Int J Clin Exp Pathol 2016;9(2):1982-1988
www.ijcep.com /ISSN:1936-2625/IJCEP0018858

Original Article

FER1L4: a potential plasma biomarker to identify gastric cancer with lymph node invasion

Ming Liang[1], Jiong Huang[2], Jiang He[1], Qianchuan Yi[1], Zuwang Zhang[1], Likuan Tu[1], Donghe Ma[1], Jia Yan[1]

[1]Center of Digestive Nerve Center, The Affiliated University City Hospital of Chongqing Medical University, Chongqing, China; [2]Dagongguan Branch of The Affiliated University City Hospital of Chongqing Medical University, Chongqing, China

Received October 30, 2015; Accepted December 26, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Objective: To explore the efficacy of lncRNA-Fer-1-like protein 4 (FER1L4) in the diagnosis and evaluation of gastric cancer (GC). Methods: 39 healthy volunteers and 121 GC patients were enrolled. Plasma FER1L4 level was measured by real-time quantitative reverse transcriptase-polymerase chain reaction. Then, the correlations between plasma FER1L4 and clinicopathological characteristics were assessed. A receiver operating characteristic (ROC) curve was conducted to assess the efficacy of plasma FER1L4 in evaluating disease status. Results: Significant difference of plasma FER1L4 was found between healthy control and GC, GC with lymph node invasion, T1 GC with lymph node invasion, gastric cancer with no metastasis and metastasis (P<0.001), while no significant difference between healthy control and GC without lymph node invasion (P=0.132) and T1 gGC without lymph node invasion (P=0.915) was found. And, plasma FER1L4 level was significantly correlated with lymph node status in GC (P<0.001) and T1 GC (P<0.001) and metastasis status in GC (P<0.001). ROC analysis indicated that FER1L4 yielded an AUC of 0.897 (95% CI: 0.821-0.949, sensitivity: 93.7%, specificity: 79.5%) in distinguishing GC with lymph node invasion form healthy control, an AUC of 0.868 (95% CI: 0.795-0.923, sensitivity: 87.3%, specificity: 77.6%) in distinguishing GC with lymph node invasion form GC without lymph node invasion, an AUC of 0.928 (95% CI: 0.791-0.987, sensitivity: 100%, specificity: 68.7%) in distinguishing lymph node invasion form no invasion in T1 GC. Conclusion: Plasma FER1L4 has potential to identify GC with lymph node invasion.

Keywords: Gastric cancer, LncRNA, FER1L4, plasma, tumor marker

Introduction

It is estimated that there were 951,600 new cases of gastric cancer and 723,100 gastric cancer-related deaths worldwide in 2012 [1]. The ideal treatment for gastric cancer should be made after fully considering the information of primary tumor features, lymph node invasion and distant metastasis [2]. It is worthy to note that endoscopic mucosal resection and submucosal resection has been developed to spate surgical morbidity for T1 gastric cancer patients without lymph node invasion [3]. At present, the tumor evaluation is mainly based on computed tomography, positron emission tomography-computed tomography, endoscopy and biopsy [2]. Sometimes, it is difficult for the above techniques to evaluate the status of lymph nodes invasion. A recent study indicated that tumor size, site, degree of differentiation, macroscopic tumor sub-classification, perineural invasion status, and depth of submucosal tumor penetration couldn't predict lymph node invasion, the only predictors of lymph node invasion was lymphovascular invasion and positive nodal status [3]. To be honest, lymphovascular invasion and nodal status are hard to evaluate before operation. Therefore, looking for sensitive and specific biomarkers for the diagnosis and evaluation of gastric cancer is an important target of cancer research.

