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Expression of SATB1 and HER2 in gastric cancer and its clinical significance

C.-L. YUAN[1], L. LI[1], X. ZHOU[1], H. LI[2], L. HAN[3]

[1]Department of Oncology, The First People's Hospital of Lianyungang, Lianyungang, Jiangsu Province, P.R. China
[2]Department of Pathology, the Affiliated Hospital of Nanjing University of TCM, Nanjing, Jiangsu, P.R. China
[3]Department of Oncology, Xuzhou Central Hospital, Xuzhou, Jiangsu Province, P.R. China

Chun-luan Yuan and Li Lei equally contributed for this work

Abstract. – OBJECTIVE: To detect expression of HER2 and SATB1 in paraffin tissues of gastric cancer, and to investigate their relationship with clinic pathological factors and prognosis, and further analyze the correlation between the expression of SATB1 and HER2.

PATIENTS AND METHODS: RT-PCR was used to determine the expression of SATB1 gene and immunohistochemistry (IHC) was used to detect the expression of HER2 protein in gastric cancer tissues. The relationships of both were evaluated with clinic pathological parameters and prognosis, and further analyzed to build a correlation between the expression of SATB1 and HER2.

RESULTS: (1) The expression level of SATB1 gene in gastric cancer tissue was related with TNM stage and distant metastasis. (2) Patients with a lower expression of SATB1 had a higher median overall survival than those with a higher expression of SATB1 (p <0.01). (3) The positive expression of HER2 in gastric cancer tissues was associated with distant metastasis (p <0.01). (4) The expression of SATB1 gene was correlated positively with HER2 protein in gastric cancer (r=0.386, p =0.002).

CONCLUSIONS: High expression of SATB1 gene predicts advanced TNM stage and possible distant metastasis in patients with GC, which was a sign of poor prognosis. The expression of SATB1 was positively correlated with HER2, indicating that SATB1 and HER2 might be in a positive regulation relationship.

Key Words: Gastric cancer, SATB1 gene, HER2 protein, Correlation, Prognosis.

Introduction

Epidemiological data show that gastric cancer is the second leading cause of cancer-related deaths in the world[1,2]. Most gastric cancers have developed into an advanced stage upon diagnosis; the advanced gastric cancers are prone to metasta-sis and invasion, which is fatal[3]. Therefore, to find out the potential predictive index for the invasion and metastasis of gastric cancer has become more and more important. In recent years, studies have shown that special AT rich sequence binding protein (SATB1) is closely related with the invasion and metastasis of the tumor. Further researches showed that SATB1 could regulate the expression of as many as 1000 genes related with the occurrence of cancer, and HER-2 gene was one of those genes[4]. The results of ToGA trial demonstrated that gastric cancer patients with positive HER2 had better survival advantage under trastuzumab + chemotherapy[5]. Another case report showed that intraperitoneal injection of Trastuzumab can improve local tumor control probability in patients with peritoneal cavity metastasis of gastric cancer[6]. Considering that in this work we detected the expression of SATB1 and HER2 in gastric cancer, and tried to investigate the relationship between the expression of SATB1 and HER2 and clinic pathological factors and prognosis, and further analyze the correlation between the expression of SATB1 and HER2.

Patients and Methods

Patients

A total of 60 patients that were confirmed with gastric cancer and accepted surgical treatment in the First Hospital of Lianyungang from April 2009 to February 2013 were enrolled. The data of all patients were complete and no one had accepted radiotherapy and chemotherapy, molecular targeted therapy or immunotherapy before surgery. Among the 60 cases, there were 47 males and 13 females, ranging from

Corresponding Author: Han Liang, MD; e-mail: hanliang801223@foxmail.com

29-72 years, the median age was 59 years. Tumor infiltration of 35 cases have affected the whole layer and reached outside serosa while 25 cases have not affected the whole layer (mucosal, submucosal and muscular layers). According to the 7th version of gastric cancer TNM staging of AJCC-21 cases were in stage I+II, 39 cases in stage III+IV. Evidence-based medicine first class recommendation scheme was applied to assist with chemotherapy. 42 cases accepted chemotherapy and had integrated follow-up data. The follow-up lasted until April 2015.

