Observational Study

Medicine®

OPEN

The diagnostic performance of serum LIM homeobox transcription factor 1 alpha in patients with gastric cancer

Dinuo Li, PhD[a], Chen Li, PhD[b,*]

Abstract

The study was conducted to investigate the diagnostic performance of serum LIM homeobox transcription factor 1 alpha (LMX1A) in patients with gastric cancer (GC).

The serum level of *LMX1A* in GC, benign, and healthy groups was measured using quantitative real time PCR (qRT-PCR) and compared with the student *t* test. The associations of serum *LMX1A* levels with clinical parameters were analyzed with chi-square test. The diagnostic value of serum *LMX1A* in GC was evaluated by receiver operating characteristic (ROC) curve.

The level of serum *LMX1A* in GC group (1.309 ± 0.553) was significantly lower than that in the benign group (2.174 ± 0.676) and healthy group (2.598 ± 0.826) ($P < .01$ for both). The decreased level of *LMX1A* was associated with large tumor size ($P = .009$), positive lymph node metastasis ($P = .027$), and advanced TNM stages ($P = .002$). Receiver operating characteristic (ROC) analysis demonstrated that serum *LMX1A* could discriminate GC patients from the healthy individuals, with the area under the curve (AUC) of 0.889 (95% confidence interval [CI] = 0.838–0.938) combining with the sensitivity and specificity of 82.68% and 82.61%. Additionally, serum *LMX1A* also exhibited high accuracy in discriminating between GC patients and benign gastric disease cases (AUC = 0.842, 95% CI = 0.782–0.901), with the sensitivity of 81.89% and specificity of 72.41%.

Serum *LMX1A* may be an effective biomarker for early detection of GC.

Abbreviations: AUC = area under the curve, CA19-9 = Carbohydrate antigen 19-9, CA72-4 = Carbohydrate antigen 72-4, CEA = carcinoembryonic antigen, GC = gastric cancer, *H pylori* = *Helicobacter pylori*, *LMX1A* = LIM homeobox transcription factor 1 alpha, qRT-PCR = quantitative real time polymerase chain reaction, ROC = receiver operating characteristic, SD = standard deviation.

Keywords: diagnosis, gastric cancer, LIM homeobox transcription factor 1 alpha, receiver operating characteristic

1. Introduction

Gastric cancer (GC), originating from gastric mucosal epithelium, is one of the most prevalent malignancies all over the world, especially in the East Asia countries.[1–3] GC represents a primary reason for cancer-related deaths worldwide, posing a severe threat to human health.[4,5] There are various available therapeutic strategies for GC patients, including surgery, chemotherapy, radiotherapy, and other treatments. However, the prognosis of the patients remains unsatisfactory, with high mortality.[6] Delay in early diagnosis may be responsible for the dismal outcomes.[7] Unfortunately, many patients do not present specific symptoms until advanced stage, consequently limited therapeutic effects, and dismal survival.[8,9] Currently, the early screening of GC mainly dependents on endoscopy and biopsy, but their application value is limited by the high cost and invasive operations.[10,11] Additionally, the commonly used serum biomarkers, such as carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), and carbohydrate antigen 72-4 (CA72-4), exhibit unsatisfactory sensitivity and specificity for GC diagnosis.[12,13] Therefore, it is in urgent need to discover novel and noninvasive biomarkers for early diagnosis of GC to improve the prognosis.

LIM homeobox transcription factor 1 alpha (*LMX1A*) is mapped to human chromosome 1q24.1, and consists of 11 exons with the length of about 151 kb.[14,15] As a member of the LIM homeobox-containing family, *LMX1A* contains a homeodomain and 2 LIM domains.[16] *LMX1A* plays important roles in various biological progresses, such as cell proliferation, apoptosis, differentiation, and neurogenesis.[17–19] Recently, growing evidences have reported that *LMX1A* may be involved in tumorigenesis. Dysregulation of *LMX1A* was observed in diverse types of cancer, such as cervical cancer and ovarian cancer.[20,21] It was reported that *LMX1A* was down-regulated in malignancy, and played inhibitory roles in multisteps of tumorigenesis. Thus, we speculated that *LMX1A* might be a candidate biomarker for early detection of GC.