Long non-coding RNAs (lncRNAs), as major factors in governing fundamental biological processes, regulates the expression of target genes at transcriptional, posttranscriptional, and epigenetic levels [4, 5]. Accumulating evidence has indicated that dysregulation of lncRNAs played an important role in the development of various malignant tumors, such as

Table 1. Pathological and clinical parameters of the enrolled subjects

Characteristic	Patients with gastric Cancer (n=121)	Healthy controls (n=39)
Age (years)	67.3±11.0	67.6±11.2 (0.876)
Gender		
Male	99	26 (0.073)
Female	22	13
Location		
Cardia	17	
Gastric body	30	
Sinuses ventriculi	74	
Diameter of tumor		
<5 cm	52	
≥5 cm	69	
Differentiation		
Well	17	
Moderate	54	
Poor	50	
General stage		
Localized cancer	36	
Advanced cancer	85	
Tumor stage (AJCC, 2010)		
T1	36	
T2	51	
T3	26	
T4	8	
Lymph node status		
Negative	58	
Positive	63	
Metastasis		
Negative	108	
Positive	13	

gastric cancer, bladder cancer, colorectal cancer and so on [6-8]. In gastric cancer, SUMO1 pseudogene 3 (SUMO1P3) and H19 are upregulated, while gastric cancer-associated transcript 1 (GACAT1) is downregulated [6, 9, 10]. What's more, lncRNAs could be detected in blood, saliva, and urine [10-12], which facilitates the application of lncRNAs in clinical practice. Long non-coding RNA Fer-1-like protein 4 (FER1L4) was found to be down-regulated in gastric cancer tissues and cells [13]. Additionally, the low expression levels of FER1L4 are associated with histological grade, tumor diameter, TNM stage, lymphatic metastasis, perineural invasion, venous invasion, and serum CA724, and plasma FER1L4 declined sharply 2 weeks after surgery [14].

In the study, we would to explore the efficacy of plasma FER1L4 in the diagnosis and evaluation of gastric cancer.

Patients and methods

Ethics statement

Ethical approval was granted by the Ethic Committee of the Affiliated University City Hospital of Chongqing Medical University, and all participators signed the written informed consent.

Patients and samples

From June 2013 to July 2015, 39 healthy volunteers and 121 gastric cancer patients were enrolled, who had no other previous malignancy, previous chemotherapy or radiotherapy and coagulation disorders. Postoperative histological examination was applied to evaluate the tumor status for gastric cancer without metastasis, and computed tomography for metastatic gastric cancer. Histological grade was assessed according to the National Comprehensive Cancer Network clinical practice guideline on gastric cancer [2]. And, the baseline characteristics of all participators were also recorded.

10 ml peripheral venous blood sample was collected in EDTA anticoagulation tubes before surgery, chemotherapy or radiotherapy. It is worthy to note that the first 3 ml of collected blood was discarded to ensure there is no contamination of epidermal cells. Then, the blood samples were transported using ice cake and centrifuged (3,000×g for 10 min) within 20 min after collection, and plasma was separated into RNase-free tubes (Axygen, Union, CA) and stored at -80°C until RNA isolation.

Total RNA extraction and reverse transcription

Total plasma RNA was extracted using TRIzol LS Reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions, the quality of which was assessed by a UV

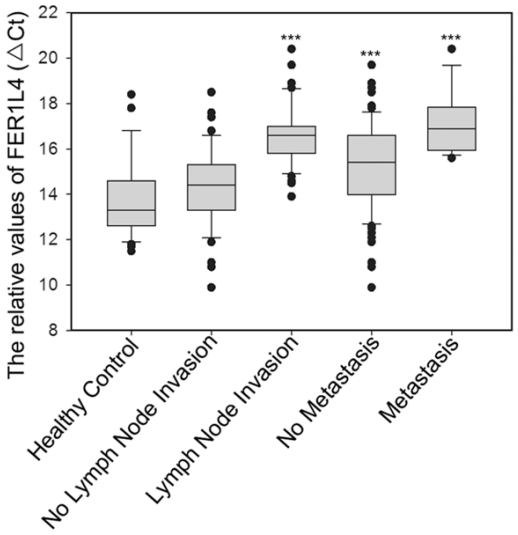

Figure 1. Plasma FER1L4 level detected by qRT-PCR. ***P< 0.001 when compared to healthy control.

spectrophotometer (BioRad, Hercules, CA, USA). Only the samples with 260/280 nm absorbance ratio between 1.8 and 2.0 were considered to be used in further analysis, which was dissolved in 10 μl diethylpyrocarbonate-treated water.

