Change of SPARC expression after chemotherapy in gastric cancer

Yong-Yin Gao, Ru-Bing Han, Xia Wang, Shao-Hua Ge, Hong-Li Li, Ting Deng, Rui Liu, Ming Bai, Li-Kun Zhou, Xin-Yuan Zhang, Yi Ba, Ding-Zhi Huang

Department of Gastrointestinal Medical Oncology, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, China

ABSTRACT

Objective: The expression of tumor biomarkers may change after chemotherapy. However, whether secreted protein acidic and rich in cysteine (SPARC) expression changes after chemotherapy in gastric cancer (GC) is unclear. This study investigated the influence of chemotherapy on SPARC expression in GC.

Methods: Immunohistochemistry was used to analyze SPARC expression in 132 GC cases (including 54 cases with preoperative chemotherapy and 78 cases without preoperative chemotherapy). SPARC expression of postoperative specimens with and without preoperative chemotherapy was assessed to analyze the influence of chemotherapy on SPARC expression.

Results: SPARC was highly expressed in GC compared with the desmoplastic stroma surrounding tumor cells and noncancerous tissues. High SPARC expression was correlated with invasion depth, lymph node, and TNM stage. After chemotherapy, a lower proportion of high SPARC expression was observed in patients with preoperative chemotherapy than in the controls. For 54 patients with preoperative chemotherapy, gross type, histology, depth of invasion, lymph node, TNM stage, and SPARC expression were related to overall survival. Further multivariate analysis showed that lymph node, histology, and SPARC expression after chemotherapy were independent prognostic factors.

Conclusion: SPARC expression may change after chemotherapy in GC. SPARC expression should be reassessed for patients with GC after chemotherapy.

KEYWORDS
Secreted protein acidic and rich in cysteine (SPARC); gastric cancer (GC); immunohistochemistry; chemotherapy
Materials and methods

Patients and tissue samples

Between January 2007 and December 2012, 132 patients with GC who underwent gastric resection at Tianjin Medical University Cancer Institute and Hospital were enrolled in this study. These 132 patients included 54 patients with preoperative chemotherapy (group A) and 78 control patients without preoperative chemotherapy (group B). All patients in group A underwent less than six cycles of preoperative chemotherapy, and subsequently, radical resection of GC. The postoperative tumor specimens of group A and group B were selected to analyze the effect of chemotherapy on SPARC expression. Among the group A patients with preoperative chemotherapy, 15 underwent taxanes-based chemotherapy and 39 underwent platinum/fluoropyrimidine-based chemotherapy. All of tumor tissues were diagnosed at the Departments of Gastrointestinal Surgery and Pathology. All of the non-cancerous tissues selected in this study were obtained far from the tumor. This study was conducted with the approval of the Hospital Ethics Committee. All patients signed a written informed consent.

Immunohistochemistry

Immunohistochemical analysis using the avidin-biotin complex (ABC) method was performed to study SPARC protein expression. Briefly, slides were baked, deparaffinization with xylene, and rehydrated. The sections were submerged into citrate antigenic retrieval buffer for antigenic retrieval, after which slides were peroxidase blocked in 3% hydrogen peroxide to quench endogenous peroxidase activity. Sections were incubated with 1:1,500 dilution of rabbit polyclonal anti-SPARC (Abcam UK) overnight at 4 °C. Sections of pancreatic cancer specimens were used as positive controls, and the sample incubated with phosphate-buffered saline rather than primary antibody was used as a negative control. After washing, tissue sections were incubated with secondary antibody. Staining was detected using DAB. Then, the specimens were counterstained with hematoxylin, dehydrated, and mounted. The cytoplasm stained with buffy was scored as SPARC positive. Sections were evaluated independently by two observers based on the proportion of positively stained tumor cells and intensity of staining. The tumor cell proportion was scored as follows: 0 (<5% positive tumor cells), 1 (6% to 25% positive tumor cells), 2 (26% to 50% positive tumor cells), and 3 (≥51% positive tumor cells). Staining intensity was graded according to the following criteria: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown). The staining index was calculated as the product of the staining intensity score and the proportion of positive tumor cells. Using this method of assessment, we evaluated SPARC expression in benign gastric epithelia and malignant lesions by determining the staining index with scores of 0, 1, 2, 3, 4, 6, or 9. The cutoff values for the high and low SPARC expression levels were selected based on the measurement of heterogeneity using the log-rank test with respect to overall survival (OS). The optimal cutoff value was identified as follows: a staining index score ≥4 was used to define tumors with high SPARC expression, and a staining index score ≤3 was used to indicate tumors with low SPARC expression.

