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Basic Study

Lysyl oxidase and hypoxia-inducible factor 1 α : biomarkers of gastric cancer

Ya-Lin Han, Li Chen, Rui Qin, Guan-Qing Wang, Xiao-Hua Lin, Guang-Hai Dai

ORCID number: Ya-lin Han (0000-0003-4552-1486); Li Chen (0000-0002-9671-7967); Rui Qin (0000-0001-6033-4393); Guan-Qing Wang (0000-0002-2269-5881); Xiao-Hua Lin (0000-0002-4494-8480); Guang-Hai Dai (0000-0001-7582-9348).

Author contributions: Dai GH and Chen L designed and provided ideas for the project; Han YL wrote and edited the manuscript; Han YL, Chen L and Qin R performed immunohistochemical staining; Han YL analyzed all the data; Han YL and Lin XH conducted RNA separation, cDNA synthesis and qRT-PCR analysis; Han YL, Qin R and Wang GQ collected clinical samples from our hospital.

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Ya-Lin Han, Li Chen, Rui Qin, Guan-Qing Wang, Guang-Hai Dai, Department of Medical Oncology, Chinese PLA General Hospital, Beijing 100853, China

Xiao-Hua Lin, Department of Oncology, the General Hospital of PLA Rocket Force, Beijing 100088, China

Corresponding author: Guang-Hai Dai, PhD, Professor, Department of Medical Oncology, Chinese PLA General Hospital, 28 Fuxing Road, Haidian District, Beijing 100853, China. handai713@126.com

Telephone: +86-10-66937231

Abstract

BACKGROUND

Gastric cancer (GC) is one of the main causes of cancer mortality worldwide. Recent studies on tumor microenvironments have shown that tumor metabolism exerts a vital role in cancer progression.

AIM

To investigate whether lysyl oxidase (LOX) and hypoxia-inducible factor 1 α (HIF1 α) are prognostic and predictive biomarkers in GC.

METHODS

A total of 80 tissue and blood samples were collected from 140 patients admitted to our hospital between August 2008 and March 2012. Immunohistochemical staining was performed to measure the expression of LOX and HIF1 α in tumor and adjacent tissues collected from patients with GC. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis was used to detect the mRNA expression levels of LOX and HIF1 α in patients with GC. In addition, single-factor analysis was applied to analyze the relationship between LOX, HIF1 α and prognosis of GC.

RESULTS

Immunohistochemical staining suggested that the expression levels of LOX and HIF1 α increased in tumor tissues from patients with GC. QRT-PCR analysis indicated that mRNA expression of LOX and HIF1 α was also upregulated in tumor tissues, which was in accordance with the above results. We also detected expression of these two genes in blood samples. The expression level of LOX and HIF1 α was higher in patients with GC than in healthy controls. Additional analysis showed that the expression level of LOX and HIF1 α was related to the clinicopathological characteristics of GC. Expression of LOX and HIF1 α increased

experiments.

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with the number of lymph node metastases, deeper infiltration depth and later tumor-node-metastasis stages. Single-factor analysis showed that high expression of LOX and HIF1α led to poor prognosis of patients with GC.

CONCLUSION

LOX and HIF1α can be used as prognostic and predictive biomarkers for GC.

Key words: Lysyl oxidase; Hypoxia-inducible factor 1α; Gastric cancer; Biomarker; Prognosis

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Core tip: Lysyl oxidase (LOX) is a secreted extracellular matrix protein that plays an important role in remodeling the extracellular matrix and promoting tumor progression. The LOX family comprises the prototypic LOX, as well as the LOX-like proteins that are involved in carcinogenesis. Hypoxia-inducible factor 1α (HIF1α) is a type of transcription factor complex that has the capacity to regulate oxygen tension. In addition, HIF1α is able to activate or bind to multiple target genes and participates in inflammatory and other diseases. HIF1α accelerates the growth and metastasis of hepatocellular carcinoma.

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INTRODUCTION

Gastric cancer (GC) is one of the main causes of cancer mortality worldwide^[1]. Current epidemiological data indicate that the incidence rate and mortality of GC rank among the top three of all malignant tumors^[2]. The morbidity of GC rises gradually with age, and people aged 50-70 years account for the majority of cases. There is no gender differences in patients with GC, and the occurrence of GC is related to geographic variation in many countries. The clinical manifestations mainly include stomach ache, abdominal distension, loss of appetite and weight loss^[3]. GC at early stages is free of symptoms, and advanced GC or metastasis sites could be pathological diagnosed by endoscopy or endoscopic ultrasound^[4-7]. Patients who suffer from GC have a poor quality of life, and the survival rates are reported to be low^[8]. As estimated in the statistics of GLOBOCAN 2012, about 410,000 patients are newly found to suffer from the pain caused by GC in China^[9]. Among them, 330,000 patients die of tumor-related causes.

