Int J Clin Exp Pathol 2012;5(7):668-673
www.ijcep.com /ISSN:1936-2625/IJCEP1206013

Original Article

High RSF-1 expression correlates with poor prognosis in patients with gastric adenocarcinoma

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Received June 30, 2012; Accepted August 3, 2012; Epub September 5, 2012; Published September 15, 2012

Abstract: Aim: To investigate the expression and prognostic significance of RSF-1 in gastric adenocarcinoma. Methods: RSF-1 expression was analyzed using immunohistochemical staining on tissue samples from a consecutive series of 287 gastric adenocarcinoma patients who underwent tumor resections between 2003 and 2006.The relationship between RSF-1 expression, clinicopathological factors, and patient survival was investigated. Results: Immunohistochemical staining indicated that RSF-1 is highly expressed in 52.6% of gastric adenocarcinomas. RSF-1 expression levels were closely associated with tumor size, histological differentiation, tumor stage, and lymph node involvement. Kaplan–Meier survival analysis showed that high RSF-1 expression exhibited a significant correlation with poor prognosis for gastric adenocarcinoma patients. Multivariate analysis revealed that RSF-1 expression is an independent prognostic parameter for the overall survival rate of gastric adenocarcinoma patients. Conclusion: Our data suggest that RSF-1 plays an important role in gastric adenocarcinoma progression and that high RSF-1 expression predicts an unfavorable prognosis in gastric adenocarcinoma patients.

Keywords: RSF-1, gastric adenocarcinoma, prognosis, survival, diagnosis

Introduction

Currently, gastric adenocarcinoma is one of the most common cancers worldwide, and is one of the leading causes of cancer-related death in China [1]. Gastric adenocarcinoma encompasses many subtypes with distinct genetic and biological features. Therefore, identification of new biological markers to determine the risk of poor prognosis is important for designing treatment strategies [2, 3].

RSF-1, also known as hepatitis B X-antigen associated protein (HBXAP), is a subunit of an ISWI chromatin remodeling complex, remodeling and spacing factor (RSF). RSF-1 (HBXAP) encodes for a cellular nuclear protein that binds to hSNF2H [4], forming a chromatin remodeling protein complex called RSF (Remodeling and Spacing Factor) [5, 6]. RSF-1 (HBXAP) has been shown to function as a histone chaperone in the nuclei while its binding partner, hSNF2H, possesses nucleosome-dependent ATPase activity [7]. The RSF-1/hSNF2H complex (RSF complex) mediates ATP-dependent chromatin remodeling, which alters the chromatin structure or positioning of nucleosomes [6]. At the cellular level, RSF participates in chromatin remodeling in response to a variety of growth signals and environmental cues. Such nucleosome remodeling is required for transcriptional activation or repression [8-10], DNA replication [11], and cell cycle progression [12].

The overexpression or amplification of the RSF-1 gene has been reported in various solid tumors such as breast,ovarian cancers and oral squamous cell carcinoma [13-15]. However, there has been no report on the expression profile of RSF-1 in gastric cancer. The purpose of the present study was to examine the expression status of RSF-1 in gastric adenocarcinoma tissues and to evaluate whether the level of

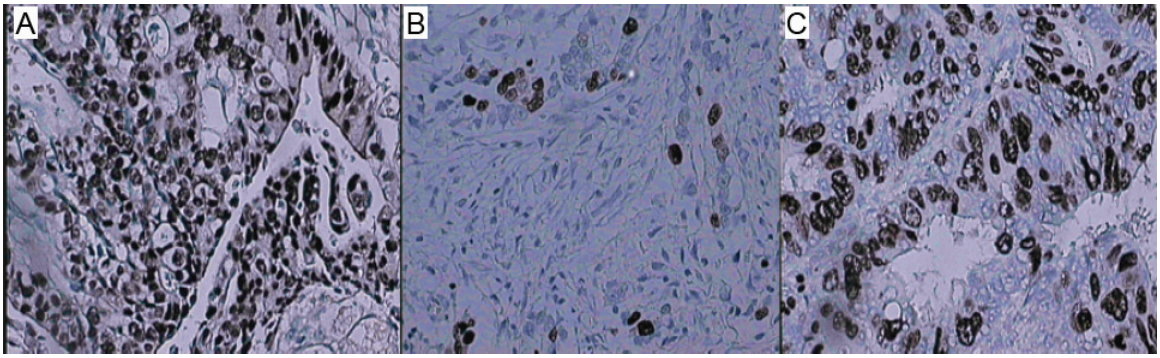

Figure 1. Immunohistochemical staining of gastric adenocarcinoma tissues. A: A representative sample showing high RSF-1 expression in gastric adenocarcinoma tissue. B: A representative sample showing low RSF-1 expression in gastric adenocarcinoma tissue. C: Nuclear expression of Ki-67 in gastric adenocarcinoma tissue (×200 magnification).