Editor: Bülent Kantarçeken.

Funded by Youth Fund of the First Affiliated Hospital of Liaoning Medical College (FY2012-11) and Natural Science Foundation of Liaoning Province (20170540334).

The authors have no conflicts of interest to disclose.

[a] Gastrointestinal Surgery, [b] Molecular Testing Center, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou City, Liaoning Province, China.

[*] Correspondence: Chen Li, Molecular Testing Center, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou City 121000, Liaoning Province, China (e-mail: rneobz@163.com).

Medicine (2019) 98:22(e15783)

Received: 30 August 2018 / Received in final form: 30 April 2019 / Accepted: 1 May 2019

http://dx.doi.org/10.1097/MD.0000000000015783

In this study, we aimed to investigate the clinical significance of serum *LMX1A* in GC. The expression pattern of *LMX1A* in GC was detected, as well as its association with clinical parameters of the patients. In addition, the diagnostic performance of serum *LMX1A* for GC was evaluated in the present study.

2. Materials and methods

2.1. Patients and specimens

A total of 127 patients, who were pathologically diagnosed with GC by 2 independent pathologists in The First Affiliated Hospital of Jinzhou Medical University were recruited in the study. All the patients were aged from 25 to 79 years old, including 62 men and 65 women. Other clinical features were listed in Table 1. In addition, 58 patients with benign gastric diseases and 69 healthy blood donors in the same hospital were enrolled in our study. All patients, both the GC and benign cases, had not received chemotherapy, radiotherapy, or other treatments before blood collection. Five milliliter peripheral blood was collected from each participant. Then the serum specimens were prepared from the peripheral blood through centrifugation. This study was supported by the Ethics Committee of The First Affiliated Hospital of Jinzhou Medical University. Written informed consent was obtained from each participant.

2.2. RNA extraction and quantitative real time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from serum samples of GC patients, benign gastric disease patients, and healthy controls using Trizol reagent (Invitrogen) following the manufacture's instructions. Then the first strand of cDNA was constructed through the reverse transcription using PrimerScript RT reagent kit (Takara, Dalian, China), and the extracted RNA samples served as temple. Finally, the real-time PCR was performed to determine the relative expression of serum *LMX1A* mRNA. The reaction was performed with SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA) on ABI Prism 7900 Sequence Detection System (Applied Biosystems). *GAPDH* was adopted as the internal reference. The primer sequences were as follows: *GAPDH* forward, 5′-ATGGGGAAGGTGAAGGTCGG-3′; reverse, 5′-GACGGTGCCATGGAATTTGC-3′. *LMX1A* forward, 5′-CCCTCAGTAACCTGGGTGATTGT-3′; reverse, 5′-TCTTCCCTGGCCTCCCTGTCCTA-3′. Each sample was repeated in 3 times, and the relative expression of *LMX1A* was calculated by $2^{-\Delta\Delta Ct}$ method.

Table 1

Relationship between serum *LMX1A* expression and clinical characteristics in GC.

Clinical feature	Case No.	*LMX1A* expression		X^2	*P* value
		Low (n = 64)	High (n = 63)		
Age, y				0.389	.533
≦50	65	31	34		
>50	62	33	29		
Gender				1.778	.182
Female	65	29	36		
Male	62	35	27		
Alcohol abuse				2.835	.092
Ever	66	38	28		
Never	61	26	35		
Smoking status				2.306	.129
Yes	59	34	25		
No	68	30	38		
Tumor size, cm				6.740	.009
≦3	70	28	42		
>3	57	36	21		
Lymph node metastasis				4.915	.027
Positive	65	39	26		
Negative	62	25	37		
TNM stage				9.643	.002
I, II	63	23	40		
III, IV	64	41	23		

GC = gastric cancer, *LMX1A* = LIM homeobox transcription factor 1 alpha.