GoScript Reverse Transcription (RT) System (Promega, Madison, WI, USA) was applied to synthesize cDNA, the 20 μl reaction system included 3 ug total RNA, 1 μl oligo$(dT)_{15}$ primer, 1 μl random primer, 4 μl GoScript 5× reaction buffer, 2 μl $MgCl_2$, 1 μl nucleotide mix, 0.5 μl recombinant RNasin ribonuclease inhibitor, and 1 μl GoScript reverse transcriptase.

Real-time quantitative polymerase chain reaction (qRT-PCR)

Plasma FER1L4 level was detected by qRT-PCR using GoTaqq PCR Master Mix (Promega, Madison, WI, USA) on an Mx3005P real-time PCR System (Stratagene, La Jolla, CA). The sequences of the PCR primers were as follows: FER1L4: 5'-CCGTGTTGAGGTGCTGTTC-3' (sense) and 5'-GGCAAGTCCACTGTCAGATG-3' (antisense); GAPDH: 5'-ACCCACTCCTCCACCTTTG-AC-3' (sense) and 5'-TGTTGCTGTAGCCAAATT-CGTT-3' (antisense) [14, 15]. The conditions of thermal cycling was as follows: 95°C at 10 min fora hot start, then 45 cycles at 95°C for 15 s, 54°C for 30 s, and 72°C for 30 s. In order to avoid bias, qRT-PCR was repeated in triplicate. Plasma FER1L4 level was calculated using the ΔCt method with GAPDH as the endogenous control for data normalization.

Statistical analysis

SPSS 17.0 software (SPSS, Chicago, IL, USA) was applied for statistical analysis, and one way analysis of variance test and Student's t test were flexibly used as appropriate. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the efficacy of FER1L4 in differentiating disease status. P<0.05 was considered statistically significant.

Results

Patients' characteristics

39 healthy volunteers and 121 gastric cancer patients were enrolled into the study, the age and gender were similar between the two groups (P>0.05, **Table 1**). 108 gastric patients received surgical resection with lymphadenectomy, and 13 patients received systemic therapy due to distant metastasis. The pathological information of gastric cancer was presented in **Table 1**.

Plasma FER1L4 expression

Compared to healthy control (ΔCt: 13.71±1.73), plasma FER1L4 expression was downregulated in gastric cancer (ΔCt: 15.48±1.93, P<0.001, **Figure 1**), gastric cancer with lymph node invasion (ΔCt: 16.60±1.29, P<0.001, **Figure 1**), gastric cancer with no metastasis (ΔCt: 15.29±1.90, P<0.001, **Figure 1**) and metastasis (ΔCt: 17.11±1.35, P<0.001, **Figure 1**). On contrast, no significant difference of FER1L4 expression was found between healthy control (ΔCt: 13.71±1.73) and gastric cancer without lymph node invasion (ΔCt: 14.26±1.76, P=0.132). Besides, significant difference of FER-1L4 expression was also found between healthy control (ΔCt: 13.71±1.73) and T1 gastric cancer with lymph node invasion (ΔCt: 16.42±1.21, P<0.001), while no significant difference between healthy control and T1 gastric cancer without lymph node invasion (ΔCt: 13.76±1.39, P=0.915). As shown in **Table 2**, plasma FER1L4 level wasn't associated with age (p=0.499), gender (P=0.306), tumor site (P=0.746), tumor diameter (P=0.690), differentiation (P=0.638), stage (P=0.371) and tumor