Statistical analysis

All statistical analyses were conducted using SPSS17.0 software. When appropriate, correlation coefficients between protein expression and clinicopathological findings were estimated using
the Pearson correlation method, $\chi^2$ test, or Fisher exact tests. OS was described as the period from the first day after diagnosis to the date of death or last follow-up. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to compute the differences between curves. Cox’s proportional hazards model was used in multivariate analysis to identify the independent predictors of survival. A $P$ value <0.05 was considered statistically significant.

**Results**

**SPARC expression in gastric cancerous tissues and noncancerous mucosa as demonstrated by immunostaining**

In normal gastric tissues, SPARC was expressed at low levels in the cytoplasm of 17 of 24 (70.8%) normal mucosal epithelial cells and 16 of 24 stromal cells (66.7%). In GC tissues, high SPARC expression was detected in 55 (70.5%) of 78 tumors (group B). However, immunoreaction was weak or absent in cells of the desmoplastic stroma surrounding cancer cells. SPARC was mainly localized in the cytoplasm of primary cancer (Figure 1).

**Correlation of SPARC expression with clinicopathological characteristics**

In 78 previously untreated GC patients (group B), higher SPARC expression in postoperative tumor tissue was significantly associated with depth of invasion, lymph node metastasis and TNM stage ($P<0.05$) (Table 1). SPARC expression did not correlate with age, gender, tumor location, tumor size, gross type, histological type, and histologic differentiation ($P>0.05$) (Table 1).

In 54 GC patients who underwent preoperative chemotherapy (group A), high SPARC expression after chemotherapy correlated with depth of invasion, lymph node metastasis, and TNM stage ($P<0.05$) (Table 2). SPARC expression did not correlate with age, gender, tumor location, tumor size, gross type, histological type, and histologic differentiation ($P>0.05$) (Table 2).

**The influence of chemotherapy on SPARC expression**

We evaluated SPARC expression with and without chemotherapy...
in specimens from 54 GC patients with preoperative chemotherapy (group A) and 78 GC patients without preoperative chemotherapy (group B). After chemotherapy, a lower proportion of high-level SPARC expression (46.3% vs. 70.5%) was observed in group A than in group B ($P=0.005$, by $\chi^2$ test) (Figure 2). Further analysis of preoperative chemotherapy in group A revealed that 15 (27.8%) of 54 GC patients in group A underwent taxanes-based chemotherapy, among which 60.0% showed low SPARC expression in post-chemotherapy specimens. Thirty-nine (72.2%) of 54 GC patients in group A underwent platinum-based chemotherapy, among which 51.2% showed low SPARC expression in post-chemotherapy specimens.

SPARC, secreted protein, acidic and rich in cysteine; GC, gastric cancer.
Correlation between the phenotype of SPARC and patient prognosis

The overall cumulative 2-year survival rate of 132 GC patients (including those in group A and group B) was 64.1% in the low SPARC expression group and 60.6% in the high SPARC expression group \( (P=0.193) \). Further analysis showed that, in 78 GC patients without preoperative chemotherapy (group B), SPARC expression did not correlate with the prognosis \( (P=0.661) \) (Figure 3). However, in 54 GC patients with preoperative chemotherapy (group A), SPARC expression after chemotherapy correlated with the prognosis. The cumulative 1-, 2-, 3-year survival rates were 81.8%, 72.7%, and 56.7%, respectively, in the group with low SPARC protein expression group; however, these rates were 43.5%, 27.0%, and 13.0%, respectively, in the group with high SPARC protein expression \( (P=0.002) \) (Figure 4).