2.3. Statistical analysis

All data were analyzed by SPSS version 18.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 5.0 software (GraphPad, San Diego, CA). The expression value of serum *LMX1A* was presented as mean ± standard deviation (SD), and compared by student *t* test. Chi-square test was conducted to analyze the relationships between *LMX1A* expression and clinical features of patents. Receiver operating characteristic (ROC) curve was used to assess the diagnostic value of serum *LMX1A* in GC with the area under the curve (AUC). *P* value $<.05$ was considered significant.

3. Results

3.1. Down-regulation of serum LMX1A mRNA in GC

To investigate the level of serum *LMX1A* mRNA in GC patients, begin gastric disease patients and healthy controls, qRT-PCR was conducted. As shown in Fig. 1, compared with the healthy individuals, *LMX1A* mRNA level was down-regulated in GC group ($P<.0001$). Moreover, the serum level of *LMX1A* was significantly different between GC patients and benign gastric diseases cases ($P<.01$). GC patients showed decreased serum levels of *LMX1A* mRNA (Fig. 1).

Figure 1. The expression of serum *LMX1A* mRNA in GC patients, benign gastric diseases patients, and healthy controls. Serum levels of *LMX1A* were lower in GC cases than that in the healthy individuals. Furthermore, compared with benign gastric disease patients, GC cases showed down-regulated serum *LMX1A* level. **: indicated $P<.01$; ***: suggested $P<.001$. GC = gastric cancer, *LMX1A* = LIM homeobox transcription factor 1 alpha.

3.2. Relationship of serum LMX1A expression and clinical characteristics of patients

To explore the association of serum *LMX1A* level with clinical parameters, patients were divided into low *LMX1A* group (n = 64) and high *LMX1A* group (n = 63) based on the median *LMX1A* expression. Chi-square test revealed that serum *LMX1A* expression exhibited negative association with tumor size ($P = .009$), lymph node metastasis ($P = .027$), and TNM stage ($P = .002$). However, there was no significant association between serum *LMX1A* expression and age, sex, alcohol abuse, or smoking status ($P > .05$ for all) (Table 1).

3.3. Diagnostic significance of serum LMX1A in GC

To evaluate the diagnostic performance of serum *LMX1A* in GC, ROC curves were constructed. As shown in Fig. 2, with the benign gastric disease population as reference, the AUC value was 0.842 (95% CI = 0.782–0.901), indicating serum *LMX1A* could discriminate GC patients from those with benign gastric disease. The optimal cutoff point was 1.725, with the sensitivity and specificity of 81.89% and 72.41%, respectively. Additionally, with the healthy individuals as reference, the AUC value of the curve was 0.889, suggesting serum *LMX1A* was a candidate for selecting GC patients from healthy population. The optimal cutoff point was 1.755, with the specificity of 82.61% and sensitivity of 82.68% (Fig. 3).

4. Discussion

GC is one of the most common malignant tumors in our countries. Several risk factors may contribute to its occurrence, such as chronic gastric diseases, dysplasia of gastric mucosa epithelial, and *Helicobacter pylori* (*H pylori*) infection.[22,23] Radical surgery is the only curative method for GC patients.[24] However, the majority of GC patents are at advanced stages when initially diagnosed, missing the operation opportunity. Tumor stage at diagnosis is the key factor for outcomes of patients with GC. Until now, the commonly used detection tools showed unsatisfactory performance for early diagnosis of GC. Therefore, it is necessary to find novel diagnostic biomarkers with high sensitivity and specificity for GC.