While several therapies have emerged in recent years, the effects of chemotherapy and surgery for GC remain limited^[10]. These interventions do not improve prognosis, increase survival rate or prolong survival time^[11]. In addition, there are disadvantages to current treatment methods, including inconvenience, increased prevalence of complications, and side-effects^[12]. It has been reported that many factors are involved in the progression of GC. More seriously, approximately 60% of patients with GC have metastases when they are diagnosed^[13]. Furthermore, the outcome of patients with late stage GC is poor, and most die within 1 year. Therefore, determination of predictive biomarkers for early diagnosis of GC is important.

Recent studies on tumor microenvironments have shown that tumor metabolism plays a vital role in cancer progression. The role of the microenvironment in tumor metabolism is currently attracting significant attention. Lysyl oxidase (LOX) and hypoxia-inducible factor 1α (HIF1α) affect the tumor microenvironment, and play a crucial role in cancer occurrence and development^[14-18]. LOX is a secreted extracellular matrix protein that plays an important role in remodeling the extracellular matrix and promoting tumor progression. The LOX family comprises the prototypic LOX, as well as LOX-like proteins that are involved in carcinogenesis. LOX is a copper-dependent monoamine oxidase, and its overexpression is related to both poor survival and the development of multiple types of cancers^[19-22]. HIF1α is a transcription factor complex

that can regulate oxygen tension^[23]. In addition, HIF1 α can activate or bind to multiple target genes, and can contribute to inflammatory and other diseases^[22,20]. HIF1 α accelerates the growth and metastasis of hepatocellular carcinoma^[17].

The present study investigated the expression of LOX and HIF1 α in patients with GC and determined whether LOX and HIF1 α act as prognostic and predictive biomarkers of GC. Research on the interaction between cancer and factors in the metabolic microenvironment is necessary to investigate the progression of GC and determine predictive biomarkers. Our study showed that LOX and HIF1 α can act as biomarkers for the diagnosis and prediction of GC.

MATERIALS AND METHODS

Patients and samples

A total of 80 tissue and blood samples were collected from 140 patients admitted to our hospital between August 2008 and March 2012. The tumor and adjacent tissues were obtained from patients with GC. The blood samples were obtained from 80 patients with GC and 80 healthy controls. The use of human tissues in this research was agreed to by the volunteers and their relatives. All participants gave written informed consent. This study was conducted with permission from the Institutional Research Ethics Committee of our hospital. GC patients were aged > 18 years, and were diagnosed pathologically and clinically with GC. Clinicians measured and analyzed the conditions according to the Response Evaluation Criteria in Solid Tumors.

RNA extraction and cDNA synthesis

Total RNA from tissues and blood samples was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA, United States). The samples were added to TRIzol for 30 min in a 4 °C refrigerator. Total RNA was extracted from samples using chloroform, isopropanol, 75% absolute ethanol (all from Beijing Shiji Tuoxin Fine Chemical Industry Co. Ltd., Beijing, China) and diethyl-pyrocabonate-treated water (Biosharp, Heifei City, China). RNA was stored in a - 80 °C freezer. Concentration and purity were measured using a Nanodrop machine (Thermo Scientific, Carlsbad, CA, United States). As described previously, to synthesize cDNAs, PrimeScript RT reagent kits (TaKaRa, Dalian City, China) were purchased and used for reverse transcription (RT)^[21]. cDNA synthesis was conducted using a RT apparatus (Applied Biosystems, Foster City, CA, United States). The cDNA samples were stored at - 20 °C, and used for additional analysis.

Real-time quantitative reverse transcription polymerase chain reaction

To determine the expression of LOX and HIF1 α in tissues and blood samples from patients with GC, qRT-PCR analysis was performed using SYBR reagents (Vazyme, Nanjing City, China) on an Applied Biosystems Real-Time PCR machine (Thermo Scientific). The cDNAs, SYBR reagents, double-distilled water and corresponding primers were used to measure expression of LOX and HIF1 α . In addition, β -actin was used as a housekeeping gene. The sequences of primers used in this study are listed in Table 1.

Immunohistochemistry

Immunohistochemical staining was performed as described previously^[22,23]. Tissue specimens were fixed in 4% paraformaldehyde, and embedded in paraffin for subsequent analysis. Antibodies against LOX and HIF1 α were purchased from Abcam (Cambridge, United Kingdom). The concentrations of LOX and HIF1 α antibodies were 1:100 and 1:400 and the antibodies were diluted in Primary antibody dilution buffer. The paraffin specimens were incubated with these two antibodies that were used to detect the expression of LOX and HIF1 α in the GC tissues. Tissue sections were incubated with antibodies against LOX or HIF1 α , and then incubated with peroxidase-conjugated goat anti-rabbit secondary antibody (Cell Signaling Technology, Boston, MA, United States). All specimens were fixed in 4% paraformaldehyde, embedded in paraffin, and stored in the Department of Pathology at our hospital.

