Correlation and expression analysis of hypoxia-inducible factor 1α, glucose transporter 1 and lactate dehydrogenase 5 in human gastric cancer

LANG-SONG HAO[1], QI LIU[2], CHUAN TIAN[2], DONG-XING ZHANG[1], BO WANG[1], DONG-XU ZHOU[1], ZHAO-PENG LI[1] and ZHI-XIANG YUAN[3]

[1]Department of General Surgery, Guizhou Provincial People's Hospital, Guiyang Medical University, Guiyang, Guizhou 550002; [2]Graduate School of Surgery, Zunyi Medical University, Zunyi, Guizhou 563003; [3]Department of Pharmacy, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan 611130, P.R. China

Received May 25, 2018; Accepted April 15, 2019

DOI: 10.3892/ol.2019.10457

Abstract. The development and identification of novel potential targeting sites for intervention therapy are essential in the search for improved treatment methods for gastric cancer (GC). Previously, it has been reported that hypoxia inducible factor-1α (HIF-1α) is a potential target gene involved in the endogenous hypoxic response and bioenergetic metabolism of GC cells. In the present study, with the assumption of a close interplay among HIF-1α, glucose transporter 1 (GLUT1) and lactate dehydrogenase-5 (LDH-5), 85 patients with GC were recruited and the protein and gene expression levels of HIF-1α, GLUT1 and LDH-5 in tumor tissues were evaluated in order to assess clinical correlations and co-expression patterns, using Immunohistochemical staining and reverse transcription-quantitative polymerase chain reaction. The results demonstrated that the protein and gene expression levels of HIF-1α were significantly associated with the depth of invasion, nodal metastasis, clinical stage, differentiation and distant metastasis. Consistent with the protein expression results, the mRNA expression levels of the genes coding for GLUT1 and LDH-5 were clearly associated with tumor size, depth of invasion, distant metastasis, clinical stage and differentiation. Correlation analysis of HIF-1α with GLUT1 and LDH-5 at the protein and mRNA expression levels in gastric carcinoma indicated that HIF-1α expression was positively correlated with the expression of GLUT1 ($P<0.01$, $r=0.765$ for mRNA expression; $P<0.01$, $r=0.697$ for protein expression) and LDH-5 ($P<0.01$, $r=0.892$ for mRNA expression; $P<0.01$, $r=0.783$ for protein expression) at the mRNA and protein levels. Therefore, it may be concluded that HIF-1α, GLUT1 and LDH-5 are potential target genes involved in the endogenous tumor response to hypoxia and the inhibition of tumor energy metabolism, highlighting a novel therapeutic target for GC.

Correspondence to: Professor Lang-Song Hao, Department of General Surgery, Guizhou Provincial People's Hospital, Guiyang Medical University, 83 Zhongshan Eastern Road, Guiyang, Guizhou 550002, P.R. China
E-mail: hls616@163.com

Professor Zhi-Xiang Yuan, Department of Pharmacy, College of Veterinary Medicine, Sichuan Agricultural University, 211 Huimin Road, Shuangliu, Chengdu, Sichuan 611130, P.R. China
E-mail: zhixiang-yuan@hotmail.com

Key words: hypoxia inducible factor-1α, glucose transporter 1, lactate dehydrogenase-5, gastric cancer, correlation analysis

Introduction

Gastric cancer (GC) is commonly diagnosed and has been identified as the leading cause of cancer-associated mortality in China since 2010; it remains a major clinical challenge due to the limited treatment options and poor prognosis (1,2). The occurrence and development of GC are complex processes involving multiple genes and mechanisms. Numerous studies have been performed to investigate potential associated genes, with the aim of identifying the pathogenic mechanisms involved in GC, which may ultimately aid in improving diagnostic and treatment methods for the disease (3,4).

Hypoxia, which serves a vital role in carcinogenesis, is able to induce metabolic reprogramming and alterations associated with glucose metabolism and glucose transport, angiogenesis, invasion and metastasis in malignant cells, facilitating adaptation to anaerobic conditions by upregulation of target genes (5,6). Hypoxia not only induces tumor cell mutations, but also promotes the survival of malignant cell clones via hypoxia-mediated anti-apoptotic effects, which are associated with malignant tumor cell invasion, proliferation, and resistance to radiotherapy and chemotherapy (5). Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric transcription factor consisting of α (HIF-1α) and β (HIF-1β) subunits (7). HIF-1 is overexpressed and exhibits enhanced activity in the hypoxic microenvironment of tumors (8,9). Notably, the protein expression levels of HIF-1α and the binding activity of HIF-1β are upregulated during hypoxia. The overexpression

of HIF-1α has been identified in numerous types of cancer and precancerous lesions, but not in normal tissues or benign lesions (10-12). Additionally, HIF-1α may contribute to hypoxia-induced drug resistance, which is a major obstacle in the development of effective cancer therapy (13). Previously, clinical studies have revealed a significant association between the expression levels of HIF-1α and prognosis in GC (14-16). The role of HIF-1α in the initiation and progression of tumors has attracted increasing attention.

GC, as is the case for most solid types of cancer, produces energy via active glycolysis, regardless of whether the conditions are aerobic or anaerobic (17). The increased energy consumption of tumor cells requires higher levels of glucose transporters (GLUTs) to be present in the cell membranes for the transport of glucose. A previous study investigated the association between glucose transporter 1 (GLUT1) and various tumor types, and identified that the abnormal expression of GLUT1 and other relevant genes may be associated with the intensive glucose metabolism in malignant cells (18). Previous studies have demonstrated that the transcription of GLUT1 is enhanced by HIF-1α in other solid types of cancer under hypoxic conditions (19,20), and glycolysis is enhanced to compensate for the increased energy demands (21,22). Therefore, HIF-1α and GLUT1 may also be associated with the regulation of certain oncogenes and growth factors in GC.

Lactate dehydrogenase-5 (LDH-5) contains four LDH-M subunits, and is one of the LDH isoenzymes that catalyze the transformation of pyruvate to lactate to provide energy under hypoxia, which may serve an important role in the development and progression of malignancies, according to the well-known Warburg effect (23). The role of LDH-5 in GC remains unclear, although recent studies have revealed certain insights (24,25). Kolev *et al* (25) demonstrated that LDH-5 expression in human GC has a positive correlation with the HIF-1α pathway at the protein expression level. Additionally, it appears that LDH-5 expression is associated with high tumoral and stromal vascular endothelial growth factor expression in GC (24).

