α shRNAs (sense: CCACCACUGAUGAAUUAATT, antisense: UUUAUUCACAGUGGUGGTT), GLUT1 shRNAs (sense: GGACCUCAAAUUUCAUGUTT, antisense: ACAUGAAAUUUGAGGUCCTT), and control siRNAs (sense: UUCUCCGAACGUGUCACGUTT, antisense: ACGUGACACGUUCGAGAATT).

Next, the Oligonucleotides were annealed in 10 mM Tris and 20 mM NaCl (pH 7.6) by heating to 94 °C for 3 min followed by slowly cooling to room temperature. Double stranded oligonucleotides were then inserted into the Plasmid vector pLVX-shRNA1 (Clontech Co., Ltd., USA) via BamHI and Hind III restriction sites. All clones were verified by DNA sequencing.

Lentivirus packaging, titer determination and construction of stable silencing cell lines

The recombinant pLVX-shRNA plasmid DNA and the lentiviral packaging mix (Clontech Co., Ltd., USA) were diluted in Xfect reaction buffer, mixed gently, and used to transfect 5 x 10⁶ Lenti-X 293T cells. Growth media was changed after 4 hours transfection and lentivirus containing supernatant was harvested 48 hours and 72 hours after transfection.

The supernatants were collected and centrifuged to harvest viruses. The titers were determined using Antibiotic Selection according to the protocol. Briefly, pLVX-shRNA-HIF-1 α , pLVX-shRNA-GLUT1 and pLVX-shRNA-NC lentiviral preparations were titrated in triplicate by serial dilutions of the concentrated vector stocks on 2x10⁵ HT-1080 cells in 6-well plates. After infection of 24 hours, the supernatants were removed and the cells were subjected to puromycin (2 μ g/ml) selection. The colonies formed within 5~10 days. Then, colonies were stained with 1% crystal violet solution, and counted. The titer value corresponded to the number of colonies generated by the highest dilution, multiplied by that

dilution factor.

HL-60/ADR cells were transduced with a multiplicity of infection (MOI) of approximately 1 when they reached 30~50% confluence on 6-well plates, and polybrene (final concentration, 4 μ g/mL) was added to the wells. Puromycin (final concentration 2 μ g/mL) was used to select stably transduced cells.

MTT assay

MTT assay was performed to assess the effect of HIF-1 α or GLUT1 on drug-resistant HL-60/ADR cells proliferation and responsiveness to ADR. The final concentrations of ADR ranged from 0.02 to 0.5 μ g/mL. Cells were seeded in 96-well plates at a density of 5x10⁴ cells/well. 10 μ l MTT dye (5 mg/ml; Sigma, USA) was added to each well at 37°C for 4 hours, then culture medium was removed. Subsequently, 200 μ l of DMSO was added and thoroughly mixed for 10 min. Spectrometric absorbance at 570 nm was measured by microplate reader. All groups were performed six times.

Statistical analysis

All the statistical analysis were performed with the statistical software package SPSS16.0. Student's t-test was used to determine the significance of the differences between the mean values. One-way ANOVA test was performed and post hoc multiple comparisons were made using LSD test under homogeneity of variance, while mean comparison of multi-group samples were analyzed by Dunnett's T3 method under homogeneity of variance. Results are presented as mean \pm standard error, $p < 0.05$ was regarded as statistically significant.

Results

Patient's characteristics

The clinical characteristics of study participants are shown in Table 1. Using the chemotherapy response

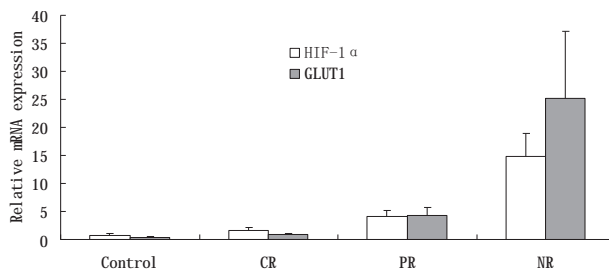


Figure 1. Expression of HIF-1α and GLUT1 mRNA in Blasts from AML Patients with Various Responsiveness to Chemotherapy. HIF-1α and GLUT1 mRNA expressions were detected by qPCR

