Int J Clin Exp Pathol 2017;10(6):6710-6718
www.ijcep.com /ISSN:1936-2625/IJCEP0054588

Original Article

Expression of 14-3-3 beta in gastric cancer and its correlation with clinicopathological features

Guanyu Zhu[1], Guodong Li[2], Ming Zhang[3], Shupeng Zhao[1], Tie Wang[1], Yuzhe Wei[1], Yingwei Xue[1]

Departments of [1]Gastroenterologic Surgery, [3]Breast Surgery, Harbin Medical University Cancer Hospital, Harbin 150040, China; [2]Department of Surgical Oncology, Cancer Treatment Center, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, China

Received April 4, 2017; Accepted May 24, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: 14-3-3 beta protein has been implicated in the initiation and progression of cancers. We had compared the protein profile separated by two-dimensional gel electrophoresis (2-DE) between two groups of gastric cancer samples with lymph node metastasis or not. Among all the changed proteins, 14-3-3 beta protein was the most elevated one in the protein profile. Then 14-3-3 beta protein expression was down-regulated by RNA interference, and a series of cytobiology functions were examined in vitro. Furthermore, 14-3-3 beta protein expression levels in gastric cancer samples were analyzed by immunohistochemistry staining, and the significance between 14-3-3 beta protein expressing levels with clinicopathological factors was explored in these samples. We have confirmed that the absent of 14-3-3 beta expression may significantly inhibit the proliferation of HGC27 and MGC803 cells and reduce the invasive rate of the MGC803 cells. Additionally, 14-3-3 beta protein was highly related to lymph node metastasis in gastric carcinoma. Overall, these results suggest that 14-3-3 beta protein has the potential to be used as a diagnostic and prognostic biomarker in gastric cancer.

Keywords: 14-3-3 beta protein, gastric cancer, lymph node metastasis, biomarker

Introduction

Despite the overall incidence of gastric cancer is declining and the prognosis patients with gastric cancer has been improved owing to early detection and extensive surgery [1-3]. However, gastric cancer is still one of the leading causes of death among malignant diseases in China today [4, 5]. Lymph node metastasis is the most important prognostic feature for gastric cancer [6]. Most of the surgeons believe that D2 lymphadenectomy is the only standard and optimal surgical procedure for patients. Although the relevant experiments have never been stopped, the molecule mechanisms of lymph node metastasis are still unclear.

In our previous study, a subgroup carcinoma proteomics approach was used to compare the protein profile between two groups of gastric cancer samples. One group of samples was with lymph node metastasis, and the other was without lymph node metastasis. The invasion depth was the same in these samples. A series of protein were separate by two-dimensional gel electrophoresis (2-DE) and 14-3-3 beta protein was identified as a novel biomarker between these two groups [7].

In the present study, 14-3-3 beta protein expression was identified in HGC27 and MGC803 gastric cancer cell lines by western blot analysis. Based on the results of 2-DE and western blot analysis, we hypothesis that 14-3-3 beta plays an important role in gastric cancer proliferation and invasion. To validate our hypothesis, 14-3-3 beta expression was down-regulated by RNA interference, and a series of cytobiology functions were examined. Furthermore, we analyzed and reviewed the 14-3-3 beta expression level in gastric cancer samples with immunohistochemistry stain and explored the significance between 14-3-3 beta protein expressing levels with clinicopathological factors.

Materials and methods

Cell lines and cell culture

SGC7901, MGC803, HGC27 gastric carcinoma cell lines were obtained from the genetics labo-

ratory of Harbin medical university. Theses cell lines were maintained in RPMI1640 medium supplement with 15% (v/v) FBS (fetal bovine serum; Sigma-Aldrich). All the cell lines were cultured at 37°C with 5% CO_2 in a humidified incubator.

Western blot assay

Whole-cell lysates of MGC803 and HGC27 were prepared by incubating cells in RIPA buffer containing protease inhibitors. Cell lysates were centrifuged at 12000 g for 30 minutes at 4°C. The supernatant was collected and the protein concentration was measured by the BCA method. Briefly, prepare the amount of BCA working solution in 50 volumes of BCA reagent A plus 1 volume of BCA reagent B (50:1) to fully mix thoroughly. Afterwards, add appropriate volume of protein sample and 200 μl of BCA working solution to each of the 96-well plate, and place the plate at 37°C for 30 minutes. The absorbance at the wavelength of 562 nm was measured by a microplate reader, and the protein concentration of the sample was calculated from the standard curve. Protein (50 μg) were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. Non-specific binding was blocked with 5% skim milk in PBS for 2 hours at room temperature. Then the membrane was incubated with mouse monoclonal antibodies to 14-3-3 beta (1:10000 Abcam) in TBST/5% skim milk at 4°C overnight. After that, the membrane was rinsed with TBST three times, and followed by incubation with anti-mouse IRDye 800 secondary antibodies, mouse anti-mouse IgG (Amersham Biosciences), for 1 hour at room temperature. Finally, after three washed with TBST, the protein bands were visualized by an Odyssey infrared imaging system (LICOR, Lincoln, NE) at wavelength of 800 nm.