Major Instruments and Teagents

Real-time fluorescence quantitative PCR (7900 HT Fast) was purchased from ABI Company (Waltham, MA, USA); nucleic acid protein detector was purchased from Eppendorf Company (Hamburg, Germany); paraffin RNA extract kit and reverse transcription kit were purchased from Qiagen (Hilden, Germany); fluorescent dyes were purchased from AB Company; primers were synthesized by the Shanghai Sangon Co., Ltd. (Shanghai, China).

SATB1 Real-Time Fluorescence Quantitative PCR Assay

Human SATB1 gene cDNA primers used for this study are shown in Table I. RNA was extracted (RNase-free FFRE Kit, Qiagen) according to kit instructions, with β-actin used as internal control. 1 μl of RNA was added to 99 μl RNase-free ddH_2O and concentration and purity was estimated with UV spectrophotometer. RT reaction (10 μl) consists of 1 μl, 6 μlRNA+DEPC H_2O, at 42°C for 2 min, followed by addition of 0.5 μl primer (10 nmol/μl), 0.5 μl RT enzyme, 2 μl Buffer at 42°C for 30 min, and finally at 95°C for 5 min. QPCR reaction (5 μl) consists of master mix- 2.5 μl Template cDNA, 1 μl SATB1 upstream and downstream premier 0.25 μl (20 pmol/μl) for each, H_2O: 1 μl. PCR amplification conditions were pre-denaturation at 95°C for 10 min, 95°C for 15 s, 60°C for 1 min, for a total of 50 cycles. The temperature of dissolution curve was 95°C for 15s, 60 °C for 15s and 95°C for 15s.

The experimental data was processed by $2^{-\Delta\Delta CT}$ on the premise that the amplification efficiency of the target gene and reference gene were similar[7,8]. The average CT value from triplicate samples and ΔCT value ($\Delta CT=CT_{SATB1}-CT_{\beta\text{-actin}}$) was calculated, and $2^{-\Delta\Delta CT}$ ($CT=CT_{\text{target sample}}-CT_{\text{reference sample}}$) was computed. The values were used to present the relative multiples of the target value relative to the reference value and calculated out the mean value of the expression amount of SATB1 gene in the 60 patients. The patients were divided into high expression group and low expression group according to whether the expression amount was higher or lower than the mean value.

HER2 Immunohistochemistry Assay

Paraffin block preserved under the formaldehyde fixation and paraffin embedding were taken, and consecutively slices of 5 μm. All procedure were followed according to instructions of kit. Cell membrane showing no staining or <10% cell membrane showed staining was defined to be negative (-); ≥10% tumor cell membrane slightly or faintly sho+wed staining was defined to be weak positive (+); ≥10% cells showed weak to moderate staining in basolateral part, lateral membrane or the whole membrane was defined to be moderate positive (++); ≥10% cells showed staining in basolateral part, lateral membrane or the whole membrane was defined to be strong positive (+++), (-)/(+) was defined to be negative expression, (++) to be moderate expression, and (+++) to be over-expression[7-10]. In our experiment, (++) and (+++) were defined as HER2 positive.

Statistical Analysis

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was applied to make a statistical analysis. Measurement data were presented by $\bar{X}\pm s$, χ^2 and *t*-test were applied in the comparison between groups, Kaplan-Meier and Log-rank methods were applied to make a survival analysis, Spearman correlation analysis was applied to determine the correlation between SATB1 and HER2, $p<0.05$ was statistically significant.

Results

Comparison of the Amplification Efficiency of SATB1 gene and Reference Gene

Mapping SATB1gene ΔCT through log value of cDNA concentration gradient. The slope of the straight line was 0.01 (slope absolute value <0.1), indicating that the amplification efficiency of the target gene and reference gene were similar. Experimental results can be processed by $2^{-\Delta\Delta CT}$ (Figure 1).

Expression of HER2 and SATB1 in Gastric cancer tissues

Both SATB1 and HER2 were highly expressed in gastric cancer tissues, and the positive rate of HER2 was 23.33% (Figures 2, 3).

Table I. Relationship between the expression of SATB1 and HER2 in gastric cancer and the clinical pathological characteristics.

