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Circulating SHIP2 mRNA as a novel biomarker in the diagnosis and prognosis of gastric cancer

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Abstract. – OBJECTIVE: Previous studies showed the aberrant expression of Src homology 2-containing inositol 5-phosphatase 2 (SHIP2) in GC tissue. However, the exact role of circulating SHIP2 in GC remains unclear. The aim of this manuscript was to analyze potential diagnostic and prognostic value of circulating SHIP2 levels in GC.

PATIENTS AND METHODS: Circulating SHIP2 expression was detected in the plasma of 156 GC patients and 60 healthy controls by qRT-PCR. The receiver operating characteristic (ROC) curves were plotted to explore the reliability of circulating SHIP2 in detecting GC. Survival curves were estimated using the Kaplan-Meier method, and differences between them were evaluated by the log-rank test. The influence of each variable on survival was examined by the Cox multivariate regression analysis.

RESULTS: Our research showed that the expression levels of circulating SHIP2 in plasma of GC patients were lower than in healthy controls ($p < 0.05$). Decreased circulating SHIP2 mRNA expression was negatively correlated with clinical stage ($p = 0.004$), lymph node metastasis ($p = 0.003$) and distant metastasis ($p = 0.025$). ROC curve analysis showed that circulating SHIP2 may be a useful marker for discriminating cases from healthy controls. In addition, patients with low circulating SHIP2 mRNA level had poorer overall survival than those with high circulating SHIP2 mRNA level ($p = 0.006$). Moreover, multivariate analysis indicated that the level of circulating SHIP2 mRNA expression was an independent prognostic indicator ($p = 0.005$) for the survival of patients with GC.

CONCLUSIONS: The present study indicated that decreased plasma SHIP2 mRNA level might be a novel diagnostic and prognostic biomarker for GC patients. This conclusion should be further assessed in randomized clinical trials.

Key Words:
Circulating SHIP2 mRNA, Gastric cancer, Prognosis, Diagnosis.

Introduction

Cancer is a broad group of diseases in which cells divide and grow uncontrollably[1]. Gastric cancer (GC) is the fifth most commonly diagnosed cancer and the third leading cause of cancer death worldwide[2]. China has the highest incidence and mortality rate of GC in the world, and the incidences keep increasing in recent decades[3]. Although significant improvement has been achieved in surgical techniques and adjuvant treatment, patients with GC have a poor 5-year survival rate because of the high invasiveness and recurrence of this malignancy[4,5]. Therefore, it is important to develop early diagnostic methods and effective therapies for GC.

SHIP2, encoded by the gene INPPL1 (inositol polyphosphate phosphatase-like 1) on human chromosome 11q13, belongs to the phosphoinositol phosphatases family, which plays important role in modulating signaling pathways relevant to both diabetes and cancer[6-8]. SHIP2 is considered to downregulate phosphatidylinositol 3'-kinase (PI3K) signaling, which underlies the development of several kinds of human cancers[9,10]. Additionally, it has been reported that abnormal expression of SHIP2 could affect a variety of biology behaviors, including cell adhesion, migration, invasion and receptor internalization[11,12]. Recently, several studies indicated that SHIP2 expression was associated with prognosis of tumor patients. In generally, SHIP2 exerted a tumor oncogene in these tumors, including colorectal cancer[13], hepatocellular cancer[14] and non-small cell lung cancer[15]. On the contrary, a recent investigation by Ye et al[16] showed that SHIP2 served as a tumor suppressor in GC. Those results indicated the complicated role of SHIP2 in development of tumors. To our best knowledge, its prognostic and diagnostic significance in GC remains unknown.

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In the present work, we firstly determine the expression pattern of circulating SHIP2 mRNA expression in GC. Then, the correlation of circulating SHIP2 mRNA expression with diagnosis and prognosis of GC patients was explored. Our work provided the first evidence that circulating SHIP2 mRNA may serve as a novel diagnostic and prognostic marker in GC.