Table 2. FER1L4 expression level (ΔCt) phenotype

Characteristic	No. of patients	FER1L4 expression	*P*
Age (years)			0.499
<60	44	14.87±2.10	
≥60	116	15.12±2.00	
Gender			0.306
Male	125	14.96±2.01	
Female	35	15.36±2.08	
Site			0.746
Cardia	17	15.35±2.04	
Gastric body	30	15.29±2.16	
Sinuses ventriculi	74	15.59±1.82	
Diameter of tumor			0.690
≥5 cm	52	15.40±2.18	
<5 cm	69	15.54±1.73	
Differentiation			0.638
Well	17	15.42±1.59	
Moderate	54	15.32±2.10	
Poor	50	15.68±1.85	
General stage			0.371
Localized cancer	36	15.24±1.85	
Advanced cancer	85	15.58±1.96	
Tumor stage			
(AJCC, 2010)			0.175
T1	36	15.02±3.66	
T2	51	14.07±3.19	
T3	26	14.42±3.96	
T4	8	16.78±0.77	
Lymph node status			
Negative	58	14.26±1.76	<0.001
Positive	63	16.60±1.29	
Lymph node status in T1 cancer			
Negative	16	14.26±1.76	<0.001
Positive	20	16.42±1.21	
Metastasis			0.001
Negative	108	15.29±1.90	
Positive	13	17.11±1.35	

stage (AJCC, 2010) (P=0.175). But plasma FER1L4 level was significantly correlated with lymph node status in gastric cancer patients (P<0.001) and T1 gastric cancer patients (P<0.001) and metastasis status in gastric cancer (P<0.001) (**Table 2**).

Efficacy of FER1L4 in differentiating disease status

Receiver operating characteristic (ROC) curve analysis was constructed to analyze the efficacy of FER1L4 in differentiating disease status. In regard of differentiating gastric cancer with lymph node invasion from healthy control, the results showed that the area under the ROC curve (AUC) was up to 0.897 (95% confidence interval (CI): 0.821-0.949, P<0.001, **Figure 2A**). The optimal cutoff value was 14.7 with which the sensitivity and specificity were 93.7% and 79.5%, respectively. It also found that FER1L4 at the cut point of 15.3 yielded an AUC of 0.868 (95% CI: 0.795-0.923) in distinguishing gastric cancer with lymph node invasion form gastric cancer without lymph node invasion, the sensitivity and specificity of which were 87.3% and 77.6%, respectively (**Figure 2B**). In T1 gastric cancer, similar results were also calculated. At the cutoff of 14.2, the AUC of FER1L4 in distinguishing lymph node invasion form no invasion was 0.928 (95% CI: 0.791-0.987), and the sensitivity and specificity were 100% and 68.7%, respectively (**Figure 2C**).

Discussion

The present study indicated that plasma FER1L4 was down-regulated in gastric cancer, and its expression was associated with lymph node invasion status and metastasis status. But no significant downregulation of FER1L4 was found in gastric cancer without lymph node invasion.

There is mounting evidence that lncRNAs are related to gastric cancer, such as FER1L4, H19, CUDR, LSINCT-5, PTENP1, LINC00152 and so on [14-17]. Song et al firstly reported that FER1L4 was down-regulated in gastric cancer compared to normal gastric specimens [4]. A recent study demonstrated that the low expression of FER1L4 in tissue samples was associated with histological grade, tumor diameter, TNM stage, lymphatic metastasis, perineural invasion, venous invasion, and serum CA724 [14]. Besides, no difference of plasma FER1L4 expression was observed between gastric cancer patients and healthy volunteers; but a sharply decline plasma FER1L4 was found