Characteristics	No.	SATB1 expression		p	HER2 expression (n)		p
		ΔCT	2-ΔΔCT		Positive	Negative	
Age (year)				0.286			0.131
≥60	28	4.89±0.68	1.38±0.61		9	19	
<60	32	4.70±0.67	1.21±0.60		5	27	
Gender				0.643			0.474
Male	47	4.82±0.72	1.31±0.63		10	37	
Female	13	4.69±0.51	1.22±0.53		4	9	
Tumor size (cm)				0.586			0.473
≥5	38	4.82±0.63	1.32±0.58		10	28	
<5	22	4.74±0.76	1.23±0.65		4	18	
Location				0.077			0.309
Cardia/fundus	8	5.05±0.64	1.64±0.52		3	5	
Body/antrum/pylorus	52	4.75±0.68	1.23±0.60		11	41	
Differentiation				0.145			0.078
Well or moderate	45	4.74±0.67	1.22±0.58		13	32	
Poor or undifferentiated	15	4.95±0.63	1.49±0.66		1	14	
Depth of invasion				0.446			0.606
T1+T2	25	4.69±0.74	1.22±0.68		5	20	
T3+T4	35	4.86±0.62	1.34±0.55		9	26	
TNM stage				0.008			0.481
I+II	21	4.48±0.67	1.01±0.56		6	15	
III+IV	39	4.95±0.63	1.44±0.58		8	31	
Lymph node metastasis				0.061			0.305
Present	37	4.92±0.65	1.40±0.60		7	30	
Absent	23	4.57±0.67	1.10±0.57		7	16	
Distant metastasis				0.000			0.003
Present	10	5.22±0.57	1.17±0.54		6	4	
Absent	50	4.70±0.67	1.88±0.59		8	42	

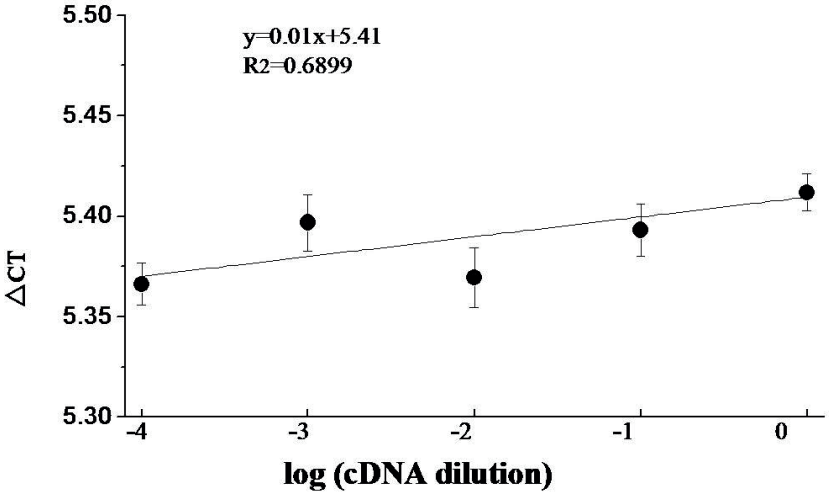

Figure 1. Amplification efficiency of the target gene (SATB1) and the internal control (b-actin)