Patients and Methods

Patients and Sample Collection

Patients who have been diagnosed as gastric tumor by histopathological evaluation at the People's Hospital of Ganzhou City between January 2009 and September 2011, were enrolled. None of the GC patients had received any anticancer treatment prior to sampling. Tumor stage and disease grade were classified according to the 6th edition of the tumor-node-metastasis (TNM) classification of the International Union against Cancer. All the patients' demographic characteristics, date of surgery, tumor stage, lymph node metastasis, survival time, and other relevant data were extracted from hospital records. Overall survival was defined as the period from initial biopsy confined diagnosis to death. All patients who died from other diseases or unexpected causes were excluded from the evaluation. Peripheral venous blood samples were obtained from patients and healthy volunteers in 8-ml blood collection tubes. The samples were centrifuged at 12,000 g for 5 min to completely remove and then stored at -80°C until further processing. All serum samples were processed within 24 h after obtained. Experiments were performed in compliance with the Chinese laws and guidelines concerning the patients' informed consent.

RNA Isolation, Reverse Transcription and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from 400 µl of plasma using the mirVana PARIS kit (Ambion, Austin, TX, USA). The purity and concentration of total RNA were determined by a dual-beam ultraviolet spectrophotometer (Eppendorf, Hamburg, Germany). Quantitation of mRNA was carried out by qRT-PCR using SYBR Premix Ex TaqTM (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. The primer sequences used to amplify the SHIP2 mRNA were as follows: Forward, 5'-AGCTGCCCACGCTCAAACCAA-3', Reverse, 5'-AGGTCAGGAACTGTTGGGCCGT-3'. GAPDH mRNA levels were used as an internal normalization control. The relative expression of circulating SHIP2 mRNA in tissues and cell lines were calculated by the $2^{-\Delta ct}$ method.

Statistical Analysis

Between-group analysis was carried out by independent *t*-test for continuous data. Associations between circulating SHIP2 mRNA expression level and clinicopathological features were determined using the x^2-test. ROC curves and AUC with 95% CI were established to illustrate the diagnostic power of serum biomarkers in GC. Survival curves were constructed with the Kaplan-Meier method and compared by log-rank tests. Multivariate analyses were performed using Cox proportional hazards model. $p < 0.05$ was considered statistically significant. Data analyses were performed with SPSS 21.0 (IBM, Armonk, NY, USA).

Results

Circulating SHIP2 mRNA Expression Decreases in GC Patients

We firstly detected the expression levels of circulating GAPDH mRNA in all GC patients and healthy controls. As shown in Figure 1A, the results showed that no significant difference was observed in terms of Ct values of GAPDH between patients with GC and healthy controls ($p < 0.05$), suggesting that GAPDH mRNA expression could be used as a suitable internal control. Then, we performed RT-PCR to detect the levels of circulating SHIP2 mRNA in plasma of all subjects. As shown in Figure 1B, we found that the expression levels of circulating SHIP2 mRNA in plasma of GC patients were lower than in healthy controls ($p < 0.05$).

Associations Between Circulating SHIP2 mRNA and the Clinicopathological Characteristics

In order to explore the effect of circulating SHIP2 mRNA in progression of GC, We divided the 156 patients with GC into a high expression group (n = 75) and a low expression group (n = 81), according to the median expression level of circulating SHIP2. The relationship between clinicopathological features and NDUFA4L2 expression levels in GC cases was shown in Table I. We observed that decreased circulating SHIP2 expression was negatively correlated with clinical stage ($p = 0.004$), lymph node metastasis ($p = 0.003$) and distant metastasis ($p = 0.025$). However, there were no differences between the two

Table I. Clinicopathological features and the expression of circulating SHIP2 mRNA in GC patients.