The development and identification of novel potential targeting sites for intervention are essential in the search for enhanced treatments for GC. Effective targeting to GC requires a considerable understanding of the associated crosstalk and pathways in cancerous and noncancerous cells. Our group previously demonstrated that HIF-1α is a potential target gene involved in the endogenous hypoxic response and bioenergetic metabolism of GC cells. HIF-1α gene silencing may disturb cellular energy metabolism and promote the apoptosis of GC cells (Hao *et al*, unpublished). Furthermore, GLUT1 and LDH-5 serve key roles in the use of pyruvate for anaerobic energy acquisition and the predominance of this metabolic pathway in cancer cells. Given the close interplay among HIF-1α, GLUT1 and LDH-5, the present study investigated the protein and gene expression levels of HIF-1α, GLUT1 and LDH-5 in patients with GC in order to assess their clinical correlation and co-expression, with the aim of providing a basis for the development of diagnostic and treatment methods for GC.

Materials and methods

Patients and samples. A total of 85 patients with GC who were recruited between March 2015 and September 2015 were included in the present study, and were treated at the General Surgery Department, West China Hospital. All individuals had a confirmed diagnosis of GC based on histopathological evaluation and underwent partial or total gastrectomy, depending on the extent of the neoplastic lesions [21 of these 85 patients with distant metastasis (M1) underwent planned gastrectomy due to bleeding, obstruction or perforation directly resulting from GC]. Clinical data describing the patient demographics (age and sex) and clinical variables (site, size of lesions and disease duration) were obtained and documented (Table I). The grading and staging classifications were made according to the 8th English edition of American Joint Committee on Cancer/Union for International Cancer Control TNM classifications (26). A written consent form approved by the Clinical Trials and Biomedical Ethics Committee of West China Hospital, Sichuan University, was signed by every patient prior to study initiation.

The tissue specimens collected in the operating room were prepared and evaluated by an experienced pathologist. Normal gastric tissues were taken from distant sites from the margins of the tumors (>5.0 cm) by individually harvesting samples from presumed noncancerous regions. Hematoxylin and eosin staining was performed for the histological confirmation of noncancerous and cancerous tissues. In brief, tissues were fixed in 4% paraformaldehyde for 24 h and embedded in paraffin. Then, tissue blocks were cut into 4-μm thick sections and incubated at 60˚C for 4 h. After the removal of the paraffin, the sections were incubated with hematoxylin for 5 min and with eosin for 1 min, respectively. The slides were observed under a Zeiss light microscope at x400 magnification (Carl Zeiss AG). Biopsies from cases were site-matched and stored at -80˚C prior to analysis.

Reagents. Mouse anti-human HIF-1α monoclonal antibody (cat. no. ab16066), rabbit anti-human GLUT1 polyclonal antibody (cat. no. ab15309) and goat anti-human LDH-5 polyclonal antibody (cat. no. ab240482) were purchased from Abcam. Horseradish peroxidase (HRP) labeled secondary antibodies (cat. nos. ZB-2305, ZB-2306 and ZB-5301) were from Origene Technologies, Inc. An UltraSensitive SP kit, liquid diaminobenzidine (DAB) enzyme substrate kit, poly-L-lysine and antigen retrieval buffer were obtained from Fuzhou Maixin Biotech Co., Ltd. TRIzol® reagent, a total RNA extraction kit and the RevertAid™ First Strand cDNA Synthesis kit were purchased from Invitrogen; Thermo Fisher Scientific, Inc. DNA Marker (Marker I) was provided by Tiangen Biotech Co., Ltd. All other chemicals and solvents were of analytical grade.

Immunohistochemical staining. Sections of tumor tissues (4-μm thickness) were deparaffinized and peroxidase was quenched with methanol and 3% hydrogen peroxide (1:1) for 30 min at 80˚C. The sections were immersed in citrate buffer, followed by microwaving for antigen retrieval (3x5 min). After neutralization with endogenous peroxidase using 3% H_2O_2 for 5 min at room temperature, sections were preincubated with 5% blocking serum for 1 h and then incubated with primary antibodies overnight at room temperature (mouse anti-human HIF-1α monoclonal antibody was diluted to 1:80; rabbit anti-human GLUT1 polyclonal antibody was diluted to 1:100; goat anti-human LDH-5 polyclonal antibody was diluted to 1:150). Following washing with 0.01 M PBS (pH=7.4), the

Table I. Demographic characteristics of patients with gastric cancer.

Characteristics	Number of patients, n (%)
Sample size	85
Median age (range)	52 (33-81)
Age (years)	
<50	36 (42.35)
≥50	49 (57.65)
Sex	
Male	52 (61.18)
Female	33 (38.82)
Tumor size	
<3 cm	32 (37.65)
≥3 cm	53 (62.35)
Site of lesions	
Fundus	28 (32.94)
Body	20 (23.53)
Antrum	37 (43.53)
Lymph node metastasis[a]	
N_0	26 (30.59)
N_1	12 (14.12)
N_2	27 (31.76)
N_3	20 (23.53)
Degree of tumor infiltration[a]	
T_1	10 (11.76)
T_2	13 (15.29)
T_3	51 (60.00)
T_4	11 (12.95)
Clinical stage[a]	
I	11 (12.95)
II	13 (15.29)
III	34 (40.00)
IV	27 (31.76)
Histologic grade[a]	
G_1	15 (17.65)
G_2	29 (34.12)
G_3	41 (48.23)

[a]The classification was made according to the 8th English edition of American Joint Committee on Cancer/Union for International Cancer Control TNM classifications (26).

sections were incubated with HRP-labeled secondary antibodies (diluted 1:200) at room temperature for 15 min and washed in PBS. Finally, the sections were incubated with streptavidin peroxidase reagent for 15 min at room temperature and washed in PBS again. The color was developed via a 15 min incubation with DAB solution and the sections were weakly counterstained with hematoxylin at room temperature for 1 min. Normal IgG was substituted for the primary antibody as a negative control (equivalent concentration to the respective test antibody) (11). The slides were observed under a Zeiss light microscope at x400 magnification (Carl Zeiss AG).

Assessment of HIF-1α, GLUT1 and LDH-5 protein expression. The percentages of GC cells with strong cytoplasmic and nuclear HIF-1α, GLUT1 and LDH-5 expression were assessed following inspection of each entire section. Blinded scoring of the specimens was performed using a Zeiss microscope (Zeiss AG) by three independent evaluators. In each x200 magnification optical field the percentage was recorded and the final score for each case was the mean value, and the blinding was removed when all scoring had been completed. Tumors were semi-quantitatively scored using a three-point system (score 0-3) according to the intensity and extent of staining.

HIF-1α expression in GC cells was assessed as score 0 (no positive or <1% of cell nuclei positive), score 1 (1-10% of cell nuclei positive), score 2 (11-50% of cell nuclei positive) and score 3 (>50% of cell nuclei positive), as previously described (27).

Cellular GLUT1 expression was considered positive only if distinct membrane staining was present. Cytoplasmic-only stained cells were not designated as positive. GLUT1 expression in GC cells was classified as score 0 (no positive cells), score 1 (<10% of cells positive), score 2 (11-50% of cells positive) and score 3 (51-100% of cells positive), as described previously (28).