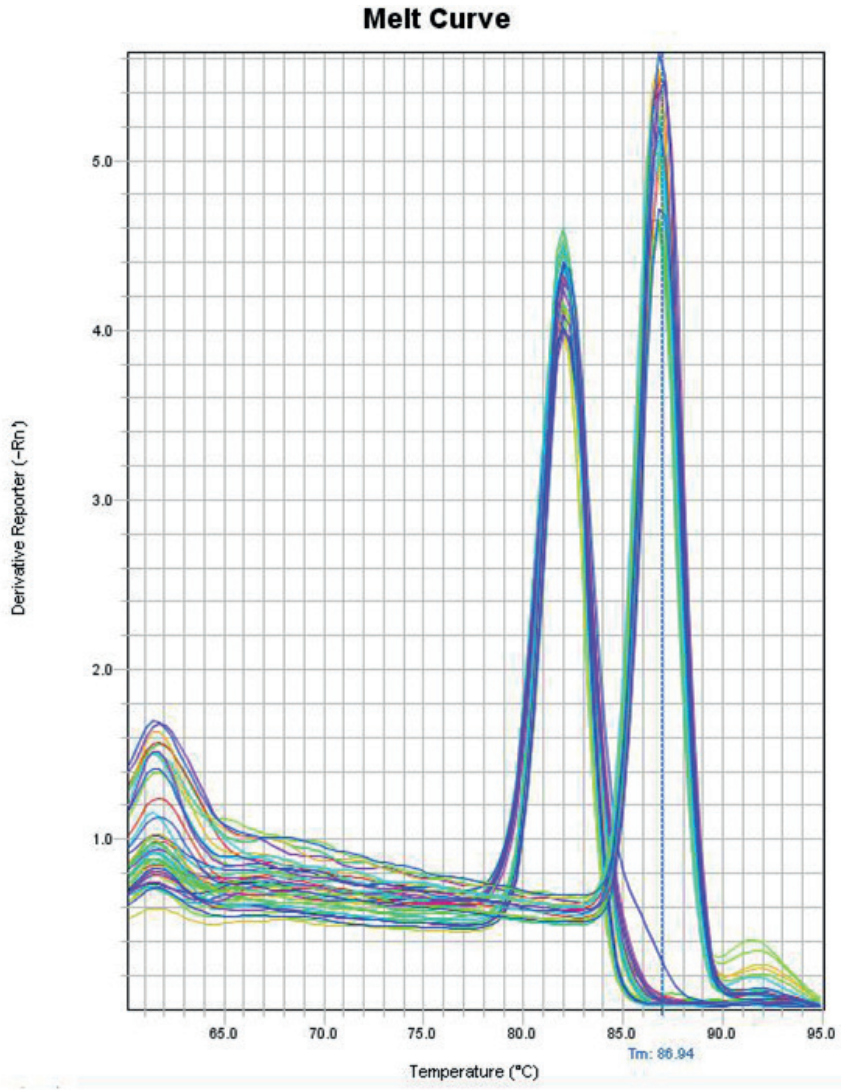

Figure 2. Real-time PCR for detecting the expression of SATB1 in the samples.