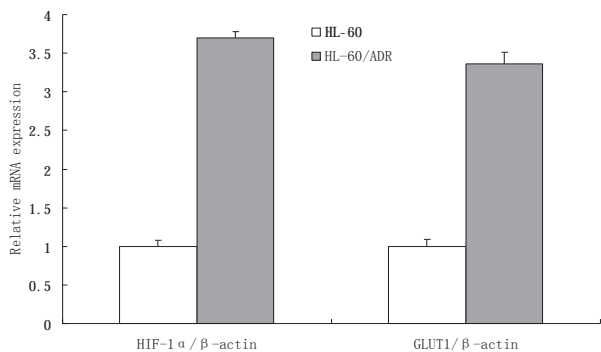


Figure 2. Expression of HIF-1α and GLUT1 mRNA in ADR-resistant and Sensitive AML Cell Lines. HIF-1α and GLUT1 mRNA expression levels in ADR-sensitive (HL-60) and ADR-resistant (HL-60/ADR) AML cell lines were detected by qPCR

criteria established by Chinese Chemotherapy Symposium of Leukemia in 1987 (Chinese Chemotherapy Symposium of Leukemia., 1988), chemotherapy response was assessed according to two induction cycles of chemotherapy. Response criteria: complete remission (CR) was defined as 5% or less blasts in a normocellular or hypercellular bone marrow with a normal peripheral count (granulocyte count more than $1 \times 10^9/L$ and a platelet count more than $100 \times 10^9/L$). Partial remission (PR) required similar criteria except for the presence of 6% to 20% marrow blasts. No remission (NR) was defined as failure to achieve CR or PR. These patients were divided into three groups (CR, PR, and NR) according to response to induction chemotherapy. In addition, 38 healthy donors were acted as negative control.

HIF-1 and GLUT1 mRNA expressions in primary AML cells and cell lines

As shown in Figure 1, the transcription level of these three genes (HIF-1α, GLUT1, and HK-II) involved in glucose metabolism showed a rising tendency in health control, CR group, PR group, and NR group ($p=0.000$). The level of HIF-1α mRNA in NR group (14.85 ± 4.16) was obviously higher than in healthy control (0.79 ± 0.22 , $p=0.000$), in CR group (1.67 ± 0.55 , $p=0.000$), and in PR group (4.02 ± 1.10 , $p=0.000$). GLUT1 mRNA expression was also markedly higher in NR group (25.17 ± 11.89) than healthy control (0.34 ± 0.11 , $p=0.000$), CR group (0.83 ± 0.24 , $p=0.000$), and PR group (4.34 ± 1.35 , $p=0.000$). Similarly, the expression of HK-II mRNA was significantly

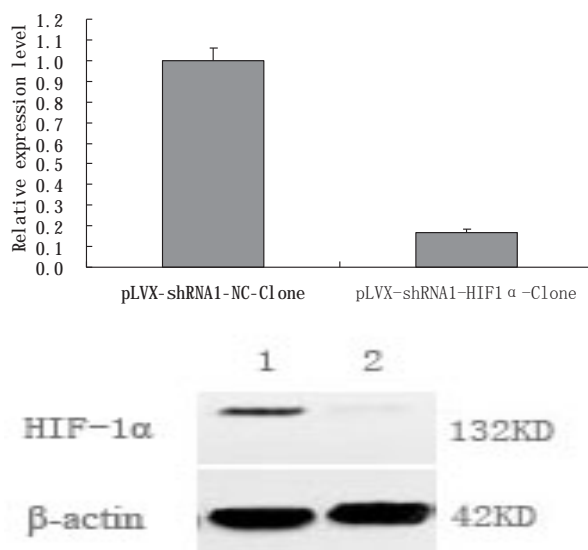


Figure 3. Effect of HIF-1α shRNA on the Expression of HIF-1α in HL-60/ADR Cells. A HIF-1α mRNA expression was suppressed in HL-60/ADR cells stably transfected by pLVX-shRNA- HIF-1α. B HIF-1α protein expression was suppressed in HL-60/ADR cells stably transfected by pLVX-shRNA- HIF-1α. (1) pLVX-shRNA-NC-clone, (2) pLVX-shRNA- HIF-1α-clone