Characteristics	Number	Circulating SHIP2 mRNA levels		*p*-value
		High (%)	Low (%)	
Gender				NS
Female	49	23 (47)	26 (53)	
Male	107	52 (49)	55 (51)	
Age (y)				NS
<50	51	27 (52.9)	24 (47.1)	
≥50	105	48 (45.7)	57 (54.3)	
Histological type				NS
Differentiated	70	34 (48.6)	36 (51.4)	
Undifferentiated	86	41 (47.7)	45 (52.3)	
Clinical stage				0.004
I-II	94	54 (57.4)	40 (42.6)	
III-IV	62	21 (33.9)	41 (66.1)	
Tumor depth				NS
T1-T2	125	61 (48.8)	64 (51.2)	
T3-T4	31	14 (45.2)	17 (54.8)	
Lymph node metastasis				0.003
N0-N1	100	57 (57)	43 (43)	
N2-N3	56	18 (32.1)	38 (67.9)	
Distant metastasis				0.025
Yes	35	11 (31.4)	24 (68.6)	
No	121	64 (52.9)	57 (47.1)	

groups in terms of ages, gender, histological type and tumor depth (all $p > 0.05$).

Diagnostic Performance of Circulating SHIP2 mRNA for GC

To verify the diagnostic value of circulating SHIP2 mRNA in identifying GC, the ROC curve was analyzed. The results showed that the circulating SHIP2 mRNA was a potential biomarker for separating GC patients from healthy controls with an AUC of 0.809 (95% CI, 0.707-0.911) (Figure 2).

Relationship Between Circulating SHIP2 mRNA Expression and Survival Outcomes in GC Patients

To investigate the relationship between circulating SHIP2 mRNA expression and clinical outcomes, we reviewed the clinical information for all 156 GC patients. Survival information

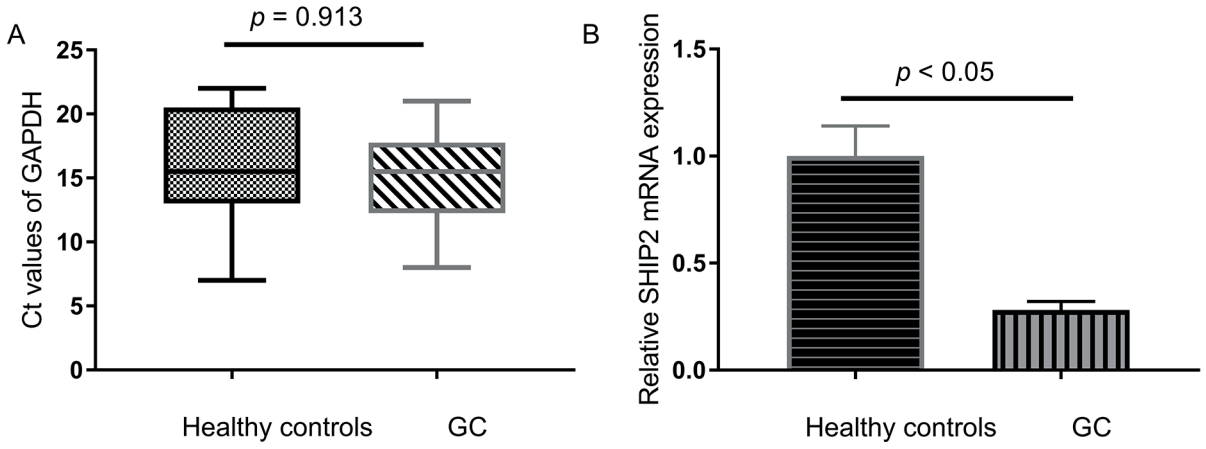

Figure 1. Circulating SHIP2 mRNA was decreased in GC patients. ***(A)*** Ct values of GAPDH between GC and healthy controls. ***(B)*** Comparison of circulating SHIP2 mRNA in GC patients and healthy controls. Bars represent the mean of three independent experiments.

Table II. Multivariate Cox's hazards model analysis for prognostic factors.