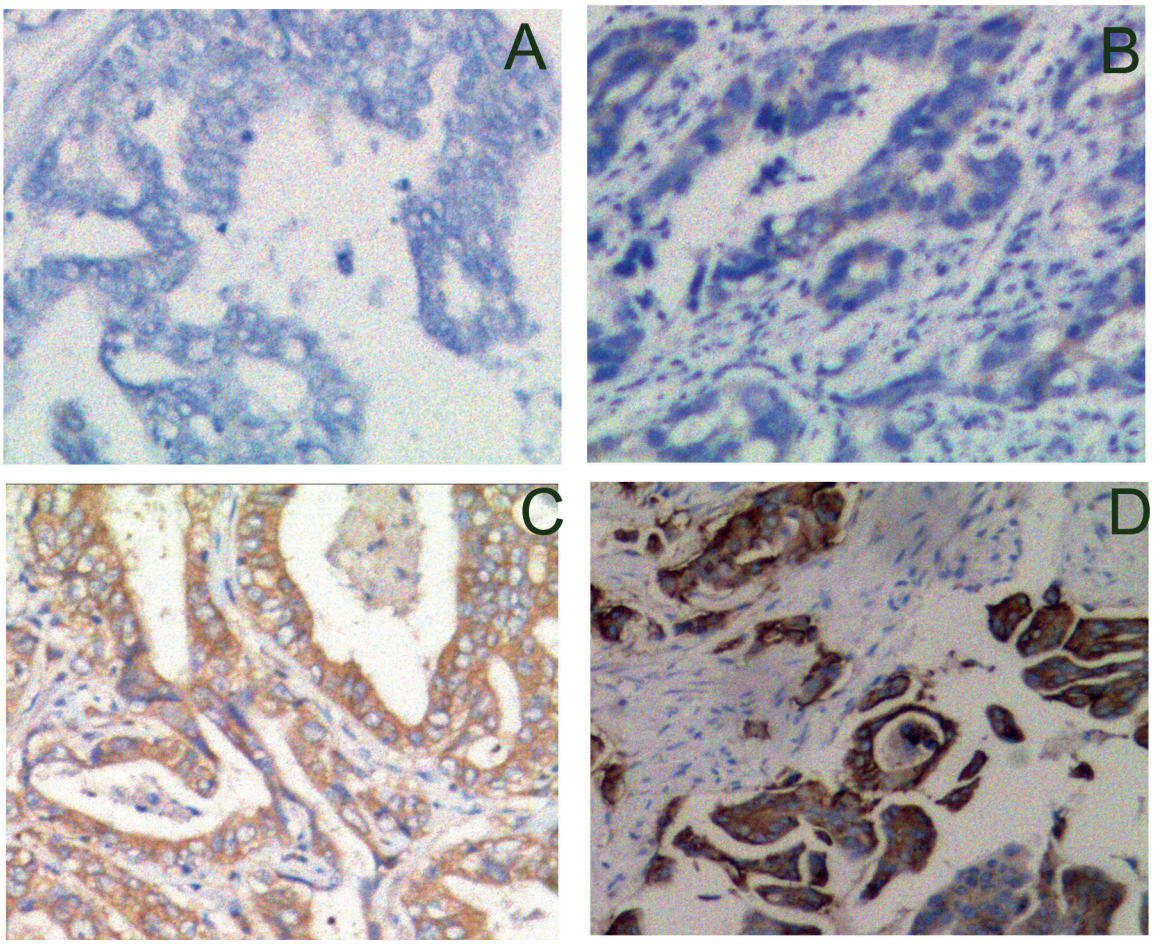

Figure 3. HER2 expression in gastric cancer tissue (×400). ***A,*** Negative expression in gastric cancer tissue (0). ***B,*** Weadly positive expression in gastric cancer tissue (+1). ***C,*** Positive expression in gastric cancer tissue (+2). ***D,*** Strongly expression in gastric cancer tissue (+3).

Relationship Between The Expression of SATB1 and HER2 in Gastric Cancer and the Clinical Pathological Characteristics

The expression level of SATB1 gene in gastric cancer tissues was significantly correlated with TNM stage and distant metastasis (p <0.01); the expression of HER2 was significantly correlated with distant metastasis (p <0.01), but not significantly correlated with TNM staging (p >0.05); differences on the expression of SATB1 and HER2 of different age, gender, tumor size, tumor location, histological differentiation degree, tumor invasion depth and lymph node metastasis were not statistically significant (p >0.05) (Table II).

Table II. The relationship between SATB1 gene and HER2 in gastric cancer tissues.

		SATB1 expression		
HER2	N	$2^{-\Delta\Delta CT}$	r	p
(-)	33	1.08±0.52	0.386	0.002
(+)	13	1.43±0.71		
(++)	8	1.57±0.63		
(+++)	6	1.76±0.34		

Correlation Between the Expressions of SATB1 Gene and HER2 in Gastric Cancer Tissues

The expressions of SATB1 gene and HER2 in gastric cancer tissues were positively correlated (r=0.386, p =0.002) (Table III).

Relationship Between the Expression of SATB1 and HER2 and the Overall Survival of 42 Gastric Cancer Patients

OS median of the 42 patients was 28.0 months. 19 patients had low expression of SATB1 while 23 patients had high expression of SATB1, and the median OS of patients with low expression was longer than those with high expression (43.0 months *vs.* 23.0 months, p <0.001). 29 patients had a positive expression of HER2 while 13 patients had a negative expression of HER2, and difference of the median survival time of the two groups was not statistically significant (28.0 months *vs.* 32.0 months, p =0.689) (Figures 4, 5).

Discussion

Gastric cancer is characterized by high malignancy, high invasiveness and also high metastasis. Its occurrence and development are subject to the regulation of multiple genes and are involved with multiple stages and phases. Most patients have reached an advanced stage when they are diagnosed with gastric cancer, thus resulting in the high fatality rate. Therefore, it has become quite important to promote individualized precision treatment, to find out the molecular biological markers for predicting prognosis and to screen out the groups with therapeutic advantages.

Table III. RT-PCR primers used in this study.