RNA interference

The specific siRNA (short interfering RNA) targeted to different region of 14-3-3 beta mRNA was designed and purchased from the Genechem in Shanghai. MGC803 and HGC27 gastric cancer cells were seeded in 24 well plates (3×10^4 per well) separately. When the cells grew in the log phase, cells were transfected with siRNA at 125 pmol each well using 150 μl LipofectaminTM2000 (Invitrogen, Carlsbad, CA, U.S.A). The efficiency of transfection was detected by microscope with fluorometric method. Cells were collected for assay at 48 hours post-transfection.

Cell proliferation assay

Normal, negative control and post-transfected HGC27, MGC803 cells were seeded into 96-well plates (1.5×10^3 per well). After culturing for various durations, cell proliferation was evaluated by MTT assay, according to the manufacture's protocol. In brief, 10 μl MTT solution (5 mg/ml) was added to each well. Followed by cultured for another 4 hours at 37°C in a 5% CO_2 humidified chamber. Then 100 μl DMSO was added to each well and mix vigorously to solubilize colored crystals. The absorbance at 490 nm was measured by using a multi-well scanning spectrophotometer.

Cell invasion assay

Normal, negative control and post-transfected (48 hours) HGC27, MGC803 cells were treated with trypsin and re-suspended as a single cell solutions. A total of 5×10^4 cells in 0.5 ml serum-free RPMI1640 medium were seeded on a 8 μm pore polycarbonate membrane Boyden chamber insert in a transwell apparatus, coated with Matrigel (BD Bioscience). 500 μl RPMI1640 containing 15% FBS was added to the lower chamber. After incubated for 24 hours at 37°C, in a 5% CO_2 humidified chamber, cells on the top surface of insert were removed by wiping with a cotton swab. Cells that have migrated to the bottom surface of the insert were fixed in 100% methanal for 2 minutes, stained by hematoxylin and eosin, washed in PBS, and then detected by microscopic inspection (×200, ×400). Values for invasion were obtained by counting five fields of per membrane and represent the average of three independent experiments.

Immunohistochemical stain and analysis

181 paraffin-embedded human gastric cancer tissues were obtained from Department of Pathology of the third affiliated hospital of Harbin medical university (Harbin, PR China) from January, 2007 to December, 2009. Consecutive 4 μm sections were cut from each paraffin-embedded block. After bake at 60°C for 2 hours, the slides were deparaffinized with xylene, and rehydrated. The slides were

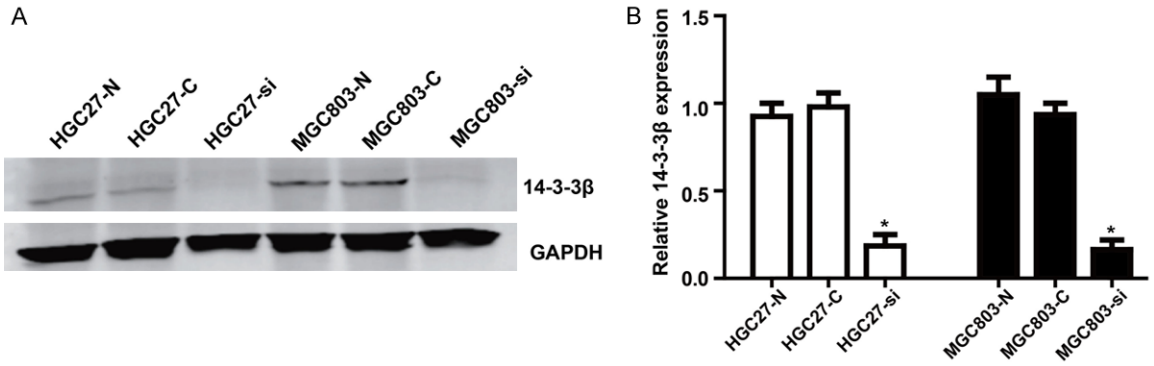

Figure 1. A. The expression of 14-3-3 beta proteins were significantly decreased when the HGC27, MGC803 cells were transfected with 14-3-3 beta-siRNA for 48 hours. B. A columnar graph of the relative 14-3-3 beta protein expression. Data are expressed as the mean ± SEM. n=3. *P<0.05 when compared to the control group (Student's t-test).