Variable	HR	95% CI	*p*-value
Variable	HR	95% CI	*p*-value
Gender	1.266	0.865-1.893	NS
Age (y)	1.123	0.721-1.544	NS
Histological type	1.644	0.593-1.992	NS
Clinical stage	3.544	1.793-5.622	0.002
Tumor depth	1.631	0.891-1.884	NS
Lymph node metastasis	2.931	1.266-4.474	0.007
Distant metastasis	2.341	1.255-3.542	0.009
Circulating SHIP2 mRNA levels	3.145	1.663-5.477	0.005

of 156 patients with GC was obtained through letters and phone calls. As shown in Figure 3, the Kaplan-Meier survival curves show that patients with higher circulating SHIP2 mRNA expression had significantly longer overall survival compared to those with lower expression levels (p = 0.006). Then, multivariate analysis was performed using the Cox proportional hazards model for all of the significant variables. As shown in Table II, the results showed that low circulating SHIP2 mRNA expression was a significant independent predictor of poor survival in GC (HR = 3.145, 95% CI: 1.663-5.477, p = 0.005).

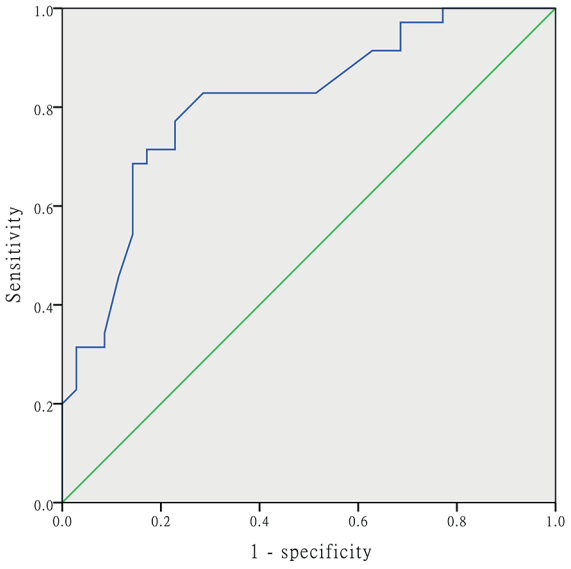

Figure 2. Receiver operating characteristic (ROC) analysis was performed to determine the sensitivity and specificity of circulating SHIP2 mRNA level using area under the ROC curve (AUC) analysis.

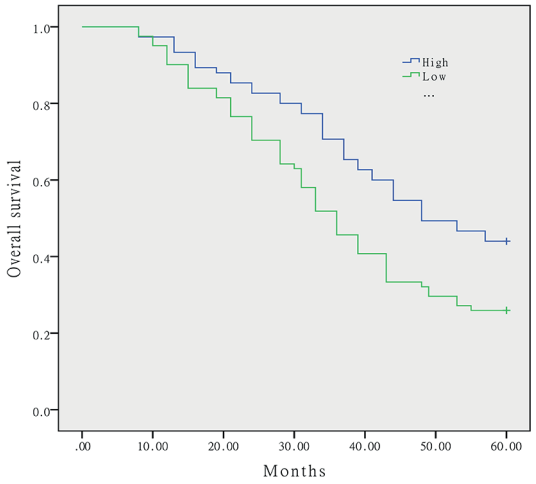

Figure 3. The 5-year OS rate of GC patients with high circulating SHIP2 mRNA expression was significantly higher than that of those patients with low circulating SHIP2 mRNA expression (p = 0.006). Corresponding p-values analyzed by log-rank tests are indicated.

Discussion

Gastric cancer is a major cause of cancer deaths in Asian countries[17]. Although many researches focused on the identification of effective biomarkers for GC prognosis and diagnosis, only a few studies were used in clinical practice[18-20]. For many tumors, blood-based proteins have been widely used as biomarkers in clinical diagnosis and prognosis due to their relatively high accuracy[21,22]. Unfortunately, common tumor biomarkers like CA125, CA199, and CEA, have exhibited poor diagnostic value in GC[23]. Thus, diagnostic methods with high accuracy are urgently needed for GC patients.