SATB1U	5'-ACGGGATGCACCAAAGTGTGTA-3'
SATB1L	5'-GCTTGTTTGAGGCTCCGGAATA-3'
β-actin U	5'-CCTGGCACCCAGCACAAT -3'
β-actin L	5'-GCCGATCCACACGGAGTACT -3'
SATB1U	5'-ACGGGATGCACCAAAGTGTGTA-3'

SATB1 is a kind of tissue-specific nuclear matrix binding protein located in chromosome 3p23 region of No. 3 chromosome. SATB1 was screened out from human cDNA library in 1992 by Dickinson et al[11]. SATB1 could specifically combine with matrix attachment regions (MARs), thus influencing the construction of DNA ring. SATB1 is involved in the processes including chromosome remodeling, histone acetylation, and methylation, and could regulate the transcriptional activity of multiple genomes[12-14]. SATB1 had an abnormal expression in most tumor cells[15]; it could regulate the expression of as many as 1000 genes related with the occurrence of malignant cancer, so it is also called "genome organizer". SATB1 could promote the occurrence, development and metastasis of the tumor, indicating that it is playing a key role in tumor progression[16,17]. Human epidermal receptor 2 (HER2) is a member of the tyrosine kinase receptor family, and also an important regulator of cell proliferation, differentiation and survival. HER 2 could affect the invasion and metastasis of a tumor in many ways. It is one of the biological markers of poor prognosis of many epithelial malignancies[18,19]. The results of a meta-analysis[20] on the relationship between HER 2 expression and patholog-

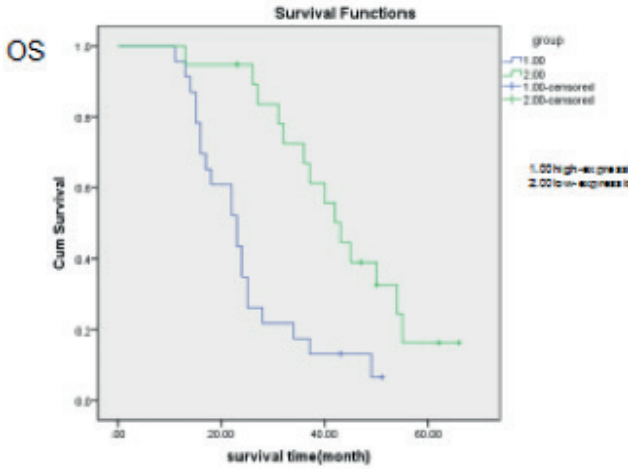

Figure 4. Kaplan-Meier survival curves of gastric cancer patients with higher or lower level expression of SATB1

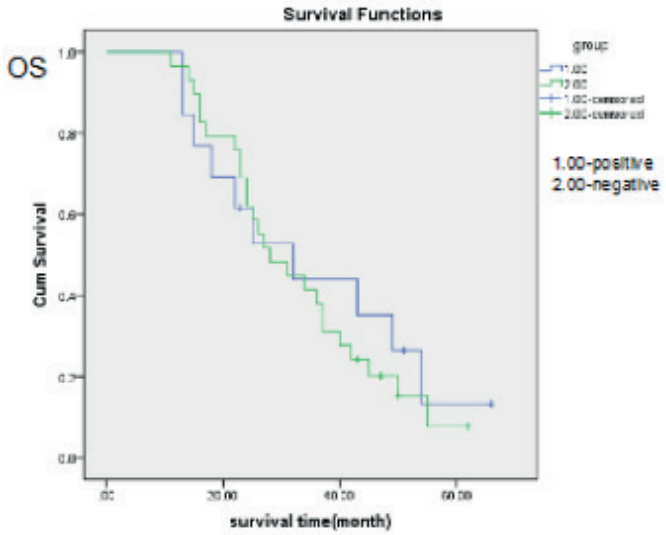

Figure 5. Kaplan-Meier survival curves of gastric cancer patients with positive or negative expression of HER2.