LDH-5 expression in GC cells was graded as score 0 (negative or weak staining in <50% of the optical fields), score 1 (weak staining in 60-100% of the optical fields), score 2 (strong staining in <50% of the optical fields) and score 3 (strong staining in 60-100% of the optical fields), as described previously (29).

Reverse transcription-quantitative PCR (RT-qPCR). Tissues stored at -80˚C were placed in a liquid nitrogen pre-cooled mortar. Following homogenization with liquid nitrogen, the total RNA of the tissues was isolated using the TRIzol® one-step extraction method at 4˚C and cDNA was synthesized using the RevertAid™ first strand cDNA synthesis kit and followed by the PCR condition protocol from Bio-Rad Laboratories, Inc. The reaction mixture was incubated at 20˚C for 10 min, 42˚C for 60 min then at 70˚C for 10 min. After centrifugation at 4˚C for 10 min, the cDNA was obtained and stored at -20˚C. The primers and probes were designed using Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table II) and synthesized by Sangon Biotech Co., Ltd. The β-actin gene was used as the internal control. The amplification reaction of the target genes, including HIF-1α, GLUT1 and LDH-M (LDH-5 contains four LDH-M subunits), was performed in a 30-µl volume containing 3 μl 10X buffer (Mg^{2+} free), 3 μl $MgCl_2$ (25 mM), 0.36 μl dNTP (25 mM), 2 μl respective primers, 1 μl probe with a FAM (10 μM), 5 μl cDNAs and 0.3 μl Taq DNA polymerase (5 U/μl). The RT-qPCR reaction was conducted under the following conditions: Denaturation for 3 min at 95˚C, followed by 33 cycles of 30 sec at 95˚C, annealing for 35 sec at 61.4˚C and extension for 1 min at 72˚C. All PCR results contained amplification products obtained in the linear range of amplification. Relative mRNA quantification was performed using the $^{\Delta}$Cq method. Relative expression ratios (R) were recalculated using the following equation (30):

$$R=(E_{target})^{\Delta CP\ target(control-sample)}/(E_{reference})^{\Delta CP\ ref(control-sample)}$$

Where E represents the corresponding real-time PCR efficiency of one cycle in the exponential phase which was

Table II. Sequences of primers and probes for the determination of HIF-1α, GLUT1 and LDH-M gene expression.

Target gene	Sequence (5'-3')	Length (bp)
HIF-1α (245 bp)		
Forward	CTGACCCTGCACTCAATCAA	20
Reverse	CTTTGCTTCTGTGTCTTCAGCA	22
Probe	FAM-CACCTGAGCCTAATAGTCCCAG	22
GLUT1 (126 bp)		
Forward	GGCATCAACGCTGTCTTCTAT	21
Reverse	CACAAACAGCGACACGACAGT	21
Probe	FAM-CAGCAGCCTGTGTATGCCACCA	22
LDH-M (195 bp)		
Forward	CCAGCGTAACGTGAACATCTT	21
Reverse	CCCATTAGGTAACGGAATCG	20
Probe	FAM-CTTGACCTACGTGGCTTGGAAGA	23
β-actin (114 bp)		
Forward	GCCAACACAGTGCTGTCT	18
Reverse	AGGAGCAATGATCTTGATCTT	21
Probe	FAM-ATCTCCTTCTGCATCCTGTC-TAMRA	20

GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH-M, lactate dehydrogenase M.

calculated according to the equation E=10[-1/slope]; CP is defined as the point at which the fluorescence rises appreciably above the background fluorescence; E_{target} is the qPCR efficiency of the target gene transcript; $E_{reference}$ is the qPCR efficiency of the reference gene transcript; $^{\Delta}CP_{target}$ is the CP deviation of the control-sample of the target gene transcript; and $^{\Delta}CP_{reference}$ is the CP deviation of control-sample of the reference gene transcript.

Statistical analysis. Statistical analysis was performed using SPSS version 14.0 software (SPSS, Inc.). The χ^2 test and Fisher's exact test were used to assess the associations between categorical tumor variables, as appropriate. Spearman's rank correlation was used to test the associations between continuous variables. Gene expression in GC and normal tissues was analyzed using the Relative Expression Software Tool (REST, version 2009) (31). RT-qPCR experiments for each sample were repeated at least twice. The data were presented as mean ± standard deviation. All P-values were two-sided and P<0.05 was considered to indicate a statistically significant difference.

Results

HIF-1α expression in GC. HIF-1α expression was semi-quantitatively examined using immunohistochemical staining. Generally, the staining was evident at the edges of the tumor invasion and necrotic areas, whereas the staining in the normal gastric mucosa was negative (Fig. 1A-D). The staining was primarily nucleoplasmic and partly cytoplasmic, and the extent of staining also varied appreciably among sections. Tumors with scores of 0-1 were considered to have negative reactivity, while tumors with scores of 2-3 were considered to exhibit HIF-1α reactivity. A total of 56/85 cancerous tissues (65.88%) exhibited HIF-1α positive reactivity using these criteria (Table III), indicating that HIF-1α expression was significantly associated with tumor invasion (P<0.01), lymph node metastasis (P<0.05), clinical stage (P<0.01), degree of differentiation (P<0.01) and distant metastasis (P<0.01), but not with age, sex, tumor location or tumor size (Table IV).

GLUT1 expression in GC. The majority of cancerous cells exhibited distinct membrane staining for GLUT1, while cytoplasmic staining was occasionally observed (Fig. 1E and F). Normal gastric mucosa exhibited non-staining with anti-GLUT1. Tumors with scores 0-1 were considered to exhibit negative reactivity, while tumors with scores 2-3 were considered to exhibit GLUT1 reactivity. The results demonstrated that 61/85 cancerous tissues (71.76%) were GLUT1-positive (Table III). The protein expression levels of GLUT1 were clearly associated with tumor size (P<0.05), depth of invasion (P<0.01), distant metastasis (P<0.05), clinical stage (P<0.01) and differentiation (P<0.05), but not with age, sex, tumor location or nodal metastasis (Table IV).