Statistical analysis

All experiments were repeated at least three times. Statistical analyses were performed with SPSS software (IBM, Armonk, NY, United States). Pearson's χ^2 tests for categorical variables. Survival rate was calculated by Kaplan-Meier method, Log-rank tests was used to confirm the relationship between LOX, HIF1 α and the development of GC. Analysis of LOX and HIF1 α expression was conducted using

Table 1 Primer sequences

Target gene		Primer sequences
HIF1 α	Forward	CATAAAGTCTGCAACATGGAAGGT
	Reverse	ATTTGATGGGTGAGGAATGGGTT
LOX	Forward	CAGGCACCGACCTGGATATGG
	Reverse	CGTACGTGGATGCCTGGATGTAGT
β -actin	Forward	TGGCAGCCAGCACAAATGAA
	Reverse	CTAAGTCATAGTCCGCCTAGAAGCA

GraphPad Prism software. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of LOX in patients with GC

Immunohistochemical staining and qRT-PCR analysis were performed to determine whether expression of LOX was dysregulated in tissues and blood samples from patients with GC versus healthy controls. Immunohistochemical staining indicated that LOX was mainly located in the cytoplasm of tumor cells. Most of the cytoplasm of tumor cells exhibited brown positively stained particles, which displayed a scattered particle distribution. The same results were seen in the extracellular matrix (Figure 1A). The high expression rate of LOX in GC tissues was 35.7% (50/140), which was significantly higher than that in adjacent tissues (18.6%, 26/140). QRT-PCR showed that mRNA expression of LOX was markedly increased in GC tissues; about four times higher than in adjacent tissues (Figure 1B). We further detected the expression level of LOX in blood samples from GC patients and healthy controls. Expression of LOX was higher in patients with GC than in the control group. Additional analysis showed that the later the clinicopathological stage, the higher the expression level of LOX in the blood samples (Figure 1C). Taken together, these results suggested that expression of LOX was significantly higher in patients with GC.

Expression of HIF1 α in patients with GC

Further analysis was performed to detect expression of HIF1 α in patients with GC. Immunohistochemistry revealed that HIF1 α was mainly expressed in the nuclei of tumor cells (Figure 2A). The nuclei of HIF1 α -positive cells presented with a brown color, and HIF1 α exhibited occasional limited expression in the cytoplasm (Figure 2A). Scattered light brown granules were revealed by immunohistochemical staining (Figure 2A). The high expression rate of HIF1 α in GC tissues was 33.6% (47/140), which was significantly higher than in adjacent tissues (12.1%, 17/140). The high expression of HIF1 α in tumor compared with adjacent tissues was measured by qRT-PCR (Figure 2B). Expression of HIF1 α in GC tissues was approximately four times higher than in adjacent tissues (Figure 2B). Additional analysis showed that the later the clinicopathological stage, the higher the expression level of HIF1 α in the blood samples (Figure 2C). Thus, we concluded that expression of HIF1 α was upregulated in tumor tissues and blood samples from patients with GC.

Correlation between LOX and clinicopathological characteristics of GC

Expression of LOX and clinicopathological characteristics are shown in Table 2. In various groups with different clinical characteristics, expression of LOX was different. Expression of the LOX gene correlated with lymph node metastasis of GC, the tumor infiltration depth and the tumor-node-metastasis (TNM) stage. The P values for the correlation between LOX expression and lymph node metastasis of GC, between LOX expression and the infiltration depth of GC, and between LOX expression and clinical stage were 0.000, 0.005 and 0.000, respectively. When the number of lymph node metastasis was >16 in patients with GC, the rate of high expression of LOX was 100.0%. When the tumor infiltration depth reached T4, the high expression rate of LOX was 54.1%. When the tumor was clinicopathological stage III, the high expression rate of LOX was 55.6%. However, expression of LOX did not show any relationship with other clinical features, such as age, gender, tumor size, and tumor location. The results showed that the expression of LOX increased with the number of lymph node metastases, deeper infiltration depth and the late TNM stage.