Univariate analysis showed that other significant prognostic factors for the survival of group A with preoperative chemotherapy included gross type \( (\text{Borrmann I/II, Borrmann III/IV}; \log -\text{rank} \ P=0.015) \), histology \( (\text{intestinal, diffuse}; \log -\text{rank} \ P=0.042) \), depth of invasion \( (T_1/T_2, T_3/T_4; \log -\text{rank} \ P=0.034) \), lymph node \( (N0, N+; \log -\text{rank} \ P=0.002) \) and TNM stage \( (I/II, III; \log -\text{rank} \ P=0.001) \) (Table 3). Multivariate analysis revealed that lymph node \( (P=0.032) \), histology \( (P=0.027) \), and SPARC expression after chemotherapy \( (P=0.024) \) were independent prognostic factors for the survival of the patients with preoperative chemotherapy (Table 4).
Discussion

SPARC overexpression was observed in GC and was correlated with invasion, metastasis, apoptosis, and prognosis. Knowledge concerning SPARC in GC above-mentioned is controversial. We tested SPARC expression in GC tissues and noncancerous tissues and explored its correlation with clinicopathological parameters to further evaluate the correlation of SPARC with the development and progression of GC.

Regarding the cellular origin of SPARC, SPARC may be expressed predominantly in tumor or stromal cells, depending on the type of malignancy. Our immunohistochemical studies showed that SPARC expression was detected mainly in GC cells and slightly in the desmoplastic stroma surrounding tumor cells, normal epithelial cells, and stromal cells. Our results were similar to those of Zhao et al. and Wang et al. However, our results differed from those of other reports demonstrating SPARC staining mainly located in stromal cells and that SPARC overexpression in stromal cells surrounding the tumor cells was negatively correlated with clinicopathological factors and Ki-67 labeling index. Therefore, we hypothesize that SPARC expression in GC cells or stromal cells might play different roles in the carcinogenesis, development, and prognosis of GC.

In this study, we revealed that SPARC overexpression in GC was associated with depth of invasion, lymph node metastasis, and TNM stage. These results are consistent with the observations of Wang et al. and Zhao et al. A previous study proposed that SPARC may play a key role during the initial steps in the process of tumor invasion and metastasis. SPARC might...
favor metastatic dissemination of malignant cells through the activation of matrix-degrading enzymes\textsuperscript{23}. Moreover, previous studies have shown that a high level of SPARC is often correlated with poor prognosis for patients with GC\textsuperscript{9,20}. The results of this study showed that, although in the whole GC patients (group A and group B) SPARC expression did not significantly relate to prognosis, subgroup analysis showed that in group A SPARC expression in post-chemotherapy specimens was associated with poor prognosis, which is probably related to the small size. However, we noted that post-chemotherapy biomarkers were associated with the outcome of patients, a similar finding that could also be observed in breast cancer\textsuperscript{24}. The exact mechanism needs to be further explored.

Effective individualized treatment depends on molecular phenotypes, with some evidence of biomarker discordance between pre-chemotherapy and post-chemotherapy tissue specimens\textsuperscript{13,14}. In this study, a lower proportion of high-level SPARC expression was observed in specimens with preoperative chemotherapy than in control specimens without preoperative chemotherapy (46.3\% vs. 70.5\%). Therefore, our results suggested that preoperative chemotherapy may alter the phenotype of SPARC. Considering the fact that we cannot reach a definite conclusion based on only a few biopsy specimens, we analyzed the effect of chemotherapy on SPARC expression by comparing SPARC expression between groups A and B, not before and after chemotherapy. A similar method was used in the MAGIC\textsuperscript{25} study. The mechanisms responsible for the discordance may be multifactorial and complicated. Possible explanations may include that chemotherapy may “enrich” to the SPARC-positive GC cells, then kill the SPARC-overexpressioning GC cells or chemotherapy may contribute to the cancer cells inner genetic and phenotypic change in cancer cells.

In addition, further analysis of the preoperative chemotherapy regimens revealed that 60.0\% of patients with taxanes-based preoperative chemotherapy showed low SPARC expression in post-chemotherapy specimens. And 51.2\% of GC patients with preoperative platinum/fluoropyrimidine-based chemotherapy showed low SPARC expression in post-chemotherapy specimens. Nab-paclitaxel has shown promising activity and showed low SPARC expression in post-chemotherapy specimens. And 51.2\% of GC patients with preoperative chemotherapy showed low SPARC expression in post-chemotherapy specimens. nab-paclitaxel plus gemcitabine plus nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369:1691-1703.

References

1. Lordick F, Allum W, Carneiro F, Mitry E, Tabernero J, Tan P, et al. Unmet needs and challenges in gastric cancer: the way forward. Cancer Treat Rev 2014;40:692-700.

2. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Kawakishi A, et al. 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.

3. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369:1691-1703.

4. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, et al. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncol 2005;23:7794-7803.

5. Sasaki Y, Nishina T, Yasui H, Goto M, Muro K, Tsuji A, et al. Phase II trial of nanoparticle albumin-bound paclitaxel as second-line chemotherapy for unresectable or recurrent gastric cancer. Cancer Sci 2014;105:812-817.

6. Yardley DA. nab-Paclitaxel mechanisms of action and delivery. J Control Release 2013;170:365-372.

7. Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest 2001;107:1049-1054.

8. Borsini J, Sage EH. Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol 2002;14:608-616.

9. Zhao ZS, Wang YY, Chu YQ, Ye ZY, Tao HQ. SPARC is associated with gastric cancer progression and poor survival of patients. Clin Cancer Res 2010;16:260-268.

10. Jeung HC, Rha SY, Im CK, Shin SJ, Ahn JB, Yang WI, et al. A randomized phase 2 study of docetaxel and S-1 versus docetaxel and cisplatin in advanced gastric cancer with an evaluation of SPARC expression for personalized therapy. Cancer 2011;117:2050-2057.

11. Sinn M, Sinn BV, Strieder JK, Lindner JL, Stieler JM, Lohneis P, et al. SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study.
12. Desai N, Trieu V, Damascelli B, Soon-Shiong P. SPARC Expression Correlates with Tumor Response to Albumin-Bound Paclitaxel in Head and Neck Cancer Patients. Transl Oncol 2009;2:59-64.
13. Bai H, Wang Z, Chen K, Zhao J, Lee JJ, Wang S, et al. Influence of chemotherapy on EGFR mutation status among patients with non-small-cell lung cancer. J Clin Oncol 2012;30:3077-3083.
14. Dawood S, Gonzalez-Angulo AM. Biomarker discordance pre and post neoadjuvant chemotherapy in breast cancer. Cancer Biomark 2012-2013;12:241-250.
15. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577-580.
16. Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma - 2nd English Edition -. Gastric Cancer 1998;1:10-24.
17. Zhang J, Wang P, Zhu J, Wang W, Yin J, Zhang C, et al. SPARC expression is negatively correlated with clinicopathological factors of gastric cancer and inhibits malignancy of gastric cancer cells. Oncol Rep 2014;31:2312-2320.
18. Sato T, Oshima T, Yamamoto N, Yamada T, Hasegawa S, Yukawa N, et al. Clinical significance of SPARC gene expression in patients with gastric cancer. J Surg Oncol 2013;108:364-368.
19. Wang CS, Lin KH, Chen SL, Chan YF, Hsueh S. Overexpression of SPARC gene in human gastric carcinoma and its clinic-pathologic significance. Br J Cancer 2004;91:1924-1930.
20. Franke K, Carl-McGrath S, Rohl FW, Lendeckel U, Ebert MP, Tänzer M, et al. Differential Expression of SPARC in Intestinal-type Gastric Cancer Correlates with Tumor Progression and Nodal Spread. Transl Oncol 2009;2:310-320.
21. Wang L, Yang M, Shan L, Qi L, Chai C, Zhou Q, et al. The role of SPARC protein expression in the progress of gastric cancer. Pathol Oncol Res 2012;18:697-702.
22. Porte H, Chastre E, Prevot S, Nordlinger B, Empereur S, Basset P, et al. Neoplastic progression of human colorectal cancer is associated with overexpression of the stromelysin-3 and BM-40/SPARC genes. Int J Cancer 1995;64:70-75.
23. Nagaraju GP, Dontula R, El-Rayes BF, Lakka SS. Molecular mechanisms underlying the divergent roles of SPARC in human carcinogenesis. Carcinogenesis 2014;35:967-973.
24. Alamgeer M, Ganju V, Kumar B, Fox J, Hart S, White M, et al. Changes in aldehyde dehydrogenase-1 expression during neoadjuvant chemotherapy predict outcome in locally advanced breast cancer. Breast Cancer Res 2014;16:R44.
25. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006;355:11-20.

Cite this article as: Gao YY, Han RB, Wang X, Ge SH, Li HL, Deng T, Liu R, Bai M, Zhou LK, Zhang XY, Ba Y, Huang DZ. Change of SPARC expression after chemotherapy in gastric cancer. Cancer Biol Med 2015;12:33-40. doi: 10.7497/jissn.2095-3941.2014.0023