LDH-5 expression in GC. Cytoplasmic expression of LDH-5 was strong and universal, while nucleoplasmic staining was an infrequent observation. In addition, nucleoplasmic expression was always accompanied by strong staining in the cytoplasm. The paracancerous tissues were occasionally weakly positive (Fig. 1G and H). There was no staining in the normal tissues. Tumors with scores of 0-1 were designated as having negative LDH-5 reactivity, and tumors with scores of 2-3 were considered to exhibit positive reactivity. A total of 65/85 cancerous tissues (76.47%) were LDH-5-positive (Table III). Consistent with GLUT1, the expression levels of LDH-5 protein were also associated with tumor size (P<0.05), depth of invasion (P<0.01), distant metastasis (P<0.05), clinical stage

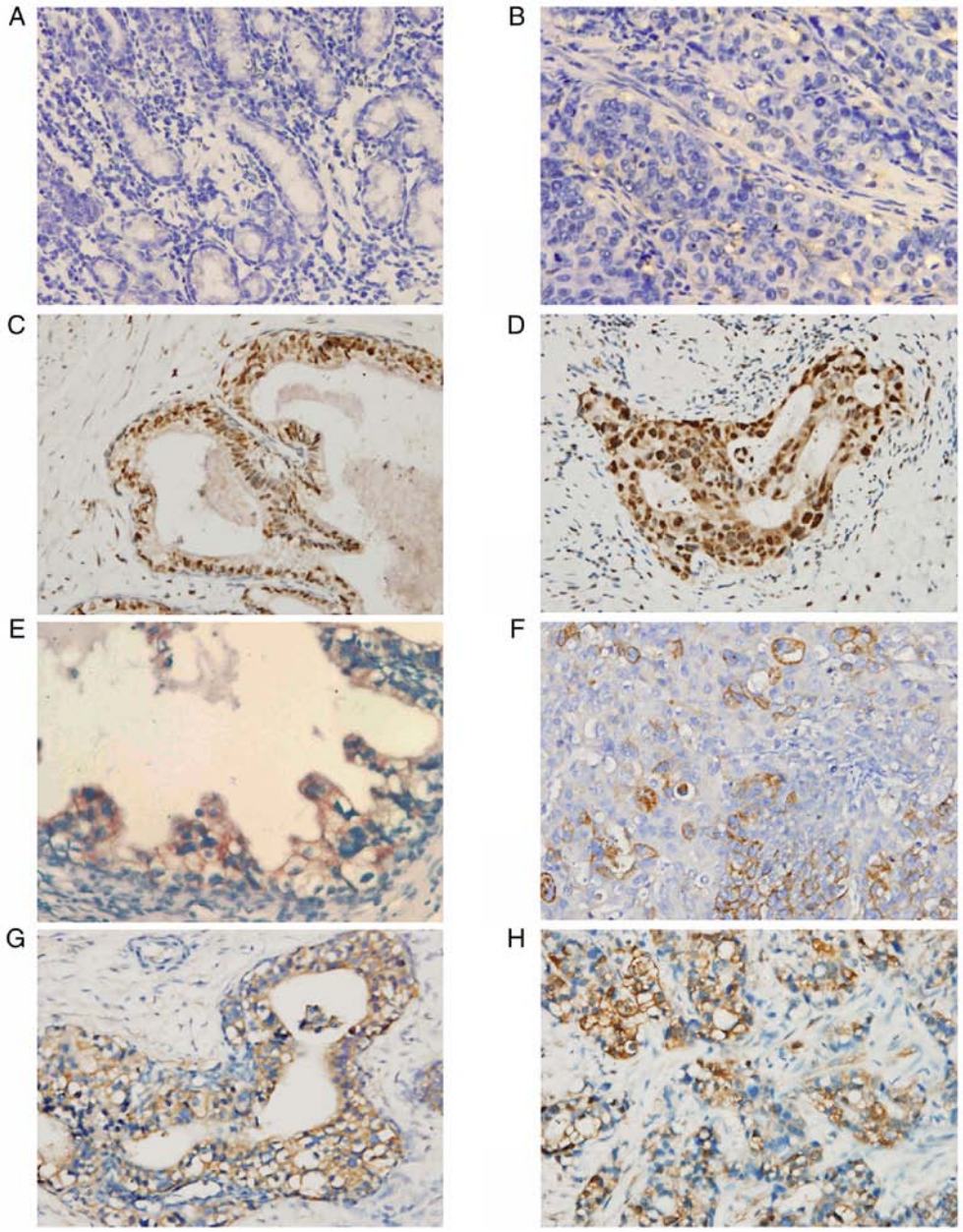

Figure 1. Immunohistochemical staining for the determination of HIF-1α, GLUT1 and LDH-5 protein expression. (A) Non-staining normal gastric tissue and (B) gastric tumor tissue with negative staining. (C and D) Tumor cells exhibited strong HIF-1α reactivity. The staining was obvious at the edge of the tumor infiltration and necrotic areas, whereas the normal gastric tissue (A) had negative staining. (E and F) The cellular membranes were strong reactive with GLUT1 while cytoplasmic staining was an occasional finding. (G and H) Cytoplasmic expression of LDH-5 was strong and universal and nucleoplasmic staining was an infrequent observation. (C, E and G) revealed well-differentiated adenocarcinoma of the stomach. (D, F and H) revealed poorly differentiated adenocarcinoma of the stomach. Magnification x400.

(P<0.01) and differentiation (P<0.05), but not with age, sex, tumor location or nodal metastasis (Table IV).

Correlation of HIF-1α expression to GLUT1 and LDH-5. Details of the correlations among HIF-1α expression and GLUT1 and LDH-5 are presented in Table V. Out of 61 cancerous tissues with GLUT1 expression, 45 cancerous tissues (73.77%) exhibited HIF-1α expression, while 11/24 cancerous tissues (45.83%) with negative expression of GLUT1 exhibited HIF-1α expression (P<0.05). Correlation analysis of GLUT1 expression with HIF-1α expression revealed a significant association (P<0.01, r=0.697). Additionally, the expression of LDH-5 in the tumor tissues was observed in 47/65 cancerous tissues (72.31%) exhibiting HIF-1α expression, whereas 9/20 cancerous tissues (45.00%) with no expression of GLUT1 exhibited HIF-1α expression (P<0.05). Therefore, LDH-5 expression was significantly correlated with HIF-1α expression (P<0.01, r=0.783).

HIF-1α, GLUT1 and LDH-M gene expression in GC. RT-qPCR analysis was performed for the determination of mRNA expression levels of HIF-1α, GLUT1 and LDH-M in cancerous and noncancerous tissues, with β-actin as a reference. As a standard practice, R of target genes was expressed as levels in samples compared with those in controls and normalized to the reference gene. R values >1 represented upregulated expression

Table III. HIF-1α, GLUT1 and LDH-5 expression in normal stomach and gastric cancer tissues.

		HIF-1α		GLUT1		LDH-5	
Specimen	(+) Number, n	(-) χ^2=83.509	(+) P<0.01	(-) χ^2=95.138	(+) P<0.01	(-) χ^2=105.238	P<0.01
Cancerous tissues	85	56	29	61	24	65	20
Normal tissues	85	0	85	0	85	0	85

GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH-5, lactate dehydrogenase-5.

Table IV. Associations of the expression levels of HIF-1α, GLUT1 and LDH-5 with clinical and pathological parameters.