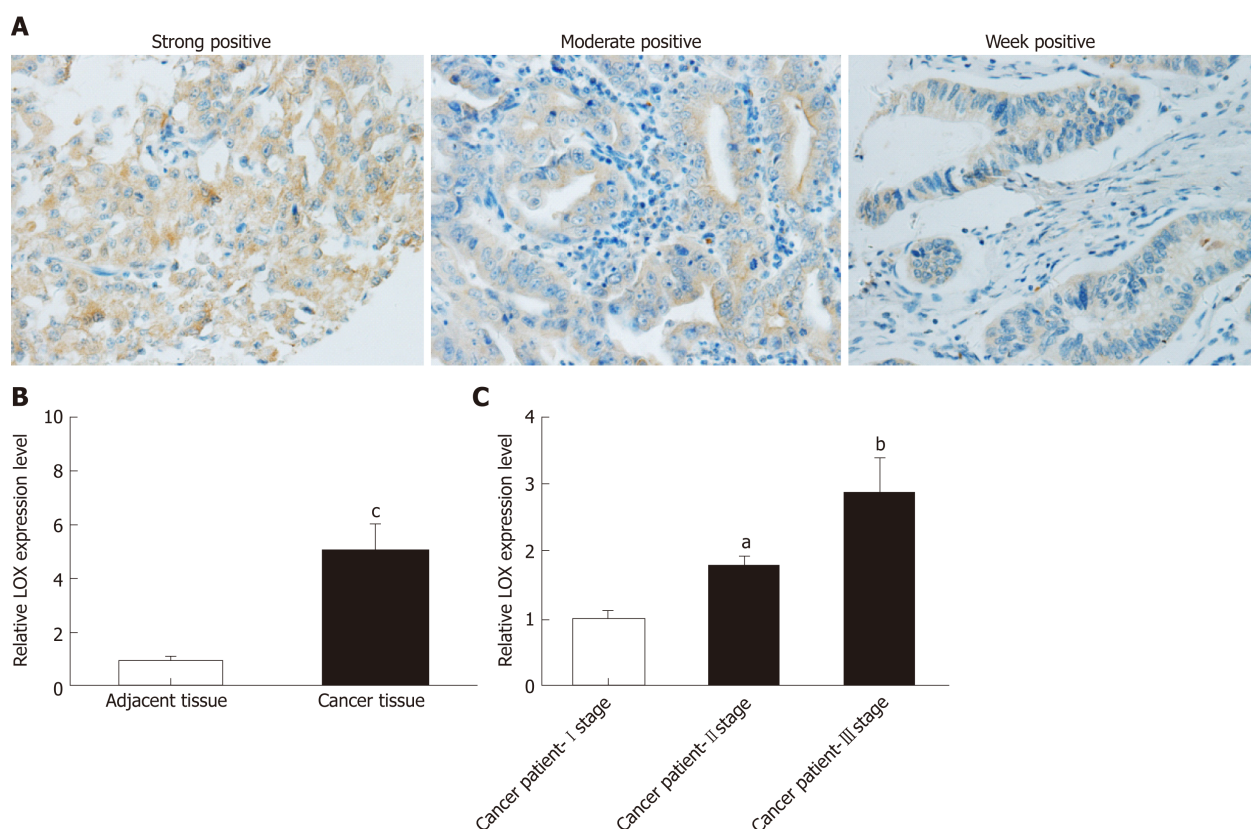


Figure 1 Expression of LOX in GC patients. A: Immunohistochemical staining was used to detect expression of LOX in tumor tissues from patients with GC; B: qRT-PCR was performed to measure mRNA expression of LOX in adjacent tissues and cancer tissues from GC patients; C: qRT-PCR was performed to examine the expression level of LOX in patients with GC. ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$ represent significant difference compared with controls. LOX: Lysyl oxidase; GC: Gastric cancer; qRT-PCR: Quantitative reverse transcription polymerase chain reaction.

Relationship between HIF1 α and clinicopathological characteristics of GC

Expression of HIF1 α and clinicopathological characteristics are shown in Table 3. Expression of HIF1 α differed among various groups. Expression of HIF1 α was related to lymph node metastasis ($P = 0.000$). When the number of lymph node metastasis was > 16 , the rate of high expression of HIF1 α was 100.0%. When the tumor infiltration depth reached T4, high expression of HIF1 α was 56.8% ($P = 0.001$). When they had stage III disease, the rate of high expression of HIF1 α was 51.9% ($P = 0.000$). These results suggested that expression of HIF1 α correlated with the number of metastatic lymph nodes, the tumor infiltration depth and the TNM stage. However, expression of HIF1 α was not associated with other clinical characteristics, such as age, gender, tumor size, and tumor location.

Relationship between expression of LOX and prognosis of GC

Single-factor analysis showed that disease-free survival (DFS) in the LOX-low expression group was 26.7 mo, which was significantly longer than in the LOX-high expression group at 15.6 mo ($P = 0.037$) (Figure 3A and Table 4). Overall survival (OS) of patients in the LOX-low expression group was significantly longer than that in the LOX-high expression group ($P = 0.033$) (Figure 3B and Table 4). These results demonstrated that high expression of LOX was associated with poor prognosis.

Relationship between expression of HIF1 α and prognosis of GC

To determine the correlation between HIF1 α expression and the prognosis of GC, we focused on DFS and OS in patients with different levels of HIF1 α expression. Single-factor survival analysis showed that DFS and OS in the HIF1 α -low expression group were 26.9 mo and 40.2 mo, respectively, which were significantly longer than in the HIF1 α -high expression group at 14.0 mo and 20.4 mo (both $P = 0.003$) (Figure 4A, 4B and Table 4). High expression of HIF1 α was associated with poor prognosis.