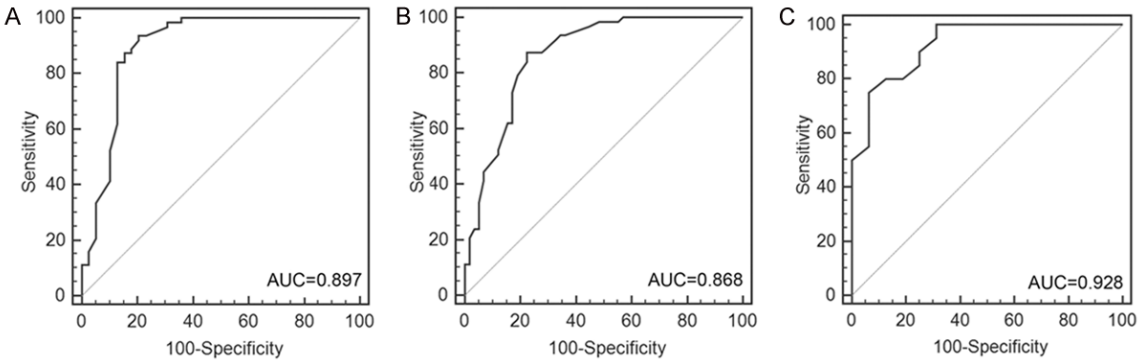

Figure 2. Receiver-operating characteristics curve analysis using FER1L4 for differentiating disease status. A: Distinguishing gastric cancer from healthy control; B: Differentiation of gastric cancer with lymph node invasion or without lymph node invasion; C: Differentiating T1 gastric cancer with lymph node invasion from no invasion.

in gastric cancer patients 14 days after operation [14]. In our study, statistical difference of plasma FER1L4 was observed between healthy control and gastric cancer, and plasma FER1L4 expression in gastric cancer with lymph node invasion was also significant lower than gastric cancer without lymph node invasion. Of course, plasma FER1L4 expression in healthy control was much higher than gastric cancer with lymph node invasion. But, there is no significant difference of plasma FER1L4 between healthy control and gastric cancer without lymph node invasion. We held the idea that the difference between healthy control and gastric cancer was mainly determined by the difference between healthy control and gastric cancer with lymph node invasion after considering the nearly equal number of patients with or without lymph node invasion in the cohort and relatively higher ΔCt value in gastric cancer with lymph node invasion. So plasma FER1L4 might fail to distinguish gastric cancer without lymph node invasion from healthy control. Besides, plasma FER1L4 expression was also considerably higher in gastric cancer without metastasis than those with metastasis. These data indicated that FER1L4 depletion may be associated with the metastatic process of gastric cancer. Similarly, another study suggested that FER1L4 expression was considerably lower in invaded lymph nodes than in primary colon cancers [18].

The role of FER1L4 in the development of malignant tumor is still in research, and available data is really limited. Doctor Xia reported that FER1L4 was involved in a cancer-associated ceRNA network to modulate tumor development and invasion in gastric cancer, which includes nine miRNAs (miR-106a-5p, miR-106b-5p, miR-139-5p, miR-19a-3p, miR-195-5p, miR-18a-5p, miR-18b-5p, miR-20b-5p and miR-31-5p) and eight lncRNAs (FER1L4, AC009499.1, H19, LINC00152, RP4-620F22, GACAT1, GACAT3 and 3AP000288.2.) [19]. miR-106a-5p, as one of the typical onco-miRNAs, is the core element in this network [20]. FER1L4 and RB1 are targeted by miR-106a-5p, and siRNA silencing of FER1L4 led to RB1mRNA level decrease [19]. Additionally, it is also reported that FER1L4 and PTEN mRNA are a pair of ceRNAs that are linked by miR-106a-5p and FER1L4 downregulation accelerated cell proliferation by promoting the G0/G1 to S phase transition [13].

Further, ROC curve analysis was conducted to analyze the efficacy of plasma FER1L4 in differentiating gastric cancer with lymph node invasion from healthy control and gastric cancer without lymph node invasion. In regard of differentiating gastric cancer with lymph node invasion from healthy control, the results showed that the area under the ROC curve (AUC) was up to 0.897 (95% confidence interval (CI): 0.821-0.949, P<0.001, **Figure 1**). The optimal cutoff value was 14.7 with which the sensitivity and specificity were 93.7% and 79.5%, respectively. And FER1L4 at the cut point of 15.3 yielded an AUC of 0.868 (95% CI: 0.795-0.923) in distinguishing gastric cancer with lymph node invasion form gastric cancer without lymph node invasion, the sensitivity and specificity of which were 87.3% and 77.6%, respectively.