immersed in 3% hydrogen peroxide for blocking endogenous peroxidase activity. Antigenic retrieval was carried out by heating the sections in citrate acid antigenic retrieval buffer (0.01 mmol/L, pH 6.0) in high pressure holder. Nonspecific binding was blocked with 10% BSA in PBS for 60 minutes, followed by sections were incubated with polyclonal anti-14-3-3 beta antibody (Biosynthesis biotechnology Co., Ltd, Beijing, China) in a dilution 1:100 overnight at 4°C. Controls without primary antibodies were also included. After washed in PBS, the sections were incubated with horseradish peroxidase-conjugated secondary antibody for 30 minutes at 37°C. Immunocomplexes were detected with 3'-diaminobenzidine. Sections were counterstained with hematoxylin, dehydrated and mounted for examination under the microscope.

All the results were judged by two pathologists independently. 14-3-3 beta was stained as a brown and yellow reaction product. Staining intensity was scored according to the following criteria: 0 (negative), 1 (weak positive, less than 10% tumor cells were stained), 2 (moderate positive, 11-50% tumor cells were stained), and 3 (strong positive, more than 50% tumor cells were stained). Moderate or strong staining was used to indicate high 14-3-3 beta expression, and negative or weak staining was used to represent low 14-3-3 beta expression.

Patients and clinicopathologic records

A consecutive series of 181 patients underwent surgery as an initial treatment for gastric cancer from January, 2007 to December, 2009 at the Third Affiliated Hospital of Harbin medical university (Harbin, PR China). Data were received from operative and pathology report. The clinicopathological parameters that were evaluated included sex, age (50 years or younger, older than 50 years), tumour size (smaller than 5 cm, 5 cm and larger), anatomical portion (upper one-third, middle, lower one-third), histological type (well and moderately differentiated, poorly differentiated, signet-ring cell and mucinous), serosal invasion (absent or present), lymph node metastasis (negative or positive). Then Based on the expression level of 14-3-3 beta we divided these sample into two groups, one is the lower expression level group (the immunohistochemical stain score is 0 or 1), the other is the higher expression level group (the immunohistochemical stain score is 2 or 3).

Statistical analysis

Quantitative data in paired groups were analyzed using a Student's t-test, the chi-square test or Fisher's exact test was used to identify statistical differences in the clinicopathologic variable analysis. Multivariate logistic regression with covariate adjustment was used to assess the association of 14-3-3 beta overexpression with metastasis. A two-sided P<0.05 was considered as statistically significant. Statistical analysis was carried out using SAS software (version 9.1.3, SAS Institute, Cary, NC).

Results

14-3-3 beta protein was expressed in gastric cancer cell lines and inhibited by transfection of 14-3-3 beta-siRNA

Based on the detection of western blot analysis, we have confirmed that 14-3-3 beta protein

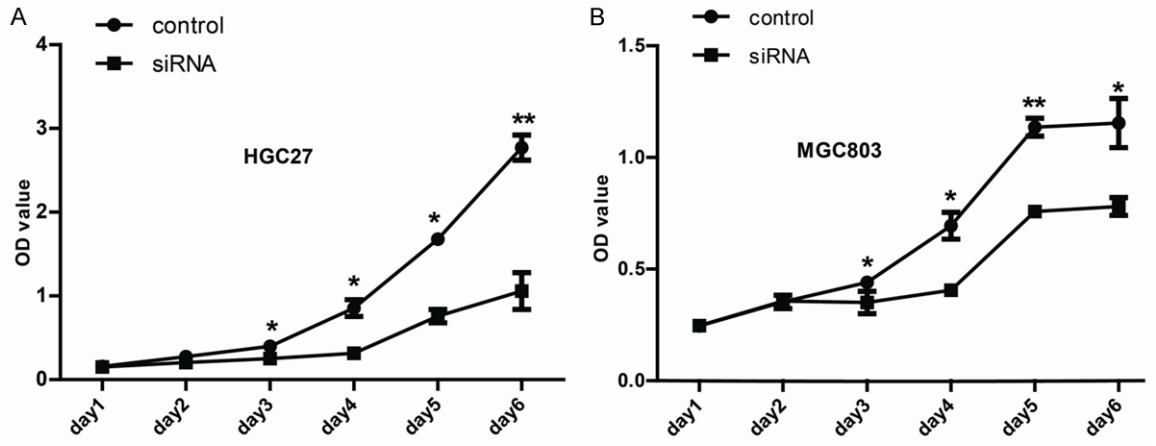

Figure 2. Knockdown of 14-3-3 beta protein inhibited the proliferation of both HGC27 and MGC803 cells. A, B. The growth curve shifted to the right and the growth rate was slowed after the HGC27 and MGC803 cells were transfected with 14-3-3 beta-siRNA. Data are expressed as the mean ± SEM. n=6. *P<0.05 or **P<0.01 when compared to the control group (Student's t-test).