DISCUSSION

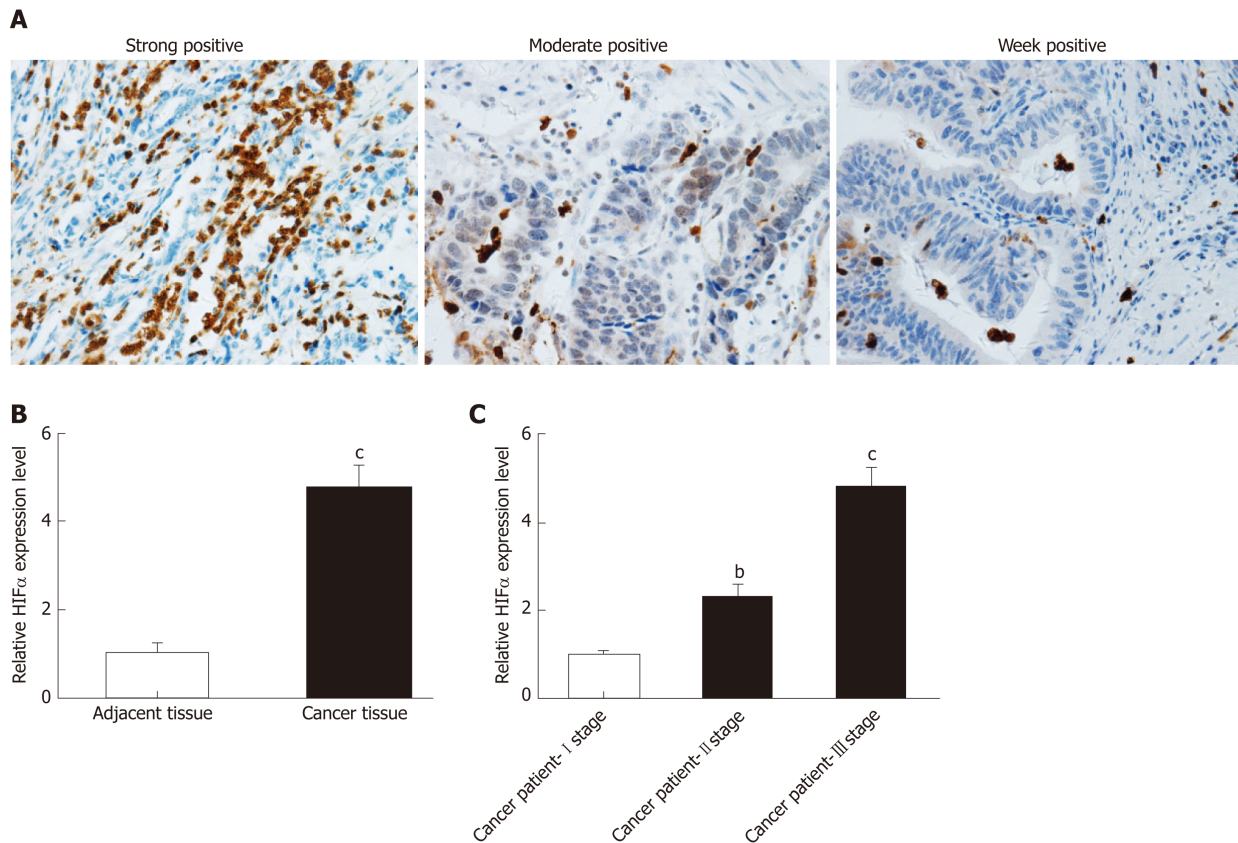


Figure 2 Expression of HIF1 α in patients with GC. A: Immunohistochemical analysis of HIF1 α in tumor tissues from patients with GC; B: qRT-PCR was applied to measure mRNA expression of HIF1 α in adjacent tissues and cancer tissues from GC patients; C: qRT-PCR was used to detect the level of HIF1 α in patients with GC. ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$ represent significant difference compared with controls. HIF1 α : Hypoxia-inducible factor 1 α ; GC: Gastric cancer; qRT-PCR: Quantitative reverse transcription polymerase chain reaction.

The occurrence of GC has been increasing rapidly worldwide^[24]. GC is related to the presence of tumor-suppressor or tumor-associated genes^[25]. There is no accurate and efficient clinical biomarker of GC. Other gastrointestinal biomarkers or early detection methods were also not applied in GC^[26-28]. Therefore, the aim of our study was to investigate whether LOX and HIF1 α could be used as biomarkers of GC.