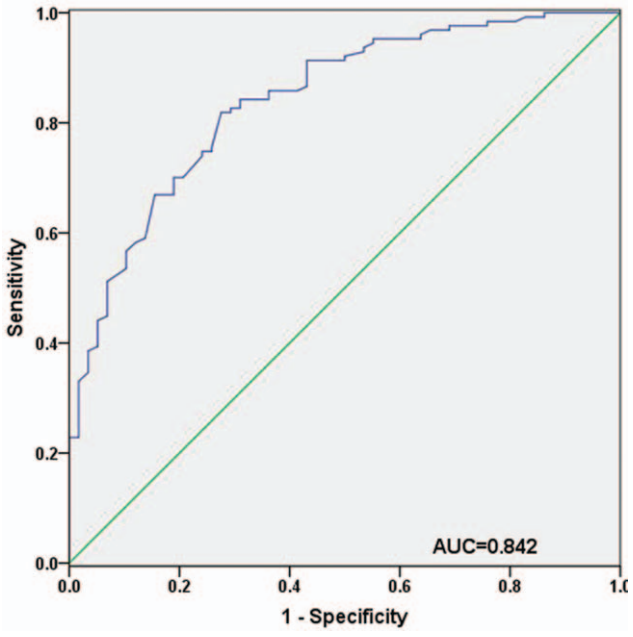

Figure 2. ROC curve was constructed based on serum *LMX1A* levels in GC patients and benign gastric disease cases. The curve demonstrated that serum *LMX1A* could discriminate between GC and benign gastric disease with the AUC value of 0.842 (95% CI = 0.782–0.901), combining with the sensitivity and specificity of 81.89% and 72.41%, respectively. The optimal cutoff point was 1.725. AUC = area under the curve, GC = gastric cancer, *LMX1A* = LIM homeobox transcription factor 1 alpha, ROC = receiver operating characteristic.

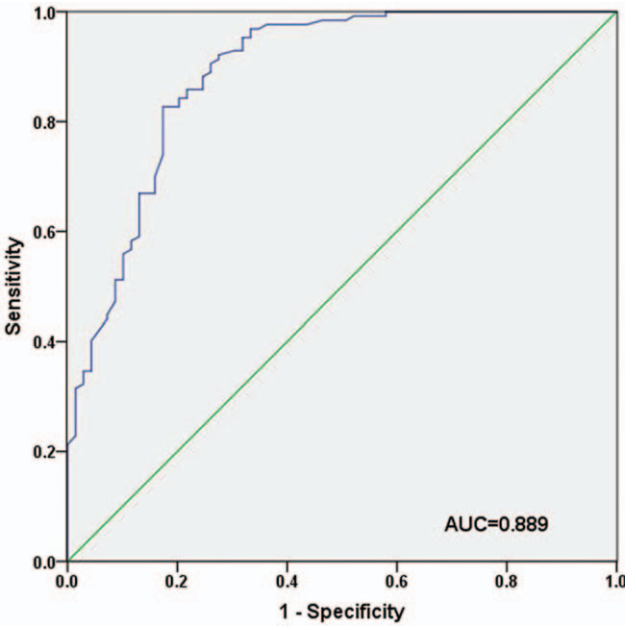

Figure 3. The accuracy of serum *LMX1A* in discriminating between GC cases and healthy individuals. The AUC value of the curve was 0.889, suggesting serum *LMX1A* might be a candidate biomarker for screening of GC. The optimal cutoff point was 1.755, with the specificity of 82.61% and sensitivity of 82.68%. AUC = area under the curve, GC = gastric cancer, *LMX1A* = LIM homeobox transcription factor 1 alpha.

The development and progression of GC is a complex process which is implicated with the activation of oncogenes and inactivation of tumor suppressor genes.[25] The molecular biomarkers which may provide new insights into the mechanisms of GC are considered as novel and promising candidate biomarkers for early diagnosis, progression prediction, and targeted therapy in management of malignancy. In the previous studies, various genetic biomarkers were confirmed for GC. He et al,[26] reported that the expression of *Tspan 5* was significantly down-regulated in GC, moreover, its expression patterns showed inverse association with clinical characteristics that might be a potential independent biomarker for prognosis of the patients. The research carried out by Kong et al[27] demonstrated that long non-coding RNA *PVT1* as a tumor oncogene was involved in malignant progression of GC, and its over-expression predicted poor outcomes of the patients. The altered genes during tumorigenesis can provide accurate information for tumor development and progression which may be candidate biomarkers for human cancers.

LMX1A, a tumor suppressor, is a newly discovered transcription factor, which is implicated in cell proliferation, differentiation, apoptosis, embryonic development, and organ formation.