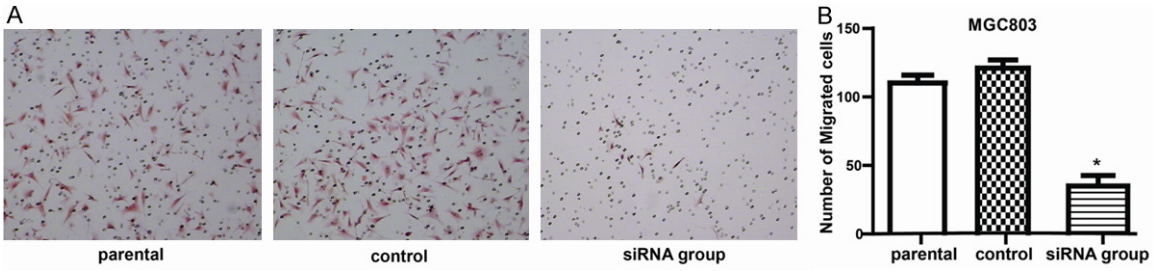

Figure 3. Knockdown of 14-3-3 beta protein inhibited the invasion of MGC803 cells determined by transwell invasion assay. A. Photographs represented the cells passing through the Matrigel by transwell invasion assay (200 magnification). B. Average invasive cell number from three independent experiments was shown in bar chart. n=6, *P<0.05 when compared with control group (Student's t-test).

was stably expressed in HGC27 and MGC803 cell lines. In order to detect the effect of 14-3-3 beta on the gastric cancer cell proliferation and invasion, we transfected 14-3-3 beta-siRNA into the HGC27 and MGC803 cells for 48 h. And the results showed that RNA interference effectively down regulated the 14-3-3 beta protein expression as shown in **Figure 1** (*P<0.05).

14-3-3 beta protein inhibited tumor cell proliferation and invasion in vitro

After transfection of 14-3-3 beta-siRNA or negative control in HGC27 and MGC803 cells, the proliferation rates of transfected cells at the 6 time points were detected by MTT assay. As shown in **Figure 2**, knock-down of 14-3-3 beta significantly decreased the proliferation rates of HGC27 and MGC803 cells compared to the negative control after 72 h (*P<0.05, **P< 0.01). Furthermore, the cell invasion was detected by the Matrigel invasion assay. There was no difference of invasiveness between the 14-3-3 beta knock-down group (siRNA group) and the negative control group in HGC27 cells. However, in MGC803 cells, the 14-3-3 beta knock-down group showed a significantly decreased invasiveness compared with the negative control group (P<0.05) (**Figure 3**). These results demonstrated that the absence of 14-3-3 beta expression may decrease the proliferation of HGC27 and MGC803 cells and reduce invasive rates of MGC803 cells obviously.

Relationship of 14-3-3 beta protein expression level with clinicopathologic factor of gastric tumor

Data from one hundred and eighty-one patients were collected for the analysis. The characteris-

Table 1. Relationship of 14-3-3β expression with clinicopathologic features of gastric carcinoma

Variables	14-3-3β		*P* value
	Low	High	
Gender			0.7302 (chi-square)
Male	48	77	
Female	20	36	
Age group			0.6765 (chi-square)
≤50	20	30	
>50	48	83	
Location			0.0594 (fisher)
Upper	45	58	
Middle	18	32	
Lower	5	17	
Whole	0	6	
Tumor size			0.0004 (chi-square)
<5 cm	30	22	
≥5 cm	38	91	
Histological type			0.8042 (fisher)
WMD	15	31	
PD	46	69	
SRC	3	4	
MC	4	9	
Serosal invasion			<.0001 (chi-square)
Absent	34	23	
Present	34	90	
Lymph node metastasis			0.0015 (chi-square)
Negative	31	26	
Positive	37	87	
Total	68	113	

tics of the enrolled patients are generalized in **Table 1**. The patient median age was 55 years (range, 24-73 years). Over half of all patients (68.5%) had lymphatic metastasis. The median tumor size was 5.5 cm. The results of univariate analysis showed that 14-3-3 beta expression level was associated with tumor size, lymph node metastasis and serosal invasion (P<0.05) (**Table 1**). **Figure 4** showed the representative immunohistochemical stain results of 14-3-3 beta expression in gastric carcinoma tissues.