up-regulated in NR group (17.47 ± 5.22) compared with healthy control (0.49 ± 0.20 , $p=0.000$), CR group (0.78 ± 0.39 , $p=0.000$), and PR group (11.61 ± 3.69 , $p=0.036$).

qPCR revealed that a rising tendency in mRNA expression of HIF-1α and GLUT1 in health control, CR group, PR group, and NR group ($p=0.000$). The transcription level of HIF-1α in NR group (14.85 ± 4.16) was obviously higher than in healthy control (0.79 ± 0.22 , $p=0.000$), in CR group (1.67 ± 0.55 , $p=0.000$), and in PR group (4.02 ± 1.10 , $p=0.000$). GLUT1 mRNA expression was also markedly higher in NR group (25.17 ± 11.89) than healthy control (0.34 ± 0.11 , $p=0.000$), CR group (0.83 ± 0.24 , $p=0.000$), and PR group (4.34 ± 1.35 , $p=0.000$) (Figure 1).

Moreover, we compared HIF-1α and GLUT1 expression levels in ADR-sensitive (HL-60) and ADR-resistant (HL-60/ADR) AML cell lines in vitro. The expression of HIF-1α and GLUT1 was considerably higher in HL-60/ADR cells than HL-60 cells (HIF-1α, 3.70 ± 0.084 vs. 1.00 ± 0.075 , $p=0.000$; GLUT1, 3.36 ± 0.149 vs. 1.0 ± 0.080 , $p=0.000$), respectively (Figure 2).

Inhibition of the glucose transport pathway by RNAi increases ADR-induced toxicity

HL-60/ADR cells are rather resistant to ADR-induced toxicity. The over-expression of HIF-1α and GLUT1 which play important roles in glucose uptake and utility has been described to be related to the resistant phenotype in AML. To demonstrate further that the glucose transport pathway is associated with ADR resistance in AML cells, HL-60/ADR cells were infected with lentiviral mediated-shRNA plasmids to stably silence HIF-1α or GLUT1 gene expression. The efficiency of RNA interference was monitored at the RNA level by qPCR and protein level by western blot. As shown in Figure 3, RNA interference

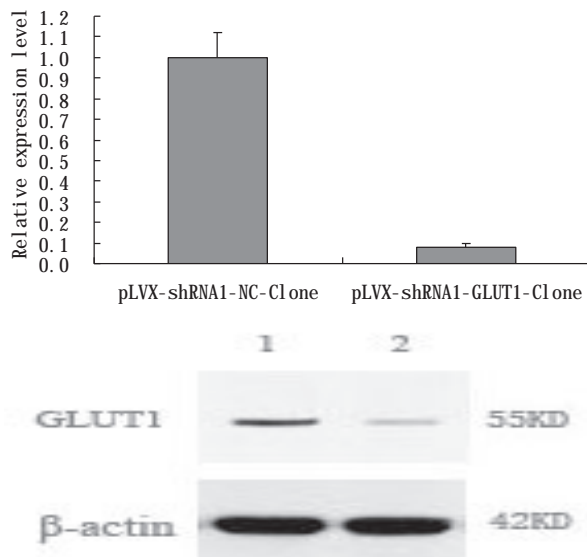


Figure 4. Effect of GLUT1 shRNA on the Expression of GLUT1 in HL-60/ADR Cells. A GLUT1 mRNA expression was suppressed in HL-60/ADR cells stably transfected by pLVX-shRNA- GLUT1. B GLUT1 protein expression was suppressed in HL-60/ADR cells stably transfected by pLVX-shRNA- GLUT1. (1) pLVX-shRNA-NC-clone, (2) pLVX-shRNA- GLUT1-clone