		HIF-1α expression		GLUT1 expression		LDH-5 expression	
Variable	Number, n (n=85)	+ (n=56)	- (n=29)	+ (n=61)	- (n=24)	+ (n=65)	- (n=20)
Sex		χ^2=0.349	P>0.05	χ^2=0.692	P>0.05	χ^2=0.015	P>0.05
Male	52	33	19	39	13	40	12
Female	33	23	10	22	11	25	8
Age (years)		χ^2=0.353	P>0.05	χ^2=0.006	P>0.05	χ^2=0.075	P>0.05
<50	36	25	11	26	10	27	9
≥50	49	31	18	35	14	38	11
Tumor location		χ^2=1.419	P>0.05	χ^2=4.361	P>0.05	χ^2=1.217	P>0.05
Upper	28	19	9	20	8	20	8
Middle	20	11	9	11	9	17	3
Lower	37	26	11	30	7	28	9
Tumor diameter (cm)		χ^2=0.967	P>0.05	χ^2=6.096	P<0.05	χ^2=5.567	P<0.05
<3	32	19	13	18	14	20	12
≥3	53	37	16	43	10	45	8
Degree of Differentiation		χ^2=11.149	P<0.01	χ^2=6.209	P<0.05	χ^2=6.272	P<0.05
Well	15	5	10	7	8	8	7
Moderate	29	18	11	21	8	22	7
Poor	41	33	8	33	8	35	6
Tumor invasion[a]		χ^2=10.040	P<0.01	χ^2=8.918	P<0.01	χ^2=10.345	P<0.01
T_1+T_2	23	9	14	11	12	12	11
T_3+T_4	62	47	15	50	12	53	9
Lymph node metastasis[a]		χ^2=4.204	P<0.05	χ^2=1.933	P>0.05	χ^2=1.091	P>0.05
N0	26	13	13	16	10	18	8
N_1+N_2+N_3	59	43	16	45	14	47	12
Distant metastasis[a]		χ^2=7.505	P<0.01	*χ^2=4.819*	P<0.05	χ^2=4.162	P<0.05
M_0	64	37	27	42	22	45	19
M_1	21	19	2	19	2	20	1
Clinical stage[a]		χ^2=8.725	P<0.01	χ^2=7.818	P<0.01	χ^2=9.246	P<0.01
I+II	24	10	14	12	12	13	11
III+IV	61	46	15	49	12	52	9

[a]The classification was made according to the 8th English edition of American Joint Committee on Cancer/Union for International Cancer Control TNM classifications (26). GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH-5, lactate dehydrogenase 5. GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor 1α; LDH-5, lactate dehydrogenase 5.

of the target genes. Upregulated expression of HIF-1α, GLUT1 and LDH-M transcripts was confirmed in 72.94 (62/85), 78.82 (67/85) and 81.18% (69/85) of cancerous tissue samples, respectively. The statistical significance of the differences in

Table V. Correlations of HIF-1α expression with expression levels of GLUT1 and LDH-5 in gastric cancer.

Marker	Number, n n=85	HIF-1α			χ^2 test		Spearman's rank correlation	
		+ (n=56)	- (n=29)	Positive ratio (%)	χ^2	P-value	*r*	P-value
GLUT1 (+)	61	45	16	73.77	5.981	P<0.05	0.697	P<0.01
GLUT1 (-)	24	11	13	45.83				
LDH-5 (+)	65	47	18	72.31	5.074	P<0.05	0.783	P<0.01
LDH-5 (-)	20	9	11	45.00				

GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH-5, lactate dehydrogenase-5.

mRNA expression levels was analyzed using REST. mRNA expression levels of HIF-1α, GLUT1 and LDH-M in GC were significantly higher compared with those in normal gastric tissues, with P-values of 0.003, 0.014 and 0.008, respectively.

The expression levels of HIF-1α, GLUT1 and LDH-M transcripts with respect to relevant clinical and pathological parameters in patients with GC are presented in Table VI. Consistent with the protein expression data, the mRNA expression levels of HIF-1α were significantly associated with depth of invasion, nodal metastasis, clinical stage, differentiation and distant metastasis, and not with age, sex, tumor location or tumor size. GLUT1 mRNA expression was associated with tumor size, depth of invasion, distant metastasis, clinical stage and differentiation, but not with age, gender, tumor location or nodal metastasis, which was consistent with the protein expression pattern of GLUT1. The same trends were observed for the mRNA expression levels of LDH-M.

Correlations of individual protein and mRNA expression levels. The present study compared the protein expression levels of HIF-1α, GLUT1 and LDH-5 with their associated mRNA expression levels in 85 GC samples (Table VII). The Spearman correlation coefficients of HIF-1α, GLUT1 and LDH-5 and their associated mRNAs were 0.648, 0.664 and 0.713, respectively, indicating statistically significant correlations among the expression levels of proteins and mRNAs (P<0.01).

Correlation of HIF-1α gene expression with GLUT1 and LDH-M gene expression levels. The R values of HIF-1α, GLUT1 and LDH-M gene expression and the results of the statistical analysis are summarized in Table VIII. Out of 67 cancerous tissues with GLUT1 gene upregulation, 54 cancerous tissues (80.60%) exhibited HIF-1α gene upregulation, while 8/18 cancerous tissues (44.44%) with downregulated expression of GLUT1 exhibited HIF-1α gene upregulation (P<0.01). A significant correlation was observed between GLUT1 and HIF-1α mRNA expression levels (P<0.01, r=0.765). In addition, upregulated expression levels of the LDH-M gene in the tumor tissues were observed in 56/69 cancerous tissues (81.16%) exhibiting HIF-1α gene upregulation, whereas 6/16 cancerous tissues (37.50%) with downregulated expression of LDH-M exhibited upregulated expression of HIF-1α (P<0.05). Correlation analysis of LDH-M with HIF-1α mRNA expression levels revealed a significant association (P<0.01, r=0.892).

Discussion

Associations among HIF expression and tumor properties have become a topic of interest in oncology research (6,32). Notably, a trend between rapid tumor proliferation and metastasis and positive HIF-1α expression in cancer cells has been identified (33). Additionally, certain studies have demonstrated that HIF-1α may be a predictive marker of a high recurrence risk in patients with Dukes B colorectal cancer, as high-level expression of HIF-1α is strongly associated with invasive subtypes (21,34,35). Furthermore, elevated expression levels of HIF-1α are considered to be a response of tumor cells to hypoxia, and may be one of the factors that induce glycolysis during hypoxia (36).

In the present study, high protein expression levels of HIF-1α in GC samples with a positive ratio of 65.88% were revealed by immunohistochemical staining. The staining was marked at the edge of the tumor invasion and necrotic areas, particularly with larger or deeply invasive tumors, far from interstitial blood vessels with poor oxygen supplementation and nutritional intake. The positively-stained cells were observed in the nucleoplasmic and cytoplasmic areas, since HIF-1α is synthesized in the cytoplasm and transferred to the nucleus, followed by binding to HIF-1β for activation (37). Additionally, Jung *et al* (38) demonstrated that HIF-1α was overexpressed in GC at the protein expression level with a ratio of 52.3%, but not in normal gastric tissues. Furthermore, in the present study, the protein expression data were consistent with the mRNA expression levels of HIF-1α in GC samples. However, there was no positive response in certain cases. The reason for this may be that hypoxia-induced apoptosis was increased while hypoxia-induced adaptation was restricted in patients, reducing the proliferation of tumor cells. This may also explain the improved prognosis of patients with negative HIF-1α expression.