RSF-1 expression correlates with clinicopathological parameters and the prognosis of gastric adenocarcinoma patients.

Materials and methods

Patients and tissue specimens

A total of 287 gastric adenocarcinoma specimens were collected from patients undergoing surgery between January 2003 and December 2006 at the Yixing People's Hospital. None of the patients had received radiotherapy or chemotherapy prior to surgery. The histomorphology of all specimens was assessed by the Department of Pathology at the Yixing People's Hospital. Gastric adenocarcinomas were graded based on the TNM stage classification (stages I–IV). Tissue samples were taken from surgically removed tumors and embedded in paraffin after fixation in 10% formalin for histological diagnosis. Clinical information, including sex, age, tumor size, TNM grade, WHO type, and lymph node involvement was also collected. Follow-up information for all participants was obtained every three months by telephone, during a visit to the clinic, or via a postal questionnaire. During the follow-up period, overall survival was measured from diagnosis to death or to the last follow-up (at five years). Death of a patient was ascertained by reporting from the family and verified by a review of public records.

Immunohistochemical analysis

Tissue specimens were subjected to immunohistochemical analysis using the avidin–biotin-peroxidase method. Sections were deparaffinized in xylene and dehydrated using a graded alcohol series before endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in methanol for 10 min. Nonspecific binding was blocked by incubating sections with 10% normal goat serum in phosphate-buffered saline (PBS) for 1h at room temperature. Without washing, sections were incubated with a polyclonal antibody against human RSF-1 (1:300; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS at 4°C overnight. Following this, biotinylated goat anti-mouse immunoglobulin G (IgG; 1:400; Sigma, St. Louis, MO, USA) was incubated with the sections for 1 h at room temperature and detected using streptavidin–peroxidase. The brown color indicative of peroxidase activity was developed by incubating sections with 0.1% 3,3ˆ-diaminobenzidine (Sigma) in PBS with 0.05% hydrogen peroxide for 5 min at room temperature. All tissue specimens were assessed separately by two pathologists under double-blind conditions, in which they had no prior knowledge of either the clinical or clinicopathological status of the specimens. RSF-1 expression in gastric adenocarcinoma specimens was evaluated by scanning the entire tissue specimen under low magnification (×40), and was confirmed under high magnification (×200 and ×400). An immunoreactivity score (IRS) system was applied as described elsewhere [16]. The percentage of positive cells was scored as: 0, <5%, negative; 1, 5–25%, sporadic; 2, 25–50%, focal; 3, >50%, diffuse. The staining intensity was scored as: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. The RSF-1

Table 1. Relationship between RSF-1 expression and clinicopathologic features of patients with gastric adenocarcinoma.

Clinicopathological variable	Number	Low RSF-1expression n = 136	High RSF-1expression n = 151	*P* value
Age(yr)				
<60	140	72	68	0.1947
≥60	147	64	83	
Gender				
Male	156	73	83	0.9056
Female	131	63	68	
Tumor size(cm)				
≤5	50	32	18	0.0046
>5	237	94	133	
Histological grade				
Well differentiated(G1)	21	17	4	0.0008
Moderately differentiated(G2)	125	64	61	
Poorly differentiated(G3)	141	55	86	
WHO type				
Tubular	223	111	122	0.6201
Mucinous	11	7	5	
Papillary	13	6	7	
Signet ring cell	40	23	17	
Lymph node involvement				
0	55	38	17	0.0003
1	57	30	27	
>1	175	68	107	
TNM stage				
I	31	25	6	<0.0001
II	43	23	20	
III	157	71	86	
IV	56	17	39	
Ki67				
0	146	81	65	0.0133
1	70	30	40	
2	71	25	46	

immunostaining score was calculated by multiplying the positive cell score by the staining intensity score, and thus ranged from 0 to 9. High RSF-1 expression was defined as a total score of ≥4 (median of total scores for RSF-1), and low RSF-1 expression level as a total score of <4. Ki-67 immunoreactivity was evaluated as follows: 0, <20% of tumor cells showing positive immunoreactivity; 1, 20–50% of tumor cells showing positive immunoreactivity; 2, >50% of tumor cells showing positive immunoreactivity.