ical characteristics of gastric cancer that included 5290 patients and 15 studies indicated that HER 2 was associated with tumor differentiation level, Lauren type, lymph node metastasis, vascular invasion, lymphatic vessel invasion, but was not associated with tumor invasion depth and staging. HER-2, as a tumor-associated antigen, was highly expressed in gastric cancer, which may be related to the high invasiveness of tumor and the prognosis of patients[20-22]. Therefore, it is suggested that the targeted treatment that could obstruct HER2 path might be able to inhibit invasion and metastasis of the tumor. ToGA study[5] was a prospective international multi-center III stage randomized controlled clinical trial. In ToGA study, 584 patients were randomly divided into two groups: trastuzumab + chemotherapy group (n=294) and pure chemotherapy group (n=290). Further study[23] found that among the 446 patients with high HER3 expression, the median survival time of patients intrastuzumab+ chemotherapy group (n=228) (16 months) were significantly superior than those in pure chemotherapy group (n=218) (11.8 months) (HR0.65, 95%CI0.51-0.83), indicating that trastuzumab molecular targeted therapy was the first choice for the treatment of advanced gastric cancer with positive HER2 expression. However, some other investigations[24] also proved that the state of HER2 had no effect on the prognosis of patients with gastric cancer. So the conclusion was still controversial.

In our study, we evaluated the expression of SATB1 and HER2 in gastric cancer tissues. We have found that there was a high expression of HER2 and SATB1 in gastric cancer tissues, the positive rate of HER2 was 23.33%, indicating that it may be related to the occurrence of gastric cancer. Further analysis indicated that the expression level of SATB1 gene in gastric cancer tissues was significantly correlated with TNM stage and distant metastasis (p <0.01), indicating that gastric cancer patients with high expression of SATB1 would be subject to an advanced clinical stage and poor prognosis. The expression of patients with lymph node metastasis was significantly higher than those without lymph node metastasis, but the difference was not statistically significant (p >0.05). The expression of HER2 was significantly correlated with distant metastasis (p <0.01), but was not significantly correlated with TNM staging (p >0.05), which was not in line with the existing results. It might be because the sample size in our study was relatively small and there were also differences in region and ethnics. The expressions of SATB1 and HER2 in gastric cancer tissues were positively correlated (r=0.386, p =0.002), suggesting that SATB1 and HER2 may be in a positive regulation relationship in gastric cancer. Our results showed that the median survival time of patients with low expression of SATB1 was significantly longer than that with high expression (p <0.01), and the difference was statistically significant. The median survival time of HER2 positive expression group and negative expression group was not significantly different (p >0.05).

Conclusions

This research suggested that joint detection of HER2 and SATB1 expression in gastric cancer can be used to screen out the patients with high-risk of gastric cancer metastasis, and also could provide a basis for developing an individualized treatment program for patients.

This study needs to be confirmed by a larger sample size and further *in-vitro* trials as well as *in vivo* experiments. Nevertheless, there is need to elucidate the molecular mechanisms for a better understanding of the expression of SATB1, HER2 and occurrence or development of gastric cancer, along with their correlation.

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Conflicts of interest

The authors declare no conflicts of interest.