The abnormal behavior of *LMX1A* has been found in GC with down-regulated expression, indicating *LMX1A* might be related with the development and progression of GC.[28] Thereby, in the present study, we explored the significance of serum *LMX1A* in the diagnosis of GC. We found that serum *LMX1A* mRNA level was significantly decreased in GC patients compared with that in healthy controls and benign gastric diseases. Furthermore, down-regulation of *LMX1A* showed close relation with larger tumor size, positive lymph node metastasis, and advanced TNM stage. All the data indicated that *LMX1A* played inhibitory roles in aggressive development and progression of GC, revealing its function as a tumor suppressor in the disease.

Given its function in tumorigenesis, we hypothesized that serum *LMX1A* might serve as a biomarker in GC diagnosis. ROC analysis showed that serum *LMX1A* could discriminate GC patients from healthy controls and individuals with benign gastric diseases with high accuracy. Serum *LMX1A* was an efficient biomarker for early detection of GC. Although we had confirmed the low expression of serum *LMX1A* in GC and identified its diagnostic role in this disease, the accurate inhibitory mechanism of *LMX1A* in GC was not known yet. The inactivation of *LMX1A* in GC might be mediated hyper-methylation.[29] In the next study, the mechanisms of anti-tumor action of *LMX1A* in GC needed to be identified. Besides, our results might be limited by the relatively small sample size in the current study. Thus, further researches were still required to investigate the application value of serum *LMX1A* for GC diagnosis in clinical setting.

In conclusion, serum *LMX1A* is significantly decreased in GC patients compared with patients with benign gastric diseases and healthy controls. Moreover, its down-regulation is obviously correlated with large tumor size, positive lymph node metastasis, and advanced TNM stage. Serum *LMX1A* may be a potential biomarker for GC diagnosis.

Author contributions

Conceptualization: Dinuo Li.
Data curation: Dinuo Li.
Formal analysis: Dinuo Li, Chen Li.
Funding acquisition: Dinuo Li, Chen Li.
Investigation: Dinuo Li, Chen Li.
Methodology: Dinuo Li, Chen Li.
Project administration: Dinuo Li, Chen Li.
Resources: Dinuo Li, Chen Li.
Software: Dinuo Li, Chen Li.
Supervision: Dinuo Li, Chen Li.
Validation: Dinuo Li, Chen Li.
Visualization: Dinuo Li, Chen Li.
Writing – original draft: Dinuo Li, Chen Li.
Writing – review & editing: Dinuo Li, Chen Li.