LOX participates in the osteoclastogenesis of breast cancer, which suggests a therapeutic tool for osteolytic bone destruction^[21]. MiRNA-31-5p inhibits expression of HIF1 α and strengthens the Warburg effect by inhibiting its target HIF-1 α inhibitor. In addition, hepatitis transactivator protein X accelerates extracellular matrix modification by activating the HIF/LOX pathway and promoting the metastasis of hepatocellular carcinoma^[13]. Therefore, we predicted that LOX and HIF1 α could act as biomarkers of GC.

In the present study, immunohistochemical staining suggested that expression of LOX and HIF1 α increased in tumor tissues from patients with GC. QRT-PCR analysis indicated that mRNA expression of LOX and HIF1 α was upregulated in tumor tissues. We also detected expression of these two genes in blood samples. The results revealed that the expression levels of LOX and HIF1 α were higher in GC patients than in healthy controls. Additional analysis showed that the expression levels of LOX and HIF1 α were related to the clinicopathological characteristics of GC. The expression of LOX and HIF1 α increased with the number of lymph node metastases, deeper infiltration depth and the later TNM stage. Single-factor analysis showed that high expression of LOX and HIF1 α led to poor prognosis of GC patients.

In conclusion, our results demonstrated that expression of LOX and HIF1 α was higher in patients with GC than in healthy controls. Expression of LOX and HIF1 α was associated with clinicopathological characteristics and prognosis of GC. Thus, we concluded that LOX and HIF1 α could be used as prognostic and predictive biomarkers for GC. Our study provided a link between LOX, HIF1 α and GC, which contributes to the development and progression of GC.

Table 2 Relationship between lysyl oxidase and clinicopathological factors in patients with gastric cancer

Characteristics	Sample size, <i>n</i>	LOX		<i>P</i>
		Low expression (%)	High expression (%)	
Gender		90 (64.3)	50 (35.7)	
Male	112	73 (65.2)	39 (34.8)	0.659
Female	28	17 (60.7)	11 (39.3)	
Age				
< 60 yr	67	44 (65.7)	23 (34.3)	0.743
≥ 60 yr	73	46 (63.0)	27 (37.0)	
Tumor location				
Upper part	57	37 (64.9)	20 (35.1)	0.978
Middle part	34	21 (61.8)	13 (38.2)	
Lower part	44	29 (65.9)	15 (34.1)	
Total stomach	5	3 (60.0)	2 (40.0)	
Tumor size				
< 5 cm	57	39 (68.4)	18 (31.6)	0.397
≥ 5 cm	83	51 (61.4)	32 (38.6)	
Depth of invasion				
T1	5	5 (100.0)	0 (0.0)	0.005
T2	13	12 (92.3)	1 (7.7)	
T3	85	56 (65.9)	29 (34.1)	
T4	37	17 (45.9)	20 (54.1)	
Lymphatic metastasis				
0	34	33 (97.1)	1 (2.9)	0.000
1-2	32	24 (75.0)	8 (25.0)	
3-6	31	21 (67.7)	10 (32.3)	
7-15	37	12 (32.4)	25 (67.6)	
≥ 16	6	0 (0.0)	6 (100.0)	
Borrmann classification				
Type I	16	12 (75.0)	4 (25.0)	0.340
Type II or III	110	67 (60.9)	43 (39.1)	
Type IV	11	8 (72.7)	3 (27.3)	
Type V	3	3 (100.0)	0 (0.0)	
WHO histological classification				
Adenocarcinoma	77	48 (62.3)	29 (37.7)	0.862
Signet-ring cell carcinoma	17	13 (76.5)	4 (23.5)	
Mucinous adenocarcinoma	8	5 (62.5)	3 (37.5)	
Mixed carcinoma	32	20 (62.5)	12 (37.5)	
Neuroendocrine carcinoma	6	4 (66.7)	2 (33.3)	
Lauren parting				
Intestinal type	65	42 (64.6)	23 (35.4)	0.909
Diffuse type	68	43 (63.2)	25 (36.8)	
Mixed type	7	5 (71.4)	2 (28.6)	
Differentiation grade				
Low and middle	107	68 (63.6)	39 (36.4)	0.744
High	33	22 (66.7)	11 (33.3)	
Cancer embolus				
Yes	51	29 (56.9)	22 (43.1)	0.165
No	89	61 (68.5)	28 (31.5)	
Affect neural				
Yes	50	29 (58.0)	21 (42.0)	0.247
No	90	61 (67.8)	29 (32.2)	

TNM staging				
I	10	10 (100.0)	0 (0.0)	0.000
II	49	44 (89.8)	5 (10.2)	
III	81	36 (44.4)	45 (55.6)	

LOX: Lysyl oxidase; WHO: World Health Organization; TNM: Tumor-node-metastasis.