SHIP2 regulates multiple oncogenic signaling pathways by dephosphorylating PIP3 to produce phosphatidylinositol-3,4-bisphosphate[24]. Recent-

ly, the role of SHIP2 has been reported in several tumors. For instance, Fu et al[15] reported that up-regulation of SHIP2 mRNA was observed in non-small cell lung cancer and its expression was related to lymph node metastasis, TNM stage, and 5-year survival rate. Prasad et al[25] found that over-expression of SHIP2 promoted breast cancer development and metastasis by via EGFR-induced Akt activation. Zhou et al[26] reported that overexpression of SHIP2 was associated with poor overall survival in patients with laryngeal squamous cell carcinoma. Recently, Ye et al[27] found overexpression of Sp1 inhibited malignant behavior of GC cells by enhancing the transcriptional activity of SHIP2 promoter. More importantly, in another work, they further confirmed that SHIP2 was commonly downregulated in GC, and reduced SHIP2 expression promoting cell proliferation and invasion in gastric cancer via activation of the PI3K/Akt signaling[16]. Those results suggested that SHIP2 played a different role in different types of tumors. For GC, SHIP2 served as a tumor suppressor. However, no study has investigated the diagnostic and prognostic performance of circulating SHIP2 mRNA for GC.

In the present study, we firstly found that circulating SHIP2 mRNA expression was decreased in plasma of GC patients compared with non-cancerous bone tissues healthy controls. Then, we observed that decreased circulating SHIP2 mRNA expression was negatively correlated with clinical stage, lymph node metastasis and distant metastasis. Moreover, the sensitivity (73.8%) and specificity (71.7%) of detecting circulating SHIP2 mRNA expression in the serum further confirmed that circulating SHIP2 mRNA level can be used as a potential tumor marker for diagnosis of GC. Furthermore, lower circulating SHIP2 mRNA was associated with shorter overall survival of GC patients. Finally, multivariate analysis indicated that the level of circulating SHIP2 mRNA expression was an independent prognostic indicator for the survival of patients with GC.

Conclusions

This is the first investigation highlighting the clinical significance of circulating SHIP2 mRNA in GC. It could serve as promising biomarker for both diagnosis and prognosis of GC. Further prospective studies with larger cohorts are required to clarify the value of circulating SHIP2 mRNA as a useful marker for diagnosis and prognosis.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Smith RA, Andrews K, Brooks D, DeSantis CE, Fedewa SA, Lortet-Tieulent J, Manassaram-Baptiste D, Brawley OW, Wender RC. Cancer screening in the United States, 2016: a review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin 2016; 66: 96-114.
2) 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.
3) Strong VE, Wu AW, Selby LV, Gonen M, Hsu M, Song KY, Park CH, Coit DG, Ji JF, Brennan MF. Differences in gastric cancer survival between the U.S. and China. J Surg Oncol 2015; 112: 31-37.
4) Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006; 355: 11-20.
5) Yuan CL, Li L, Zhou X, Liz H, Han L. Expression of SATB1 and HER2 in gastric cancer and its clinical significance. Eur Rev Med Pharmacol Sci 2016; 20: 2256-2264.
6) Gorgani-Firuzjaee S, Meshkani R. SH2 domain-containing inositol 5-phosphatase (SHIP2) inhibition ameliorates high glucose-induced de-novo lipogenesis and VLDL production through regulating AMPK/mTOR/SREBP1 pathway and ROS production in HepG2 cells. Free Radic Biol Med 2015; 89: 679-689.
7) Hao YM, Liu QJ, Wang RY, Cao YP, Zhang Y, Zuo LF. Single nucleotide polymorphisms on SHIP2 is associated with Type 2 diabetes mellitus in Chinese Han population. Eur Rev Med Pharmacol Sci 2015; 19: 129-137.
8) Arai Y, Shibata T, Matsuoka S, Sato MJ, Yanagida T, Ueda M. Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. Proc Natl Acad Sci U S A 2010; 107: 12399-12404.
9) Awad A, Gassama-Diagne A. PI3K/SHIP2/PTEN pathway in cell polarity and hepatitis C virus pathogenesis. World J Hepatol 2017; 9: 18-29.
10) Krause M, Gautreau A. Steering cell migration: lamellipodium dynamics and the regulation of directional persistence. Nat Rev Mol Cell Biol 2014; 15: 577-590.
11) Gorgani-Firuzjaee S, Ahmadi S, Meshkani R. Palmitate induces SHIP2 expression via the ceramide-mediated activation of NF-κB, and JNK in skeletal muscle cells. Biochem Biophys Res Commun 2014; 450: 494-499.
12) Elong Edimo W, Schurmans S, Roger PP, Erneux C. SHIP2 signaling in normal and pathological situa-

tions: its impact on cell proliferation. Adv Biol Regul 2014; 54: 142-151.