References

1) Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. Methods Mol Biol 2009; 472: 467-477.
2) Patani N, Jiang W, Mansel R, Newbold R, Mokbel K. The mRNA expression of SATB1 and SATB2 in human breast cancer. Cancer Cell Int 2009; 9: 18.
3) Wang CW, Fang XH. The role of targeted agents in the treatment of advanced gastric cancer: a metaanalysis of randomized controlled trials. Eur Rev Med Pharmacol Sci 2016; 20: 1725-1732.
4) Han HJ, Russo J, Kohwi Y, Kohwi-Shigematsu T. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature 2008; 452: 187-193.
5) Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376: 687-697.
6) Berretta M, Fisichella R, Borsatti E, Lleshi A, Ioffredo S, Meneguzzo N, Canzonieri V, Di Grazia A, Cannizzaro R, Tirelli U, Berretta S:Feasibility of intraperitoneal Trastuzumab treatment in a patient with peritoneal carcinomatosis from gastric cancer. Eur Rev Med Pharmacol Sci 2014; 18: 689-692.
7) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001; 25: 402-408.
8) Padda RS, Gkouvatsos K, Guido M, Mui J, Vali H, Pantopoulos K. A high-fat diet modulates iron metabolism but does not promote liver fibrosis in hemochromatotic Hjv$^{-/-}$ mice. Am J Physiol Gastrointest Liver Physiol 2015; 308: G251-261.
9) Mrklic I, Bendic A, Kunac N, Bezic J, Forempoher G, Durdov MG, Karaman I, Prusac IK, Pisac VP, Vilovic K, Tomic S. Her-2/neu assessment for gastric carcinoma: validation of scoring system. Hepatogastroenterology 2012; 59: 300-303.
10) Rüschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, van de Vijver M, Viale G. HER2 testing in gastric cancer: a practical approach. Mod Pathol 2012; 25: 637-650.
11) Dickinson LA, Joh T, Kohwi Y, Kohwi-Shigematsu T. Atissue specific MAR SARDNA binding protein with unusual binding site recognition. Cell 1992; 70: 631-645.
12) Liliane AD, Craig DD, Terumi KS. An atypical homeodomain in SATB1 promotes specific recognition of the key structural element in a matrix attachment region. J Biol Chem 1997; 272: 11463-11470.
13) Galande S, Purbey PK, Notani D, Kumar PP. The third dimension of gene regulation: organization of dynamic chromatin loopscape by SATB1. Curr Opin Genet Dev 2007; 17: 408-414.
14) Wen J, Huang SM, Rogers H, Dickinson LA, Kohwi-Shigematsu T, Noguchi CT. SATB1 family protein expressed during early erythroid differentiation modifies globin gene expression. Blood 2005; 105: 3330-3339.
15) Mai JC, Ellenbogen RG. SATB1: the convergence of carcinogenesis and chromatin conformation. Neurosurgery 2008; 63: N6.
16) Huang YK, Fan XG, Qiu F. Genomics of hepatitis B virus-related hepatocellular carcinoma and adjacent noncancerous tissues with cDNA microarray. Chin Med J (Engl) 2011; 124: 2057-2064.
17) Tu W, Luo M, Wang Z, Yan W, Xia Y, Deng H, He J, Han P, Tian D. Upregulation of SATB1 promotes tumor growth and metastasis in liver-cancer. Liver Int 2012; 32: 1064-1078.
18) Sirica AE. Role of ErbB family receptor tyrosine kinases in intrahepatic cholangiocarcinoma. World J Gastroenterol 2008; 14: 7033-7058.
19) Hartman ZC, Wei J, Osada T, Glass O, Lei G, Yang XY, Peplinski S, Kim DW, Xia W, Spector N, Marks J, Barry W, Hobeika A, Devi G, Amalfitano A, Morse MA, Lyerly HK, Clay TM. An adenoviral vaccine encoding full-length inactivated human Her2 exhibits potent immunogenicty and enhanced therapeutic efficacy without oncogenicity. Clin Cancer Res 2010; 16: 1466-1477.

20) Liang JW, Zhang JJ, Zhang T, Zheng ZC. Clinicopathological and prognostic significance of HER2 overexpression in gastric cancer: a meta-analysis of the literature. Tumour Biol 2014; 35: 4849-4858.

21) Jørgensen JT, Hersom M. HER2 as a prognostic marker in gastric cancer – A systematic analysis of data from the literature. J Cancer 2012; 3: 137-144.

22 Chen C, Yang JM, Hu TT, Xu TJ, Yan G, Hu SL, Wei W, Xu WP. Prognostic role of human epidermal growth factor receptor in gastric cancer: a systematic review and meta-analysis. Arch Med Res 2013; 44: 380-389.

23) Qiu MZ, Li Q, Wang ZQ, Liu TS, Liu Q, Wei XL, Jin Y, Wang DS, Ren C, Bai L, Zhang DS, Wang FH, Li YH, Xu RH. HER2-positive patients receiving trastuzumab treatment have a comparable prognosis with HER2-negative advanced gastric cancer patients: a prospective cohort observation. Int J Cancer 2014; 134: 2468-2477.

24) Terashima M, Kitada K, Ochiai A, Ichikawa W, Kurahashi I, Sakuramoto S, Katai H, Sano T, Imamura H, Sasako M; ACTS-GC Group. Impact of expression of human epidermal growth factor receptors EGFR and ERBB2 on survival in stage II/III gastric cancer. Clin Cancer Res 2012; 18: 5992-6000.