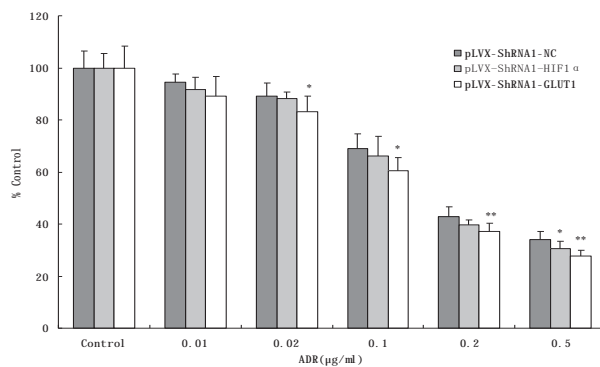


Figure 5. Inhibition of HIF-1 α or GLUT1 Expression by RNA Interference on ADR-induced Cytotoxicity. ADR-induced cytotoxicity of the inhibition of HIF-1 α or GLUT1 expression by RNA interference on HL-60/ADR cells was measured by MTT assay. Survival rate of HL-60/ADR cells infected with nonsilencing shRNA sequences was set at 100%. After treatment with ADR for 48 hours, GLUT1 gene silencing increased sensitivity to ADR in drug-resistant HL-60/ADR cell line (* $p < 0.05$ versus control shRNA, ** $p < 0.01$ versus control shRNA)

with the shRNA sequences targeting GLUT1 resulted in a reduction in gene expression of approximately 90% (Figure 3A) and protein expression of approximately 70% (Figure 3B). RNA interference for HIF-1 α gene decreased gene expression of approximately 80% (Figure 4A) and protein expression of approximately 90% (Figure 4B). Inhibition of GLUT1 by RNA interference increased ADR sensitivity in drug-resistant HL-60 cells, confirming the link between the over-expression of GLUT1 and chemoresistance in AML (Figure 5). knockdown of another gene that was differentially regulated in glucose transport, HIF-1 α , did not significantly affect ADR sensitivity in HL-60/ADR cells (Figure 5).

Discussion

Chemoresistance has emerged as an important clinical issue in the cancer treatment. Studies have revealed genetic and epigenetic alterations that cancer cells acquire to adapt to chemotherapeutic stress for survival purposes (Zhou et al., 2012). Such alterations include increases in drug efflux, enhanced drug inactivation, enhanced DNA damage repair, mutations of survival-related genes, deregulated growth factor signaling pathways, increases in anti-apoptotic gene expressions, and activation of intracellular survival signaling (Zhou et al., 2012). Although the enhanced glucose uptake and aerobic glycolysis in cancer cells is suspected to contribute to chemoresistance, but the precise mechanisms remain to be elucidated (Zhou et al., 2012).

It is found that 5-Fluorouracil (5-FU)-resistant colorectal cancer (CRC) cells obviously up-regulated the expressions of glycolytic enzymes, including GAPDH, pyruvate kinase M2 (PKM2), transketolase, and NADP (1)-dependent malic enzyme 1 (Papageorgis et al., 2011). In particular, the level of PKM2 showed an increasing tendency in both sera and tissues from CRC patients displaying no response to 5-FU-based chemotherapy, compared with that in complete or partial responders to 5-FU-based chemotherapy (Papageorgis et al., 2011). There is an evidence that the control of glucose-substrate flux is an important mechanism of the anti-proliferative action of imatinib because imatinib-resistant gastrointestinal stromal KIT-positive tumors reveal highly elevated glucose uptake in radiologic images (Kominsky et al., 2009). Similar results were also found in the imatinib-resistant K562-r and LAMA84-r cells who maintained a highly glycolytic metabolic phenotype with elevated glucose uptake and lactate production (Kominsky et al., 2009). The changes in glucose uptake and metabolism were accompanied by intracellular translocation of GLUT-1 from the plasma membrane into the intracellular fraction in sensitive cells treated with imatinib, whereas GLUT-1 remained located at the plasma membrane in LAMA84-r and K562-r cells (Kominsky et al., 2009). In addition, ALL cells resistant to glucocorticoids have been reported to aberrant change in the expression of glycolytic pathway-associated genes and increased glucose consumption compared with sensitive cells (Hulleman et al., 2009). Therefore, inhibition of glycolysis is suggested to be able to effectively target drug-resistant tumor cells with a high metabolic state.