References

[1] Cai H, Ye F, Michel A, et al. Helicobacter pylori blood biomarker for gastric cancer risk in East Asia. Int J Epidemiol 2016;45:774–81.
[2] Arhan M, Yilmaz H, Onal IK, et al. DR-70 as a novel diagnostic biomarker for gastric cancer. Turk J Gastroenterol 2015;26:480–3.
[3] Gao J, Cao R, Mu H. Long non-coding RNA UCA1 may be a novel diagnostic and predictive biomarker in plasma for early gastric cancer. Int J Clin Exp Pathol 2015;8:12936–42.
[4] Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
[5] Wu X, Tan X, Fu SW. May circulating microRNAs be gastric cancer diagnostic biomarkers? J Cancer 2015;6:1206–13.
[6] Duraes C, Almeida GM, Seruca R, et al. Biomarkers for gastric cancer: prognostic, predictive or targets of therapy? Virchows Arch 2014;464:367–78.
[7] Shao J, Fang PH, He B, et al. Downregulated microRNA-133a in gastric juice as a clinicopathological biomarker for gastric cancer screening. Asian Pac J Cancer Prev 2016;17:2719–22.
[8] Chan AW, Mercier P, Schiller D, et al. (1)H-NMR urinary metabolomic profiling for diagnosis of gastric cancer. Br J Cancer 2016;114:59–62.
[9] Beeharry MK, Liu WT, Yan M, et al. New blood markers detection technology: a leap in the diagnosis of gastric cancer. World J Gastroenterol 2016;22:1202–12.
[10] Liu WL, Liu D, Cheng K, et al. Evaluating the diagnostic and prognostic value of circulating cathepsin S in gastric cancer. Oncotarget 2016;7:28124–38.
[11] Zhang Z, Dou M, Yao X, et al. Potential biomarkers in diagnosis of human gastric cancer. Cancer Investig 2016;34:115–22.
[12] Pan YQ, Ruan YY, Peng JB, et al. Diagnostic significance of soluble human leukocyte antigen-G for gastric cancer. Hum Immunol 2016;77:317–24.
[13] Yang Z, Guo X, Li G, et al. Long noncoding RNAs as potential biomarkers in gastric cancer: opportunities and challenges. Cancer Lett 2016;371:62–70.
[14] Wu N, Yuan S, Liu J, et al. Association of LMX1A genetic polymorphisms with susceptibility to congenital scoliosis in Chinese Han population. Spine (Phila Pa 1976) 2014;39:1785–91.
[15] Tsai WC, Lee HS, Lin CK, et al. The association of osteopontin and LMX1A expression with World Health Organization grade in meningiomas and gliomas. Histopathology 2012;61:844–56.
[16] Steffes G, Lorente-Canovas B, Pearson S, et al. Mutanlallemand (mtl) and Belly Spot and Deafness (bsd) are two new mutations of Lmx1a causing severe cochlear and vestibular defects. PLoS One 2012;7:e51065.
[17] Tsai WC, Lin CK, Yang YS, et al. The correlations of LMX1A and osteopontin expression to the clinicopathologic stages in pancreatic adenocarcinoma. Appl Immunohistochem Mol Morphol 2013;21:395–400.
[18] Hoekstra EJ, von Oerthel L, van der Linden AJ, et al. Lmx1a is an activator of Rgs4 and Grb10 and is responsible for the correct specification of rostral and medial mdDA neurons. Eur J Neurosci 2013;37:23–32.
[19] Nefzger CM, Su CT, Fabb SA, et al. Lmx1a allows context-specific isolation of progenitors of GABAergic or dopaminergic neurons during neural differentiation of embryonic stem cells. Stem Cells 2012;30:1349–61.
[20] Chang CC, Huang RL, Wang HC, et al. High methylation rate of LMX1A, NKX6-1, PAX1, PTPRR, SOX1, and ZNF582 genes in cervical adenocarcinoma. Int J Gynecol Cancer 2014;24:201–9.
[21] Chao TK, Yo YT, Liao YP, et al. LIM-homeobox transcription factor 1, alpha (LMX1A) inhibits tumourigenesis, epithelial-mesenchymal transition and stem-like properties of epithelial ovarian cancer. Gynecol Oncol 2013;128:475–82.
[22] Werner S, Chen H, Butt J, et al. Evaluation of the diagnostic value of 64 simultaneously measured autoantibodies for early detection of gastric cancer. Sci Rep 2016;6:25467.
[23] Plummer M, Franceschi S, Vignat J, et al. Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer 2015;136:487–90.
[24] Xiao H, Zhang Y, Kim Y, et al. Differential proteomic analysis of human saliva using tandem mass tags quantification for gastric cancer detection. Sci Rep 2016;6:22165.
[25] Dammann R, Schagdarsurengin U, Strunnikova M, et al. Epigenetic inactivation of the Ras-association domain family 1 (RASSF1A) gene and its function in human carcinogenesis. Histol Histopathol 2003;18:665–77.
[26] He P, Wang S, Zhang X, et al. Tspan5 is an independent favourable prognostic factor and suppresses tumour growth in gastric cancer. Oncotarget 2016;7:40160–73.
[27] Kong R, Zhang EB, Yin DD, et al. Long noncoding RNA PVT1 indicates a poor prognosis of gastric cancer and promotes cell proliferation through epigenetically regulating p15 and p16. Mol Cancer 2015;14:82.
[28] Feng L, Xie Y, Zhao Z, et al. LMX1A inhibits metastasis of gastric cancer cells through negative regulation of beta-catenin. Cell Biol Toxicol 2016;32:133–9.
[29] Dong W, Feng L, Xie Y, et al. Hypermethylation-mediated reduction of LMX1A expression in gastric cancer. Cancer Sci 2011;102:361–6.