Table 3 Relationship between hypoxia-inducible factor 1 α and clinicopathological factors in patients with gastric cancer

Characteristics	Sample size, <i>n</i>	HIF1 α		<i>P</i>
		Low expression (%)	High expression (%)	
Gender		93 (66.4)	47 (33.6)	0.474
Male	112	76 (67.9)	36 (32.1)	
Female	28	17 (60.7)	11 (39.3)	
Age				0.372
< 60 yr	67	47 (70.1)	20 (29.9)	
≥ 60 yr	73	46 (63.0)	27 (37.0)	
Tumor location				0.702
Upper part	57	41 (71.9)	16 (28.1)	
Middle part	34	19 (55.9)	15 (44.1)	
Lower part	44	30 (68.2)	14 (31.8)	
Total stomach	5	3 (60.0)	2 (40.0)	
Tumor size				0.253
< 5 cm	57	41 (71.9)	16 (28.1)	
≥ 5 cm	83	52 (62.7)	31 (37.3)	
Depth of invasion				0.001
T1	5	5 (100.0)	0 (0.0)	
T2	13	12 (92.3)	1 (7.7)	
T3	85	60 (70.6)	25 (29.4)	
T4	37	16 (43.2)	21 (56.8)	
Lymphatic metastasis				0.000
0	34	29 (85.3)	5 (14.7)	
1-2	32	24 (75.0)	8 (25.0)	
3-6	31	23 (74.2)	8 (25.8)	
7-15	37	17 (45.9)	20 (54.1)	
≥ 16	6	0 (0.0)	6 (100.0)	
Borrmann classification				0.183
Type I	16	11 (68.8)	5 (31.2)	
Type II or III	110	76 (69.1)	34 (30.9)	
Type IV	11	4 (36.4)	7 (63.6)	
Type V	3	2 (66.7)	1 (33.3)	
WHO histological classification				0.725
Adenocarcinoma	77	55 (71.4)	22 (28.6)	
Signet-ring cell carcinoma	17	12 (70.6)	5 (29.4)	
Mucinous adenocarcinoma	8	6 (75.0)	2 (25.0)	
Mixed carcinoma	32	20 (62.5)	12 (37.5)	
Neuroendocrine carcinoma	6	4 (66.7)	2 (33.3)	
Lauren parting				0.155
Intestinal type	65	42 (64.6)	23 (35.4)	
Diffuse type	68	44 (64.7)	24 (35.3)	
Mixed type	7	7 (100.0)	0 (0.0)	
Differentiation grade				0.698
Low and middle	107	72 (67.3)	35 (32.7)	

High	33	21 (63.6)	12 (36.4)	
Cancer embolus				
Yes	51	32 (62.7)	19 (37.3)	0.485
No	89	61 (68.5)	28 (31.5)	
Affect neural				
Yes	50	30 (60.0)	20 (40.0)	0.230
No	90	63 (70.0)	27 (30.0)	
TNM staging				
I	10	10 (100.0)	0 (0.0)	0.000
II	49	44 (89.8)	5 (10.2)	
III	81	39 (48.1)	42 (51.9)	

HIF1 α : Hypoxia-inducible factor 1 α ; WHO: World Health Organization; TNM: Tumor-node-metastasis.

Table 4 Univariate analyses of disease-free survival and overall survival in patients with gastric cancer

Gene	Expression	Cases, <i>n</i>	Disease free survival			Overall survival		
			mo	<i>P</i>		mo	<i>P</i>	
LOX	High	50	15.6	0.037	4.352	22.9	0.033	4.524
	Low	90	26.7			40.2		
HIF1 α	High	47	14.0	0.003	8.712	20.4	0.003	8.992
	Low	93	26.9			40.2		

LOX: Lysyl oxidase; HIF1 α : Hypoxia-inducible factor 1 α .