As increased glycolysis plays an important role in chemoresistance of tumor cell, interest in glucose uptake by tumor cells has increased. In this study, GLUT1 mRNA expression in NR group showed significantly higher than in health control, in CR and PR group. This suggested that over-expression of GLUT1 may contribute to the development of chemoresistance in AML. Glucose is a major source of energy, and increased GLUT1 expression may indicate an increased utilization of energy, which in turn may correlate with poor prognosis (Amann et al., 2009). In the in vitro studies, 3-bromopyruvic acid (3-BrPA) as a glycolysis inhibitor significantly suppressed the proliferation of colon cancer cells with high GLUT1

gene expression, compared to those with low expression (Saigusa et al., 2012). Over-expression of GLUT1 gene conferred two to five fold higher drug resistance in SW620 and K562 to ADR (Cao et al., 2007). However, inhibition of glucose uptake by phloretin sensitized cancer cells to ADR for its anticancer activity and apoptosis to overcome drug resistance (Cao et al., 2007). Thus, we hypothesized that high GLUT1 expression led to increased glucose metabolism, and herewith, promoted drug resistance in tumor cells.

It has been described that GLUT1 expression is regulated by the transcription factor HIF-1 α (Amann et al., 2009). HIF-1 α is induced in response to stress and hypoxia. In cancer tissue, it may also be up-regulated under aerobic conditions. Of note, HIF-1 α was up-expressed in leukemic cells, especially in drug-resistant leukemic cells (Deeb et al., 2011; Kluza et al., 2011). Since GLUT1 expression was associated with chemoresistance in AML, it is suggested that GLUT1 expression in these leukemic cells is dependent on HIF-1 α expression. Much evidence has shown that hypoxia is closely associated with tumor resistance to anticancer drugs, and HIF-1 α is one of the major regulators contribute to the mechanisms (Song et al., 2006; Hao et al., 2008). As the most important transcription factor, HIF-1 plays important roles in the expression of many genes associated with glycolysis and antiapoptosis, which allow the cancer cells to survive and even induction of drug resistance (Hao et al., 2008). In the study, we found that HL-60/ADR cells up-regulated the levels of HIF-1 α and GLUT1 mRNA in response to ADR, suggesting that increased glucose uptake and metabolism change may account, at least in part, for the potentiation insensitivity in ADR-treated HL-60/ADR cells.

Pharmacological inhibition of glucose metabolism has been shown to exhibit promising anticancer activity in vitro and in vivo, alone or in combination with other therapeutic modalities (Pelicano et al., 2006). Comparing with inhibiting glucose metabolism in its entirety, inhibition of expression or functionality of GLUT1 or HIF-1 α may more specifically target those cells within the tumor that depend on a high rate of glucose uptake and glycolysis. Our study showed that disruption of the glucose uptake specifically affected ADR-resistant leukemia cells with high glycolytic activity, while no sensitizing effect was observed in cells already sensitive to ADR. Down-regulation of GLUT1 by use of RNA interference also modulated ADR sensitivity, confirming a direct link between chemoresistance and glucose metabolism. However, inhibition of HIF-1 α by RNA interference showed no obvious alteration in the sensitivity for ADR in HL-60/ADR cell lines in vitro. This indicated that ADR resistance in AML was not due to up-regulation of HIF-1 α , at least at the transcriptional level. Nevertheless, HIF-1 α played the pathophysiologic role in tumors (Hu et al., 2009). Inhibition of HIF-1 α function resulted in enhanced sensitivity to melphalan in myeloma cells. However, HIF-1 α knockdown could reverse hypoxia-related resistance to cisplatin and apoptotic resistance only in HT1080 cells, but had little effect on drug-resistant HT1080-6TG cells (Hao et al., 2008). Thus, HIF-1 α targeted therapy to reverse hypoxia-

related cisplatin resistance depends on normal p53 status. Changes of Bid expression levels under hypoxia might contribute in part to the differential response to HIF-1 α silencing in cells with different p53 status.