HIF-1α is able to upregulate glucose-associated receptors, including GLUT1, GLUT4 and GLUT8 in the cell membrane, in addition to the expression of key enzyme genes, in order to facilitate the intake and metabolism of glucose in tumor cells, and even to biosynthesize nutrients for the proliferation and differentiation of tumor cells via the glycolysis pathway (39,40). In a rapidly growing tumor tissue, the hypoxic cells tend to consume more glucose to satisfy their energy requirements as a consequence of enhanced glycolytic

Table VI. Associations of the gene expression levels of HIF-1α, GLUT1 and LDH-5 with clinical and pathological parameters.

		HIF-1α mRNA		GLUT1 mRNA		LDH-5 mRNA	
Variable	Number, n (n=85)	R>1 (n=62)	R≤1 (n=23)	R>1 (n=67)	R≤1 (n=18)	R>1 (n=69)	R≤1 (n=16)
Sex		χ^2=0.217	P>0.05	χ^2=0.304	P>0.05	χ^2=0.201	P>0.05
Male	52	37	15	42	10	43	9
Female	33	25	8	25	8	26	7
Age (years)		χ^2=0.134	P>0.05	χ^2=0.547	P>0.05	χ^2=0.016	P>0.05
<50	36	27	9	27	9	29	7
≥50	49	35	14	40	9	40	9
Tumor location		χ^2=2.311	P>0.05	χ^2=3.592	P>0.05	χ^2=1.718	P>0.05
Upper	28	21	7	22	6	21	7
Middle	20	12	8	13	7	18	2
Lower	37	29	8	32	5	30	7
Tumor diameter (cm)		χ^2=0.030	P>0.05	χ^2=8.192	P<0.01	χ^2=5.186	P<0.05
<3	32	23	9	20	12	22	10
≥3	53	39	14	47	6	47	6
Degree of differentiation		χ^2=8.686	P<0.05	χ^2=7.824	P<0.05	χ^2=6.674	P<0.05
Well	15	7	8	8	7	9	6
Moderate	29	20	9	23	6	23	6
Poor	41	35	6	36	5	37	4
Tumor invasion[a]		χ^2=4.307	P<0.05	χ^2=4.704	P<0.05	χ^2=6.785	P<0.01
T_1+T_2	23	13	10	14	9	14	9
T_3+T_4	62	49	13	53	9	55	7
Lymph node metastasis[a]		χ^2=4.41	P<0.05	χ^2=2.065	P>0.05	χ^2=0.935	P>0.05
N_0	26	15	11	18	8	19	7
N_1+N_2+N_3	59	47	12	49	10	50	9
Distant metastasis[a]		χ^2=7.025	P<0.01	χ^2=*5.903*	P<0.05	χ^2=4.935	P<0.05
M_0	64	42	22	46	18	48	16
M_1	21	20	1	21	0	21	0
Clinical stage[a]		χ^2=5.972	P<0.05	χ^2=8.412	P<0.01	χ^2=6.626	P<0.05
I+II	24	13	11	14	10	15	9
III+IV	61	49	12	53	8	54	7

[a]The classification was made according to the 8th English edition of American Joint Committee on Cancer/Union for International Cancer Control TNM classifications (26). GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH-5, lactate dehydrogenase-5; R, relative expression ratio.

flux and accumulation of pyruvate (40). Consistent with the studies by Kawamura *et al* (41) and Jung *et al* (38), the results of the present study suggested that GLUT1 is essential for the tumorigenesis, progression, invasion and metastasis of GC. From the perspective of glycolytic flux control analysis, it has been demonstrated that GLUT and key glycolytic enzymes provide the ideal targeting sites for therapeutic intervention at the level of energy metabolism in hypoxic and glycolytic tumors (42).

LDH-5 is the most important enzyme for promoting anaerobic glycolysis via transformation of pyruvate to lactate, and the upregulation of LDH-5 in cancerous cells guarantees a predominant glycolytic metabolism that reduces tumor dependence in the presence of oxygen (25). The clinical importance of high LDH-5 expression in tumors has attracted extensive attention and thorough investigation (25,43,44). The correlation of a high LDH-5 level with aggressive forms of several different tumor types have been observed (23-25,27). Notably, HIF-1α upregulates LDH-5 expression favoring enhanced glycolytic flux (42). Under hypoxia, lactate, as the end product of anaerobic glycolysis catalyzed by lactate dehydrogenase enzyme, is released to acidify the cellular matrix, which may further trigger aggressive behavior. In accordance with the study of Kolev *et al* (25), the present study demonstrated that cytoplasmic expression of LDH-5 was strong and universal, with a positive ratio of 76.47% (65/85 cases), while nucleoplasmic staining was an infrequent observation. The results demonstrated that LDH-M mRNA and LDH-5 protein were

Table VII. Correlations of protein expression levels with mRNA expression in gastric cancer (n=85).

	Protein		Gene		Spearman's rank correlation	
Protein/Gene name	Positive	Negative	R>1	R≤1	*r*	P-value
HIF-1α	56	29	62	23	0.648	<0.01
GLUT1	61	24	67	18	0.664	<0.01
LDH-5/LDH-M	65	20	69	16	0.713	<0.01

GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH, lactate dehydrogenase; R, relative expression ratio.

Table VIII. Correlations of HIF-1α mRNA expression with GLUT1 and LDH-M mRNA expression in gastric cancer.

		HIF-1α mRNA			χ^2 test		Spearman's rank correlation	
Marker	Number, n (n=85)	R>1(n=62)	R≤1 (n=23)	Upregulation ratio, %	χ^2	P-value	*r*	P-value
GLUT1 R>1	67	54	13	80.60	7.653	<0.01	0.765	<0.01
GLUT1 R≤1	18	8	10	44.44				
LDH-M R>1	69	56	13	81.16	10.43	<0.01	0.892	<0.01
LDH-M R≤1	16	6	10	37.50				

GLUT1, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LDH, lactate dehydrogenase; R, relative expression ratio.

specifically upregulated in GC, indicating the induction of energy metabolism in tumor cells via the glycolysis pathway, which confers a dominant proliferation during cancer evolution. At early stages in carcinogenesis, LDH-5 may represent a promising target for cancer treatment.