Relationship between lymphnode metastasis and clinicopathologic factors in gastric carcinoma

Results of univariate analysis to identify risk factor for lymph node metastasis in gastric tumor are shown in **Table 2**. When the correlation between clinicopathological variables and lymph node metastasis were analyzed by univariate analysis, the present of tumor diameter, serosal invasion and expression level of 14-3-3 beta was associated with lymph node metastasis (P<0.05).

Multivariate analysis of potential risk factors for lymph node metastasis

Results of multivariate analysis to identify risk factors for lymph node metastasis in gastric tumor are shown in **Table 3**. When the correlation between clinicopathological variables and lymphnode metastasis were analyzed by multivariate analysis, the present of serosal invasion was significantly related to a higher risk of lymph node metastasis (P<0.05), which demonstrated that serosal invasion was an independent risk factor for lymph node metastasis.

Discussion

14-3-3 proteins is a highly conserved family of dimeric protein and includes seven isoforms [8, 9]. Functioning as phosphoserine/phosphothreonine-binding modules, 14-3-3 protein interacts with various signal transduction proteins and eventually regulates cell cycle, apoptosis, stress response, malignant transformation within all eukaryotic cells [1]. In addition, 14-3-3 beta isoform is mainly distributed in cytoplasm and expressed highly in lymph node s and spleen [2]. Nagashima et al. had found that suppressed 14-3-3 beta RNA could inhibit the tumor cell proliferation [10]. Takihara et al. had declared highly expression of 14-3-3 beta protein could induce fibroblasts tumorigenesis in nude mouse models [11]. Moreover, Somanath et al. had reported that 14-3-3 beta-Rac1-p21 could regulate Akt1-mediated cytoskeletal organization, lamellipodia formation [12].

However, the function of 14-3-3 beta in gastric cancer is still not clear. We have confirmed in our previous research that, the expression of 14-3-3 beta was significantly elevated in gastric cancer tissues with lymph node metastasis than that in tissues without lymph node metastasis. Based on these results, we tried to investigate the cytobiology function of 14-3-3 beta in gastric carcinoma. Our present research showed that, 14-3-3 beta is stably expressed in HGC27 and MGC803 gastric cancer cell lines and plays an important role in the proliferation and invasion of gastric cancer cells. These

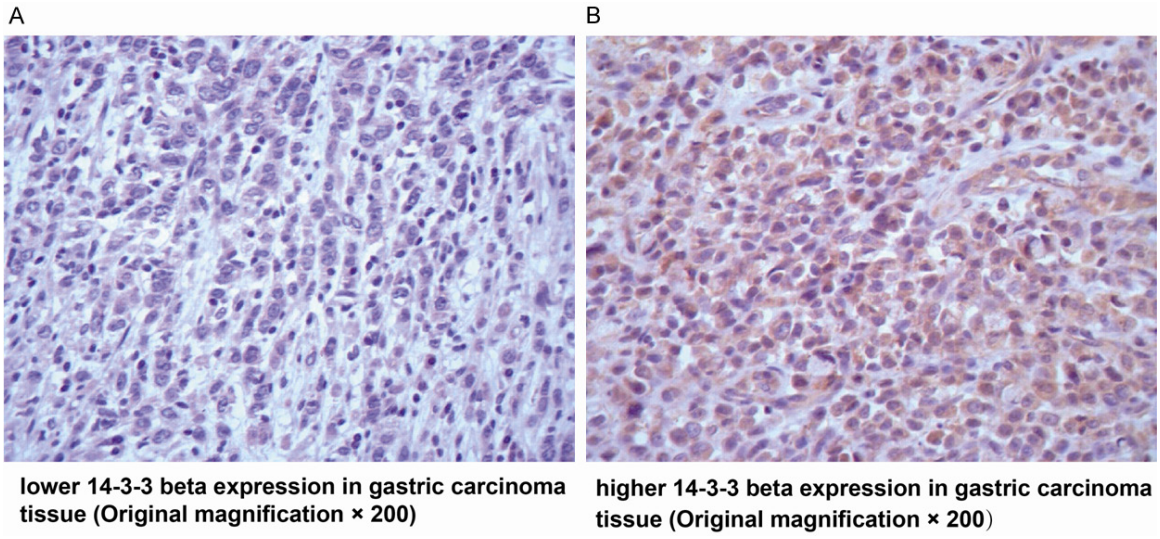

Figure 4. The representative immunohistochemical stain results of 14-3-3 beta expression in gastric carcinoma tissues. A. Relatively lower 14-3-3 beta expression in gastric carcinoma tissue (original magnification ×200). B. Relatively higher 14-3-3 beta expression in gastric carcinoma tissue (original magnification ×200).