Additionally, early gastric cancer is defined as malignant tissue limited to the mucosa or sub-

mucosa, no matter of whether there is lymph node invasion or not [21]. Even though gastrectomy is still the gold standard for the management of gastric cancer, endoscopic resection has already provided an alternative treatment with minimally morbidity and similar efficacy [22]. A previous report indicated that the complete resection rates for endoscopic mucosal resection and endoscopic submucosal dissection were 54% and 89%, respectively [23]. Of note, the premise of endoscopic resection is there is no risk of lymph node invasion [22, 24]. Therefore, we analyzed the expression profile of FER1L4 in T1 gastric cancer, and found that FER1L4 was downregulated in T1 gastric cancer with lymph node invasion than in T1 gastric cancer without lymph node invasion. Additionally, ROC curve analysis indicated that the AUC of FER1L4 in distinguishing T1 gastric cancer with lymph node invasion form no invasion was up to 0.928 (95% CI: 0.791-0.987), the sensitivity and specificity were 100% and 68.7%, respectively. The optimal cutoff point was 14.2. These data showed the great potential of FER1L4 in screening invasive lymph node in T1 gastric cancer, but it still needs more validation.

Even though the result is encouraging, several limitations need to be considered. First, all participators were from a single institute, whether they could represent the general population is still in doubt. Second, the sample size of the study is limited, studies with larger cohort is in urgent need. Third, the efficiency of FER1L4 in predicting survival wasn't discussed in the present study due to missed follow-up information. Fourth, the mechanism of FER1L4 in regulating the development of gastric cancer wasn't further explored.

Conclusion

Plasma FER1L4 fails to distinguish gastric cancer without lymph node invasion from healthy control, but it could serve as an independent biomarker for differentiating gastric cancer with lymph node from gastric cancer without lymph node invasion and healthy control. The efficacy of FER1L4 in distinguishing T1 gastric cancer with lymph node invasion from T1 gastric cancer without lymph node invasion is also verified, it can be taken into account when considering whether endoscopic resection is suitable to a patient with early gastric cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jiong Huang, Dagongguan Branch of The Affiliated University City Hospital of Chongqing Medical University, No. 50, Seventh Cultural Village, Xiejiawan, Shapingba District, Chongqing 400050, P. R. China. Tel: 0086-23-23518223; E-mail: huangjiong1101@163.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] NCCN. The NCCN gastric cancer clinical practice guidelines in oncology (version 3.2015). Fort Washington: NCCN, 2015. http://wwwnccnorg/professionals/physician_gls/pdf/gastricpdf.

[3] Ahmad R, Setia N, Schmidt BH, Hong TS, Wo JY, Kwak EL, Rattner DW, Lauwers GY, Mullen JT. Predictors of Lymph Node Metastasis in Western Early Gastric Cancer. J Gastrointest Surg 2016; 20; 531-8.

[4] Song H, Sun W, Ye G, Ding X, Liu Z, Zhang S, Xia T, Xiao B, Xi Y, Guo J. Long non-coding RNA expression profile in human gastric cancer and its clinical significances. J Transl Med 2013; 11: 225.

[5] Qi P, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. Mod Pathol 2013; 26: 155-65.

[6] Yang F, Bi J, Xue X, Zheng L, Zhi K, Hua J, Fang G. Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. FEBS J 2012; 279: 3159-65.

[7] Li HJ, Li X, Pang H, Pan JJ, Xie XJ, Chen W. Long non-coding RNA UCA1 promotes glutamine metabolism by targeting miR-16 in human bladder cancer. Jpn J Clin Oncol 2015; 45: 1055-63.

[8] Ding J, Lu B, Wang J, Wang J, Shi Y, Lian Y, Zhu Y, Wang J, Fan Y, Wang Z, De W, Wang K. Long non-coding RNA Loc554202 induces apoptosis in colorectal cancer cells via the caspase cleavage cascades. J Exp Clin Cancer Res 2015; 34: 100.