Cancer cells heavily rely on glucose transporters for glucose uptake to facilitate a high-rate glycolysis under hypoxia for their survival and drug resistance. Combination of glucose transporter inhibitors and chemotherapeutic drugs may provide a preferential novel therapeutic strategy to overcome drug resistance. In summary, our studies showed that over-expressions of HIF-1 α and GLUT1 may play important role in chemoresistance of AML. GLUT1 could be a potential target to reverse drug resistance in AML patients refractory to chemotherapy.

Acknowledgements

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References

- Amann T, Hellerbrand C (2009). GLUT1 as a therapeutic target in hepatocellular carcinoma. *Expert Opin Ther Targets*, **13**, 1411-27.
- Amann T, Maegdefrau U, Hartmann A, et al (2009). GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *Am J Pathol*, **174**, 1544-52.
- Bayley JP, Devilee P (2012). The Warburg effect in 2012. *Curr Opin Oncol*, **24**, 62-7.
- Buentke E, Nordström A, Lin H, et al (2011). Glucocorticoid-induced cell death is mediated through reduced glucose metabolism in lymphoid leukemia cells. *Blood Cancer J*, **1**, e31.
- Cao X, Fang L, Gibbs S, et al (2007). Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother Pharmacol*, **59**, 495-505.
- Chen CY, Jia JH, Zhang MX, et al (2005). Proteomic analysis on multi-drug resistant cells HL-60/DOX of acute myeloblastic leukemia. *Chin J Physiol*, **48**, 115-20.
- Chen EI (2012). Mitochondrial dysfunction and cancer metastasis. *J Bioenerg Biomembr*, **44**, 619-22.
- Chinese Chemotherapy Symposium of Leukemia (1988). The chemotherapy response criteria of acute leukemia. *Chin J Hematol*, **9**, 183-4.
- Chung FY, Huang MY, Yeh CS, et al (2009). GLUT1 gene is a potential hypoxic marker in colorectal cancer patients. *BMC Cancer*, **9**, 241.
- Comito G, Calvani M, Giannoni E, et al (2011). HIF-1 α stabilization by mitochondrial ROS promotes Met-dependent invasive growth and vasculogenic mimicry in melanoma cells. *Free Radic Biol Med*, **51**, 893-904.
- Deeb G, Vaughan MM, McInnis I, et al (2011). Hypoxia-inducible factor-1 α protein expression is associated with poor survival in normal karyotype adult acute myeloid leukemia. *Leuk Res*, **35**, 579-84.
- Deeb G, Vaughan MM, McInnis I, et al (2011). Hypoxia-inducible factor-1 α protein expression is associated with poor survival in normal karyotype adult acute myeloid leukemia. *Leuk Res*, **35**, 579-84.