Table 2. Relationship between lymph node metastasis and clinicopathologic factors in gastric carcinoma

Variables	Lymph node metastasis		*P* value
	Negative	Positive	
Gender			0.5713 (chi-square)
Male	41	84	
Female	16	40	
Age group			0.9275 (chi-square)
≤50	16	34	
>50	41	90	
Location			0.2462 (fisher)
Upper	36	67	
Middle	13	37	
Lower	8	14	
Whole	0	6	
Tumor size			0.0002 (chi-square)
<5 cm	27	25	
≥5 cm	30	99	
Histological type			0.0514 (fisher)
WMD	18	28	
PD	31	84	
SRC	5	2	
MC	3	10	
Serosal invasion			<.0001 (chi-square)
Absent	41	16	
Present	16	108	
14-3-3β			0.0015 (chi-square)
Low	31	37	
High	26	87	
Total	57	124	

WMD, well or moderately differentiated adenocarcinoma; PD, poorly differentiated adenocarcinoma; SRC, signet ring cell carcinoma; MC, mucinous adenocarcinoma.

results are consistent with previous findings that 14-3-3 beta is expressed in most of tumor tissues and plays a pivotal role in the pathophysiological process [13-16].

14-3-3 beta protein is involved in multiple processes including cell growth and differentiation. It regulates cell growth through the interaction with Raf-1 and regulation of gene expression in the MAPK signaling cascade [17-19]. We used RNA interference method to down-regulate 14-3-3 beta expression in MGC803 and HGC27 cells in order to investigate the function of 14-3-3 beta in the proliferation and invasion of gastric cancer cells. The results showed that the absent of 14-3-3 beta expression inhibited the proliferation of HGC27 and MGC803 gastric cancer cells. The invasion study showed that MGC803 cells displayed a lower invasive tendency when 14-3-3 beta was decreased by si-RNA. However, HGC27 cells showed insensitivity ability of invasion when 14-3-3 beta was decreased. Moreover, we had confirmed by immunohistochemical staining assay, the expression level of 14-3-3 beta protein in gastric cancer tissues with lymph node metastasis was significantly higher than that without lymph node metastasis. However, the

Table 3. Multivariate analysis of potential risk factors for lymph node metastasis using multivariate logistic regression with covariate adjustment

Variables	$\hat{\beta}$	$SE(\hat{\beta})$	χ^2	*P*	OR (95% CI)
Age					
<50					
≥50	-0.2551	0.4858	0.2759	0.5994	0.775 (0.299, 2.008)
Gender					
Male					
Female	0.4431	0.467	0.9004	0.3427	1.558 (0.624, 3.89)
Tumor size					
<5 cm					
≥5 cm	0.4276	0.4712	0.8235	0.3642	1.534 (0.609, 3.861)
Histological type					
WMD					
PD	0.991	0.4834	4.2026	0.0404	2.694 (1.045, 6.948)
SRC	0.1141	1.0668	0.0114	0.9148	1.121 (0.139, 9.07)
MC	0.4238	0.8823	0.2307	0.631	1.528 (0.271, 8.611)
Serosal invasion					
Absent					
Present	2.7904	0.4671	35.6861	<.0001	16.288 (6.52, 40.688)
14-3-3β					
Low					
High	0.3882	0.4338	0.8006	0.3709	1.474 (0.63, 3.45)

molecule mechanisms of 14-3-3 beta involved in the metastasis of gastric cancer are still far from understood. Recently, Some researches had showed that the altered expression level or activity of 14-3-3 beta protein may change the cell characteristics or target protein activity to affect cell growth, apoptosis and tumorigenesis [16, 20, 21].

Lymph node metastasis is the most important and complex factor in diagnosis and treatment of gastric cancer. The molecule mechanisms of lymph node metastasis are the theoretical foundation of diagnosis and treatment of gastric cancer. Yet, the mechanisms underlying the lymph node metastasis of gastric cancer is still not clear. Several researches in the molecular biology field had shown that vascular endothelial growth factor, extracellular matrix protease, metastatic gene, cytokine and adhesion molecule are associated with the lymph node metastasis of gastric cancer [22-25]. Okayama et al. had reported that positive expression of both CD44v6 and MMP-7 and negative expression of nuclear Cdx2 may lead to lymph node metastasis in gastric cancer [26]. Other researchers had reported metastasis-related gene (nm23, KISS1 and KAI1) were involved in gastric cancer with the progress of metastasis [27]. TRIM29 has also been reported as a novel biomarker for gastric cancer with lymph node metastasis [28]. Meanwhile, Ang-2 has been shown as a useful marker for lymph node metastasis in patients with early gastric cancer [29]. Furthermore, Wang et al. had reported that the overall survival rate of the patients with high expression of PRL-3 in the LNM was significantly lower than those with moderate/low expression [30]. Based on our previous 2DE study and present *in vitro* and immunohistochemical staining results, we hypothesis that 14-3-3 beta is involved in the lymph node metastasis procession of gastric cancer. To investigate the correlation between 14-3-3 beta and lymph node metastasis relevant clinicopathologic factors, we analyzed 181 cases of gastric cancer. The immunohistochemical staining results and the correlation with clinicopathological factors were analyzed. The results showed the expression level of 14-3-3 beta was correlated with tumor diameter serosal invasion and lymph node metastasis.