[9] Mei D, Song H, Wang K, Lou Y, Sun W, Liu Z, Ding X, Guo J. Up-regulation of SUMO1 pseudogene 3 (SUMO1P3) in gastric cancer and its clinical association. Med Oncol 2013; 30: 709.

[10] Sun W, Wu Y, Yu X, Liu Y, Song H, Xia T, Xiao B, Guo J. Decreased expression of long noncoding RNA AC096655.1-002 in gastric cancer and its clinical significance. Tumour Biol 2013; 34: 2697-701.

[11] Tang H, Wu Z, Zhang J, Su B. Salivary lncRNA as a potential marker for oral squamous cell carcinoma diagnosis. Mol Med Rep 2013; 7: 761-6.
[12] Hessels D, Klein Gunnewiek JM, van Oort I, Karthaus HF, van Leenders GJ, van Balken B, Kiemeney LA, Witjes JA, Schalken JA. DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 2003; 44: 8-15; discussion 15-6.
[13] Xia T, Chen S, Jiang Z, Shao Y, Jiang X, Li P, Xiao B, Guo J. Long noncoding RNA FER1L4 suppresses cancer cell growth by acting as a competing endogenous RNA and regulating PTEN expression. Sci Rep 2015; 5: 13445.
[14] Liu Z, Shao Y, Tan L, Shi H, Chen S, Guo J. Clinical significance of the low expression of FER1L4 in gastric cancer patients. Tumour Biol 2014; 35: 9613-7.
[15] Li Q, Shao Y, Zhang X, Zheng T, Miao M, Qin L, Wang B, Ye G, Xiao B, Guo J. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. Tumour Biol 2015; 36: 2007-12.
[16] Arita T, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Shoda K, Kawaguchi T, Hirajima S, Nagata H, Kubota T, Fujiwara H, Okamoto K, Otsuji E. Circulating long non-coding RNAs in plasma of patients with gastric cancer. Anticancer Res 2013; 33: 3185-93.
[17] Dong L, Qi P, Xu MD, Ni SJ, Huang D, Xu QH, Weng WW, Tan C, Sheng WQ, Zhou XY, Du X. Circulating CUDR, LSINCT-5 and PTENP1 long noncoding RNAs in sera distinguish patients with gastric cancer from healthy controls. Int J Cancer 2015; 137: 1128-35.
[18] Yue B, Sun B, Liu C, Zhao S, Zhang D, Yu F, Yan D. Long non-coding RNA FER1L4 suppresses oncogenesis and exhibits prognostic value by associating with miR-106a-5p in colon cancer. Cancer Sci 2015; 106: 1323-32.
[19] Xia T, Liao Q, Jiang X, Shao Y, Xiao B, Xi Y, Guo J. Long noncoding RNA associated-competing endogenous RNAs in gastric cancer. Sci Rep 2014; 4: 6088.
[20] Xiao B, Guo J, Miao Y, Jiang Z, Huan R, Zhang Y, Li D, Zhong J. Detection of miR-106a in gastric carcinoma and its clinical significance. Clin Chim Acta 2009; 400: 97-102.
[21] Gotoda T. Endoscopic resection of early gastric cancer: the Japanese perspective. Curr Opin Gastroenterol 2006; 22: 561-9.
[22] Espinel J, Pinedo E, Ojeda V, Del Rio MG. Treatment modalities for early gastric cancer. World J Gastrointest Endosc 2015; 7: 1062-9.
[23] Oka S, Tanaka S, Higashiyama M, Numata N, Sanomura Y, Yoshida S, Arihiro K, Chayama K. Clinical validity of the expanded criteria for endoscopic resection of undifferentiated-type early gastric cancer based on long-term outcomes. Surg Endosc 2014; 28: 639-47.
[24] Ono H, Yao K, Fujishiro M, Oda I, Nimura S, Yahagi N, Iishi H, Oka M, Ajioka Y, Ichinose M, Matsui T. Guidelines for ESD and EMR for Early Gastric Cancer. Dig Endosc 2016; 28; 3-15.