- Hao J, Song X, Song B, et al (2008). Effects of lentivirus-mediated HIF-1 α knockdown on hypoxia-related cisplatin resistance and their dependence on p53 status in fibrosarcoma cells. *Cancer Gene Ther*, **15**, 449-55.
- Herst PM, Howman RA, Neeson PJ, et al (2011). The level of glycolytic metabolism in acute myeloid leukemia blasts at diagnosis is prognostic for clinical outcome. *J Leukoc Biol*, **89**, 51-5.
- Hu Y, Kirito K, Yoshida K, et al (2009). Inhibition of hypoxia-inducible factor-1 function enhances the sensitivity of multiple myeloma cells to melphalan. *Mol Cancer Ther*, **8**, 2329-38.
- Huang XD, Wang ZF, Dai LM, et al (2012). Microarray analysis of the hypoxia-induced gene expression profile in malignant C6 glioma cells. *Asian Pac J Cancer Prev*, **13**, 4793-9.
- Hulleman E, Kazemier KM, Holleman A, et al (2009). Inhibition of glycolysis modulates prednisolone resistance in acute lymphoblastic leukemia cells. *Blood*, **113**, 2014-21.
- Kluza J, Jendoubi M, Ballot C, et al (2011). Exploiting mitochondrial dysfunction for effective elimination of imatinib-resistant leukemic cells. *PLoS One*, **6**, e21924.
- Kominsky DJ, Klawitter J, Brown JL, et al (2009). Abnormalities in glucose uptake and metabolism in imatinib-resistant human BCR-ABL-positive cells. *Clin Cancer Res*, **15**, 3442-50.
- Lu CW, Lin SC, Chen KF, et al (2008). Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J Biol Chem*, **283**, 28106-14.
- Manel N, Battini JL, Sitbon M (2005). Human T cell leukemia virus envelope binding and virus entry are mediated by distinct domains of the glucose transporter GLUT1. *J Biol Chem*, **280**, 29025-9.
- Matsunaga T, Imataki O, Torii E, et al (2012). Elevated HIF-1 α expression of acute myelogenous leukemia stem cells in the endosteal hypoxic zone may be a cause of minimal residual disease in bone marrow after chemotherapy. *Leuk Res*, **36**, e122-4.
- Moreno-Sánchez R, Rodríguez-Enríquez S, Marín-Hernández A, et al (2007). Energy metabolism in tumor cells. *FEBS J*, **274**, 1393-418.
- Nakajima EC, Van Houten B (2013). Metabolic symbiosis in cancer: refocusing the Warburg lens. *Mol Carcinog*, **52**, 329-37.
- Papageorgis P, Cheng K, Ozturk S, et al (2011). Smad4 inactivation promotes malignancy and drug resistance of colon cancer. *Cancer Res*, **71**, 998-1008.
- Patiar S, Harris AL (2006). Role of hypoxia-inducible factor-1 α as a cancer therapy target. *Endocr Relat Cancer*, **13**, S61-75.
- Pelicano H, Martin DS, Xu RH, et al (2006). Glycolysis inhibition for anticancer treatment. *Oncogene*, **25**, 4633-46.
- Robey IF, Lien AD, Welsh SJ, et al (2005). Hypoxia-inducible factor-1 α and the glycolytic phenotype in tumors. *Neoplasia*, **7**, 324-30.
- Saigusa S, Toiyama Y, Tanaka K, et al (2012). Prognostic significance of glucose transporter-1 (GLUT1) gene expression in rectal cancer after preoperative chemoradiotherapy. *Surg Today*, **42**, 460-9.
- Song X, Liu X, Chi W, et al (2006). Hypoxia-induced resistance to cisplatin and doxorubicin in non-small cell lung cancer is inhibited by silencing of HIF-1 α gene. *Cancer Chemother Pharmacol*, **58**, 776-84.
- Suh DH, Kim MK, No JH, et al (2011). Metabolic approaches to overcoming chemoresistance in ovarian cancer. *Ann NY Acad Sci*, **1229**, 53-60.
- Takubo K, Goda N, Yamada W, et al (2010). Regulation of the HIF-1 α level is essential for hematopoietic stem cells. *Cell Stem Cell*, **7**, 391-402.
- Tamada M, Nagano O, Tateyama S, et al (2012). Modulation of glucose metabolism by CD44 contributes to antioxidant status and drug resistance in cancer cells. *Cancer Res*, **72**, 1438-48.
- Wynn ML, Merajver SD, Schnell S (2012). Unraveling the complex regulatory relationships between metabolism and signal transduction in cancer. *Adv Exp Med Biol*, **736**, 179-89.
- Zhou Y, Tozzi F, Chen J, et al (2012). Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells. *Cancer Res*, **72**, 304-14.
- Zhou Y, Tozzi F, Chen J, et al (2012). Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells. *Cancer Res*, **72**, 304-14.