Taken together, high expression of 14-3-3 beta has a positive effect on the lymph node metastasis. Furthermore, 14-3-3 beta may play a role in lymph node metastasis of gastric cancer and tumor growth. Our study indicated that 14-3-3 beta could be a potential biomarker for diagnostic and prognostic in gastric cancer, which may provide new ideas for future clincal research.

Acknowledgements

This work was supported by the Office of Education of Heilongjiang Province, China (Grant number: 11551299) and Heilongjiang Post-

doctoral Fund (Grant number: LBH-Z16158). We wish to thank Professor Yingwei Xue for help with the manuscript; the Heilongjiang Cancer Institute for the immunohistology study and cell biology experiments.

Disclosure of conflict of interest

None.

Address correspondence to: Yingwei Xue, Department of Gastroenterologic Surgery, Harbin Medical University Cancer Hospital, 6 Baojian Road, Harbin 150040, China. Tel: +86-0451-86694307; E-mail: hljzlyyfsf@163.com

References

[1] Wilker E and Yaffe MB. 14-3-3 proteins--a focus on cancer and human disease. J Mol Cell Cardiol 2004; 37: 633-642.

[2] Chen XQ, Chen JG, Zhang Y, Hsiao WW and Yu AC. 14-3-3gamma is upregulated by in vitro ischemia and binds to protein kinase Raf in primary cultures of astrocytes. Glia 2003; 42: 315-324.

[3] Tian J, Hu X, Gao W, Zhang J, Chen M, Zhang X, Ma J and Yuan H. Identification of the long noncoding RNA LET as a novel tumor suppressor in gastric cancer. Mol Med Rep 2017; 15: 2229-2234.

[4] Song Y, Wang Y, Tong C, Xi H, Zhao X and Chen L. A unified model of the hierarchical and stochastic theories of gastric cancer. Br J Cancer 2017; 116: 973-989.

[5] Du C, Yang S, Zhao X and Dong H. Pathogenic roles of alterations in vitamin D and vitamin D receptor in gastric tumorigenesis. Oncotarget 2017; 8: 29474-29486.

[6] Shida A, Mitsumori N, Nimura H, Takano Y, Iwasaki T, Fujisaki M, Takahashi N and Yanaga K. Prediction of lymph node metastasis and sentinel node navigation surgery for patients with early-stage gastric cancer. World J Gastroenterol 2016; 22: 7431-7439.

[7] Ma Y, Li YF, Wang T, Pang R, Xue YW and Zhao SP. Identification of proteins associated with lymph node metastasis of gastric cancer. J Cancer Res Clin Oncol 2014; 140: 1739-1749.

[8] Aitken A, Collinge DB, van Heusden BP, Isobe T, Roseboom PH, Rosenfeld G and Soll J. 14-3-3 proteins: a highly conserved, widespread family of eukaryotic proteins. Trends Biochem Sci 1992; 17: 498-501.

[9] Hermeking H. The 14-3-3 cancer connection. Nat Rev Cancer 2003; 3: 931-943.

[10] Sugiyama A, Miyagi Y, Komiya Y, Kurabe N, Kitanaka C, Kato N, Nagashima Y, Kuchino Y and Tashiro F. Forced expression of antisense 14-3-3beta RNA suppresses tumor cell growth in vitro and in vivo. Carcinogenesis 2003; 24: 1549-1559.

[11] Takihara Y, Matsuda Y and Hara J. Role of the beta isoform of 14-3-3 proteins in cellular proliferation and oncogenic transformation. Carcinogenesis 2000; 21: 2073-2077.

[12] Somanath PR and Byzova TV. 14-3-3beta-Rac1-p21 activated kinase signaling regulates Akt1-mediated cytoskeletal organization, lamellipodia formation and fibronectin matrix assembly. J Cell Physiol 2009; 218: 394-404.

[13] Qi W, Liu X, Qiao D and Martinez JD. Isoform-specific expression of 14-3-3 proteins in human lung cancer tissues. Int J Cancer 2005; 113: 359-363.

[14] Nagappan A, Venkatarame Gowda Saralamma V, Hong GE, Lee HJ, Shin SC, Kim EH, Lee WS and Kim GS. Proteomic analysis of selective cytotoxic anticancer properties of flavonoids isolated from citrus platymamma on A549 human lung cancer cells. Mol Med Rep 2016; 14: 3814-3822.