At present, limited data are available regarding the linkage of HIF-1α with GLUT-1 and LDH-5 co-expression to bioenergetic metabolism in GC. In the present study, correlation analysis of HIF-1α with GLUT1 and LDH-5 protein and gene expression levels in GC indicated that HIF-1α expression was positively correlated with the mRNA and protein expression levels of GLUT1 and LDH-5 (LDH-M). GLUT1 and LDH-M (LDH-5), as downstream target genes, are upregulated by HIF-1α, increasing the transmembrane transport of glucose, promoting the conversion of pyruvate to lactate and enhancing the activity of the LDH isoenzyme (45). The increased glycolysis in proliferating cells results in high expression levels of a number of glycolytic enzymes, which serve an important role in the energy metabolism of tumor cells. HIF-1 upregulates glucose transporters and glycolysis enzymes in tumors, directing tumors toward anaerobic glycolysis in hypoxic conditions. Due to genetic mutations in protein-coding and tumor suppressor genes causing structural alterations in HIF-1, tumor cells preferentially convert pyruvate into lactate even under conditions of sufficient oxygen (46). Additionally, the metabolic switch from oxidative phosphorylation to glycolysis in tumor cells is able to reduce the formation of oxygen free radicals during the destruction of DNA to facilitate proliferation. The enhanced structural regulation of glycolysis and the formation of the acidic tumor microenvironment serve key roles in invasive tumor growth. Upregulation of glycolysis leads to microenvironmental acidosis, requiring tumor cells to evolve phenotypes resistant to acid-induced cell toxicity. Subsequent cell populations with acid resistance and upregulated glycolysis have a powerful growth advantage, promoting unconstrained proliferation and invasion (47). However, the present study had several limitations. The use of immunohistochemistry for the evaluation of protein expression levels is not always sufficient to reflect protein structure and functionality. Immunofluorescence staining and other methods should be performed in future studies. Nonetheless, a strength of the present study was that HIF-1α, GLUT1 and LDH-5 expressions were examined at the protein and gene levels to examine their correlations.

In conclusion, the results of the present study provided evidence for a possible key role of the HIF-1α, GLUT1 and LDH-5 pathway in the occurrence, development, metastasis and poor prognosis of gastric tumors. These proteins may be used as markers for the diagnosis and prognosis of GC. HIF-1α, GLUT1 and LDH-5 are capable of acting as an important reference for the evaluation of hypoxia in GC prior to and following treatment. Additionally, the results of the present study suggested that HIF-1α, GLUT1 and LDH-5 may be potential target genes involved in the endogenous tumor response to hypoxia and the inhibition of tumor energy metabolism, and thus highlighted novel therapeutic targets for GC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the project (grant no. 2016M602706) funded by China Postdoctoral Science Foundation and the International Exchange and Collaboration Project (grant no. 2018HH0057) funded by Science and Technology Department of Sichuan Province.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Authors' contributions

LSH, QL, CT and DXZha collected the samples, recorded the information and conducted the experiments. BW, DXZho and ZPL analyzed the data. ZXY contributed to the project design and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Human samples used in the present study were obtained from patients who provided written informed consent. The present study was approved by the Ethics Committee of West China Hospital, Sichuan University and was conducted according to The Declaration of Helsinki.

Patient consent for publication

Consent for publication was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
2. Rugge M, Fassan M and Graham DY: Epidemiology of gastric cancer. In: Gastric Cancer: Principles and Practice. Strong VE (eds). Springer International Publishing, Cham, pp23-34, 2015.
3. Xuan Y, Hur H, Ham IH, Yun J, Lee JY, Shim W, Kim YB, Lee G, Han SU and Cho YK: Dichloroacetate attenuates hypoxia-induced resistance to 5-fluorouracil in gastric cancer through the regulation of glucose metabolism. Exp Cell Res 321: 219-230, 2014.
4. Lordick F, Allum W, Carneiro F, Mitry E, Tabernero J, Tan P, Van Cutsem E, van de Velde C and Cervantes A: Unmet needs and challenges in gastric cancer: The way forward. Cancer Treat Rev 40: 692-700, 2014.
5. Muz B, de la Puente P, Azab F and Azab AK: The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia (Auckl) 3: 83-92, 2015.
6. Zeng W, Liu P, Pan W, Singh SR and Wei Y: Hypoxia and hypoxia inducible factors in tumor metabolism. Cancer Lett 356: 263-267, 2015.
7. Semenza GL: HIF-1: Upstream and downstream of cancer metabolism. Curr Opin Genet Dev 20: 51-56, 2010.
8. Esfandiary A, Taherian-Esfahani Z, Abedin-Do A, Mirfakhraie R, Shirzad M, Ghafouri-Fard S and Motevaseli E: Lactobacilli modulate hypoxia-inducible factor (HIF)-1 regulatory pathway in triple negative breast cancer cell line. Cell J 18: 237-244, 2016.
9. Koyasu S, Kobayashi M, Goto Y, Hiraoka M and Harada H: Regulatory mechanisms of hypoxia-inducible factor 1 activity: Two decades of knowledge. Cancer Sci 109: 560-571, 2018.
10. Li M, Wang YX, Luo Y, Zhao J, Li Q, Zhang J and Jiang Y: Hypoxia inducible factor-1α-dependent epithelial to mesenchymal transition under hypoxic conditions in prostate cancer cells. Oncol Rep 36: 521-527, 2016.
11. Isobe T, Aoyagi K, Koufuji K, Shirouzu K, Kawahara A, Taira T and Kage M: Clinicopathological significance of hypoxia-inducible factor-1 alpha (HIF-1α) expression in gastric cancer. Int J Clin Oncol 18: 293-304, 2013.
12. Qian J, Hao B, Gao Z, Dong YU, Pei J, Ma M and Han B: Downregulation of HIF-1α inhibits the proliferation and invasion of non-small cell lung cancer NCI-H157 cells. Oncol Lett 11: 1738-1744, 2016.
13. Liu L, Ning X, Sun L, Zhang H, Shi Y, Guo C, Han S, Liu J, Sun S, Han Z, *et al*: Hypoxia-inducible factor-1 alpha contributes to hypoxia-induced chemoresistance in gastric cancer. Cancer Sci 99: 121-128, 2008.
14. Wang J, Ni Z, Duan Z, Wang G and Li F: Altered expression of hypoxia-inducible factor-1α (HIF-1α) and its regulatory genes in gastric cancer tissues. PLoS One 9: e99835, 2014.
15. Chen J, Li T, Liu Q, Jiao H, Yang W, Liu X and Huo Z: Clinical and prognostic significance of HIF-1α, PTEN, CD44v6, and survivin for gastric cancer: A meta-analysis. PLoS One 9: e91842, 2014.
16. Zhu CL, Huang Q, Liu CH, Lin XS and Xie F: Prognostic value of HIF-1α expression in patients with gastric cancer. Mol Biol Rep 40: 6055-6062, 2013.
17. Liu X, Wang X, Zhang J, Lam EK, Shin VY, Cheng AS, Yu J, Chan FK, Sung JJ and Jin HC: Warburg effect revisited: An epigenetic link between glycolysis and gastric carcinogenesis. Oncogene 29: 442-450, 2010.
18. Szablewski L: Expression of glucose transporters in cancers. Biochim Biophys Acta 1835: 164-169, 2013.
19. Fan R, Hou WJ, Zhao YJ, Liu SL, Qiu XS, Wang EH and Wu GP: Overexpression of HPV16 E6/E7 mediated HIF-1α upregulation of GLUT1 expression in lung cancer cells. Tumor Biol 37: 4655-4663, 2016.
20. Masoud GN and Li W: HIF-1α pathway: Role, regulation and intervention for cancer therapy. Acta Pharm Sin B 5: 378-389, 2015.
21. Rajaganeshan R, Prasad R, Guillou PJ, Poston G, Scott N and Jayne DG: The role of hypoxia in recurrence following resection of Dukes' B colorectal cancer. Int J Colorectal Dis 23: 1049-1055, 2008.
22. Oh S, Kim H, Nam K and Shin I: Glut1 promotes cell proliferation, migration and invasion by regulating epidermal growth factor receptor and integrin signaling in triple-negative breast cancer cells. BMB Rep 50: 132-137, 2017.
23. Kayser G, Kassem A, Sienel W, Schulte-Uentrop L, Mattern D, Aumann K, Stickeler E, Werner M, Passlick B and zur Hausen A: Lactate-dehydrogenase 5 is overexpressed in non-small cell lung cancer and correlates with the expression of the transketolase-like protein 1. Diagn Pathol 5: 22, 2010.
24. Kim HS, Lee HE, Yang HK and Kim WH: High lactate dehydrogenase 5 expression correlates with high tumoral and stromal vascular endothelial growth factor expression in gastric cancer. Pathobiology 81: 78-85, 2014.
25. Kolev Y, Uetake H, Takagi Y and Sugihara K: Lactate dehydrogenase-5 (LDH-5) expression in human gastric cancer: association with hypoxia-inducible factor (HIF-1alpha) pathway, angiogenic factors production and poor prognosis. Ann Surg Oncol 15: 2336-2344, 2008.
26. Brierley JD, Gospodarowicz MK and Wittekind C (eds): TNM classification of malignant tumours, 8th edition. John Wiley & Sons, Published in affiliation with the Union for International Cancer Control (UICC), 2016.
27. Guan G, Zhang Y, Lu Y, Liu L, Shi D, Wen Y, Yang L, Ma Q, Liu T, Zhu X, *et al*: The HIF-1α/CXCR4 pathway supports hypoxia-induced metastasis of human osteosarcoma cells. Cancer Lett 357: 254-264, 2015.
28. Yasuda M, Miyazawa M, Fujita M, Kajiwara H, Iida T, Hirasawa T, Muramatsu T, Murakami M, Mikami M, Saitoh K, *et al*: Expression of hypoxia inducible factor-1alpha (HIF-1alpha) and glucose transporter-1 (GLUT-1) in ovarian adenocarcinomas: Difference in hypoxic status depending on histological character. Oncol Rep 19: 111-116, 2008.
29. Koukourakis MI, Giatromanolaki A and Sivridis E; Tumour and Angiogenesis Research Group: Lactate dehydrogenase isoenzymes 1 and 5: Differential expression by neoplastic and stromal cells in non-small cell lung cancer and other epithelial malignant tumors. Tumour Biol 24: 199-202, 2003.

30. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45, 2001.
31. Pfaffl MW, Horgan GW and Dempfle L: Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30: e36, 2002.
32. Kitajima Y and Miyazaki K: The critical impact of hif-1a on gastric cancer biology. Cancers (Basel) 5: 15-26, 2013.
33. Semenza GL: Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 29: 625-634, 2010.
34. Schmitz KJ, Müller CI, Reis H, Alakus H, Winde G, Baba HA, Wohlschlaeger J, Jasani B, Fandrey J and Schmid KW: Combined analysis of hypoxia-inducible factor 1 alpha and metallothionein indicates an aggressive subtype of colorectal carcinoma. Int J Colorectal Dis 24: 1287-1296, 2009.
35. Cleven AH, van Engeland M, Wouters BG and de Bruïne AP: Stromal expression of hypoxia regulated proteins is an adverse prognostic factor in colorectal carcinomas. Cell Oncol 29: 229-240, 2007.
36. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.
37. Lee K, Zhang H, Qian DZ, Rey S, Liu JO and Semenza GL: Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. Proc Natl Acad Sci USA 106: 17910-17915, 2009.
38. Jung JH, Im S, Jung ES and Kang CS: Clinicopathological implications of the expression of hypoxia-related proteins in gastric cancer. Int J Med Sci 10: 1217-1223, 2013.
39. Baumann MU, Zamudio S and Illsley NP: Hypoxic upregulation of glucose transporters in BeWo choriocarcinoma cells is mediated by hypoxia-inducible factor-1. Am J Physiol Cell Physiol 293: C477-C485, 2007.
40. Courtnay R, Ngo DC, Malik N, Ververis K, Tortorella SM and Karagiannis TC: Cancer metabolism and the Warburg effect: The role of HIF-1 and PI3K. Mol Biol Rep 42: 841-851, 2015.
41. Kawamura T, Kusakabe T, Sugino T, Watanabe K, Fukuda T, Nashimoto A, Honma K and Suzuki T: Expression of glucose transporter-1 in human gastric carcinoma: Association with tumor aggressiveness, metastasis, and patient survival. Cancer 92: 634-641, 2001.
42. Marín-Hernández A, Gallardo-Pérez J, Ralph SJ, Rodríguez-Enríquez S and Moreno-Sánchez R: HIF-1alpha modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. Mini Rev Med Chem 9: 1084-1101, 2009.
43. Danner BC, Didilis VN, Wiemeyer S, Stojanovic T, Kitz J, Emmert A, Füzesi L and Schöndube FA: Long-term survival is linked to serum LDH and partly to tumour LDH-5 in NSCLC. Anticancer Res 30: 1347-1351, 2010.
44. Augoff K, Hryniewicz-Jankowska A and Tabola R: Lactate dehydrogenase 5: An old friend and a new hope in the war on cancer. Cancer Lett 358: 1-7, 2015.
45. Sevinc A, Sari R and Fadillioglu E: The utility of lactate dehydrogenase isoenzyme pattern in the diagnostic evaluation of malignant and nonmalignant ascites. J Natl Med Assoc 97: 79-84, 2005.
46. Li XB, Gu JD and Zhou QH: Review of aerobic glycolysis and its key enzymes-new targets for lung cancer therapy. Thorac Cancer 6: 17-24, 2015.
47. Gillies RJ and Gatenby RA: Adaptive landscapes and emergent phenotypes: Why do cancers have high glycolysis? J Bioenerg Biomembr 39: 251-257, 2007.