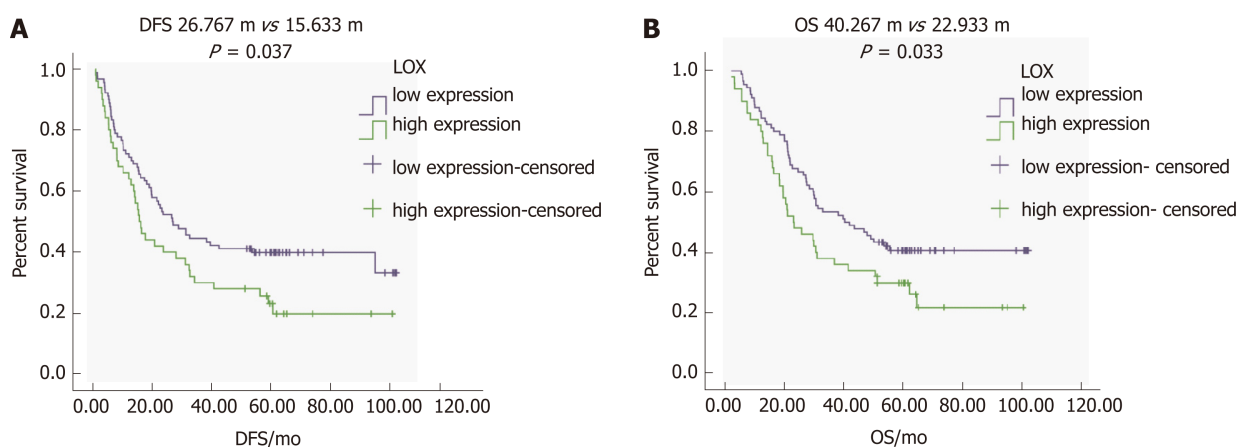


Figure 3 LOX expression is associated with DFS and OS. A: DFS curve of patients with GC with regards to lysyl oxidase expression, $^aP < 0.05$; B: Survival analysis of overall survival in patients with GC, $^aP < 0.05$. LOX: Lysyl oxidase; GC: Gastric cancer; DFS: Disease-free survival; OS: Overall survival.

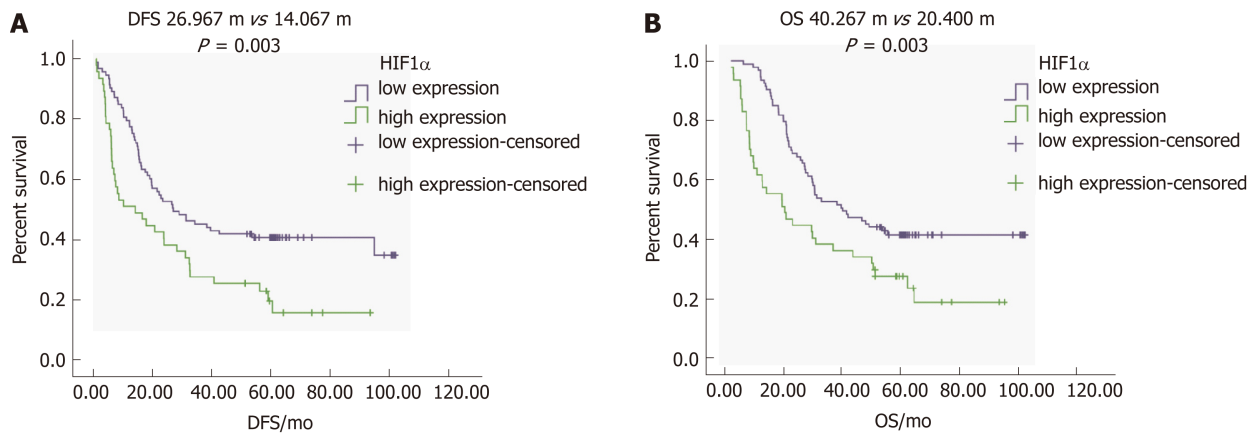


Figure 4 HIF1 α correlates with DFS and OS of patients with GC. A: DFS curve of patients with GC with respect to HIF1 α expression, $^aP < 0.05$; B: OS curve of patients with GC expressing low and high HIF1 α levels. $^aP < 0.05$. GC: Gastric cancer; DFS: Disease-free survival; OS: Overall survival; HIF1 α : Hypoxia-inducible factor 1 α .

ARTICLE HIGHLIGHTS

Research background

To study whether lysyl oxidase (LOX) and hypoxia-inducible factor 1 α (HIF1 α) can be used as prognostic and predictive biomarkers in gastric cancer (GC).

Research motivation

To provide the prognostic and predictive biomarkers for treating GC.

Research objectives

To explore the interaction between cancer and factors in the metabolic microenvironment and determine predictive biomarkers of GC.

Research methods

Patients and samples: This work is difficult and requires patient approval.

RNA extraction and cDNA synthesis: This work requires a lot of time, and so the manipulator should be patient. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR): The manipulator should add the samples accurately during qRT-PCR analysis. Immunohistochemistry: The experiments take a long time, and the manipulator should perform the experiments step by step. **Statistical analysis:** All of the experiments were repeated at least three times, and the statistical analysis was then performed.

Research results

The expression levels of LOX and HIF1 α increased in the tumor tissues and blood samples of patients with GC. The expression levels of LOX and HIF1 α were related to the clinicopathological characteristics and prognosis of GC.

Research conclusions

LOX and HIF1 α can be used as prognostic and predictive biomarkers for the treatment of GC. Our study indicated that the expression of LOX and HIF1 α was upregulated in patients with GC compared with the control group. In addition, the expression of LOX and HIF1 α was related to the clinicopathological characteristics and prognosis of GC.

Research perspectives

The present study suggested that LOX and HIF1 α might be used as both prognostic and predictive biomarkers for GC, and provided a link between LOX, HIF1 α and GC.

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