[15] Chai Y, Wang G, Fan L and Zhao M. A proteomic analysis of mushroom polysaccharide-treated HepG2 cells. Sci Rep 2016; 6: 23565.

[16] Gong X, Yan L, Gu H, Mu Y, Tong G and Zhang G. 14-3-3epsilon functions as an oncogene in SGC7901 gastric cancer cells through involvement of cyclin E and p27kip1. Mol Med Rep 2014; 10: 3145-3150.

[17] Ghorbel M, Cotelle V, Ebel C, Zaidi I, Ormancey M, Galaud JP and Hanin M. Regulation of the wheat MAP kinase phosphatase 1 by 14-3-3 proteins. Plant Sci 2017; 257: 37-47.

[18] Wu YJ, Jan YJ, Ko BS, Liang SM and Liou JY. Involvement of 14-3-3 proteins in regulating tumor progression of hepatocellular carcinoma. Cancers (Basel) 2015; 7: 1022-1036.

[19] Cohen JD, Labenski M, Mastrandrea NJ, Canatsey RD, Monks TJ and Lau SS. Transcriptional and post-translational modifications of B-Raf in quinol-thioether induced tuberous sclerosis renal cell carcinoma. Mol Carcinog 2016; 55: 1243-1250.

[20] Panigrahi SK, Manterola M and Wolgemuth DJ. Meiotic failure in cyclin A1-deficient mouse spermatocytes triggers apoptosis through intrinsic and extrinsic signaling pathways and 14-3-3 proteins. PLoS One 2017; 12: e0173926.

[21] Seo SB, Lee JJ, Yun HH, Im CN, Kim YS, Ko JH and Lee JH. 14-3-3beta depletion drives a senescence program in glioblastoma cells through the ERK/SKP2/p27 pathway. Mol Neurobiol 2017; [Epub ahead of print].

[22] Yan L, Jiao D, Hu H, Wang J, Tang X, Chen J and Chen Q. Identification of lymph node metasta-

sis-related microRNAs in lung adenocarcinoma and analysis of the underlying mechanisms using a bioinformatics approach. Exp Biol Med (Maywood) 2017; 242: 709-717.
[23] Lv C, Liu D and Wei X. Down syndrome critical region 1 positively correlates with angiogenesis in hypopharyngeal cancer. Mol Med Rep 2017; 15: 263-270.
[24] Zhu XJ, Liu J, Xu XY, Zhang CD and Dai DQ. Novel tumor-suppressor gene epidermal growth factor-containing fibulin-like extracellular matrix protein 1 is epigenetically silenced and associated with invasion and metastasis in human gastric cancer. Mol Med Rep 2014; 9: 2283-2292.
[25] Wei L, Wang H, Yang F, Ding Q and Zhao J. Interleukin-17 potently increases non-small cell lung cancer growth. Mol Med Rep 2016; 13: 1673-1680.
[26] Okayama H, Kumamoto K, Saitou K, Hayase S, Kofunato Y, Sato Y, Miyamoto K, Nakamura I, Ohki S, Sekikawa K and Takenoshita S. CD44v6, MMP-7 and nuclear Cdx2 are significant biomarkers for prediction of lymph node metastasis in primary gastric cancer. Oncol Rep 2009; 22: 745-755.
[27] Guan-Zhen Y, Ying C, Can-Rong N, Guo-Dong W, Jian-Xin Q and Jie-Jun W. Reduced protein expression of metastasis-related genes (nm23, KISS1, KAI1 and p53) in lymph node and liver metastases of gastric cancer. Int J Exp Pathol 2007; 88: 175-183.
[28] Kosaka Y, Inoue H, Ohmachi T, Yokoe T, Matsumoto T, Mimori K, Tanaka F, Watanabe M and Mori M. Tripartite motif-containing 29 (TRIM29) is a novel marker for lymph node metastasis in gastric cancer. Ann Surg Oncol 2007; 14: 2543-2549.
[29] Jo MJ, Lee JH, Nam BH, Kook MC, Ryu KW, Choi IJ, Kim YW and Bae JM. Preoperative serum angiopoietin-2 levels correlate with lymph node status in patients with early gastric cancer. Ann Surg Oncol 2009; 16: 2052-2057.
[30] Wang Z, He YL, Cai SR, Zhan WH, Li ZR, Zhu BH, Chen CQ, Ma JP, Chen ZX, Li W and Zhang LJ. Expression and prognostic impact of PRL-3 in lymph node metastasis of gastric cancer: its molecular mechanism was investigated using artificial microRNA interference. Int J Cancer 2008; 123: 1439-1447.