13) Yang J, Fu M, Ding Y, Weng Y, Fan W, Pu X, Ge Z, Zhan F, Ni H, Zhang W, Jin F, Xu N, Li J, Qiu L, Wang J, Gu X. High SHIP2 expression indicates poor survival in colorectal cancer. Dis Markers 2014; 2014: 218968.

14) Sumie S, Kawaguchi T, Komuta M, Kuromatsu R, Itano S, Okuda K, Taniguchi E, Ando E, Takata A, Fukushima N, Koga H, Torimura T, Kojiro M, Sata M. Significance of glucose intolerance and SHIP2 expression in hepatocellular carcinoma patients with HCV infection. Oncol Rep 2007; 18: 545-552.

15) Fu M, Fan W, Pu X, Ni H, Zhang W, Chang F, Gong L, Xiong L, Wang J, Gu X. Elevated expression of SHIP2 correlates with poor prognosis in non-small cell lung cancer. Int J Clin Exp Pathol 2013; 6: 2185-2191.

16) Ye Y, Ge YM, Xiao MM, Guo LM, Li Q, Hao JQ, Da J, Hu WL, Zhang XD, Xu J, Zhang LJ. Suppression of SHIP2 contributes to tumorigenesis and proliferation of gastric cancer cells via activation of Akt. J Gastroenterol 2016; 51: 230-240.

17) Taylor VM, Ko LK, Hwang JH, Sin MK, Inadomi JM. Gastric cancer in Asian American populations: a neglected health disparity. Asian Pac J Cancer Prev 2014; 15: 10565-10571.

18) Jia S, Cai J. Update on biomarkers in development of anti-angiogenic drugs in gastric cancer. Anticancer Res 2016; 36: 1111-1118.

19) Li J. Safety and effectiveness of endoscopic mucosal resection combined with chemotherapy for early gastric cancer. Eur Rev Med Pharmacol Sci 2016; 20: 2265-2270.

20) Chen Y, Lin J, Yang M, Xu CW, Chen BZ, Li XJ, Zhu WF, Fu WD, Yu H, Ying MG, Chen G. Expression and prognostic roles of magnesium-dependent phosphatase-1 in gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 2617-2625.

21) Yörüker EE, Holdenrieder S, Gezer U. Blood-based biomarkers for diagnosis, prognosis and treatment of colorectal cancer. Clin Chim Acta 2016; 455: 26-32.

22) Holdhoff M, Yovino SG, Boadu O, Grossman SA. Blood-based biomarkers for malignant gliomas. J Neurooncol 2013; 113: 345-352.

23) Chen XZ, Zhang WH, Chen HN, Liu JP, He D, Liu Y, Liu K, Chen XL, Mo XM, Zhou ZG, Hu JK. Associations between serum CA724 and HER2 overexpression among stage II-III resectable gastric cancer patients: an observational study. Oncotarget 2016; 7: 23647-2357.

24) Suwa A, Kurama T, Shimokawa T. SHIP2 and its involvement in various diseases. Expert Opin Ther Targets 2010; 14: 727-737.

25) Prasad NK. SHIP2 phosphoinositol phosphatase positively regulates EGFR-Akt pathway, CXCR4 expression, and cell migration in MDA-MB-231 breast cancer cells. Int J Oncol 2009; 34: 97-105.

26) Zhou X, Liu Y, Tan G. Prognostic value of elevated SHIP2 expression in laryngeal squamous cell carcinoma. Arch Med Res 2011; 42: 589-595.

27) Ye Y, Qian XY, Xiao MM, Shao YL, Guo LM, Liao DP, Da J, Zhang LJ, Xu J. Decreased Sp1 expression mediates downregulation of SHIP2 in gastric cancer cells. Int J Mol Sci 2017; 18: E220.