Statistical analysis

All statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Associations between RSF-1 expression and clinicopathological variables were analyzed using the Mann–Whitney and Kruskal–Wallis tests. Survival curves were plotted using the Kaplan–Meier product-limit method, and differences between survival curves were tested using the log-rank test. Cox's proportional hazards model was used to identify

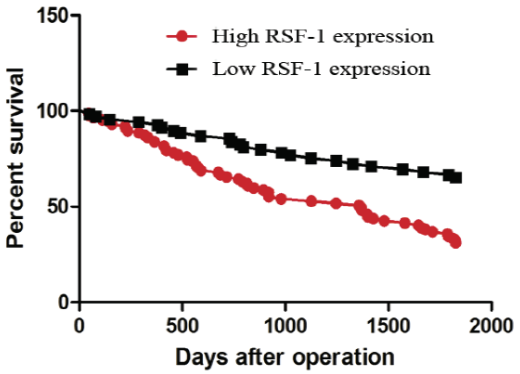

Figure 2. Overall survival rate of gastric adenocarcinoma patients relative to RSF-1 expression levels in gastric adenocarcinoma tissue samples (Kaplan–Meier method), determined by immunohistochemical staining ($P < 0.001$).

factors that had a significant influence on survival. Sta-tistical significance was set at $P < 0.05$.

Results

The degree of RSF-1 immunohistochemical staining correlates with clinicopathological characteristics

To gain further insight into the effects and prognostic value of increased RSF-1 expression in gastric adenocarcinoma patients, paraffin-embedded sections (n = 287) of gastric adenocarcinomas confirmed by histopathology were examined by immunohistochemistry. RSF-1 expression was mostly localized in nuclei of almost tumor cells. Overall, RSF-1 expression was positive in 237 (82.6%) gastric adenocarcinoma samples and negative in 50 (17.4%) gastric adenocarcinoma samples. RSF-1 was highly expressed in 151 (52.6%) and was expressed at lower levels in 136 (47.4%) gastric adenocarcinoma patients (**Figure 1**). The level of RSF-1 expression in gastric adenocarcinoma correlated with tumor size, histological differentiation, tumor stage, and lymph node involvement but not with age or gender (**Table 1**).

RSF-1 protein expression correlates with Ki-67 expression in gastric adenocarcinoma

As shown in **Figure 1**, immunoreactive Ki-67 localized to the nuclei of tumor cells. We found that 49.1% in gastric adenocarcinomas were positive for Ki-67 protein expression, and that there was a strong association between RSF-1 overexpression and a high Ki-67 labeling index ($r_s = 0.327$, $P = 0.033$).

Correlation between RSF-1 expression levels and patient survival

The prognostic effect of RSF-1 on the overall survival rate of gastric adenocarcinoma patients was investigated by comparing the survival rate of patients with high or low levels of RSF-1 protein expression in tumors using Kaplan–Meier survival curves and the log-rank test. These tests showed that high expression of RSF-1 protein was a significant prognostic factor for poor overall survival of gastric adenocarcinoma patients. The 5-year survival rate of gastric adenocarcinoma patients with high or low RSF-1 proteinexpression level was 34.4% and 62.5%, respectively. This difference was statistically significant ($P < 0.001$, log-rank test; **Figure 2**).

Univariate and multivariate analyses

Univariate Cox regression analysis also showed that clinical variables, including tumor size, histological differentiation, tumor stage, lymph node involvement, and RSF-1 expression significantly associated with overall survival (**Table 2**). Furthermore, multivariate Cox regression analyses were performed to evaluate the potential of RSF-1 expression as an independent predictor for the overall survival of patients with gastric adenocarcinoma. Although other parameters failed to demonstrate independence, tumor size, histological differentiation, lymph node involvement, tumor stage, and RSF-1 expression may play a role in predicting overall survival in patients with gastric adenocarcinoma (**Table 2**).

Discussion

Despite many advances in diagnostic tumor imaging, combination chemotherapy, and radiation therapy, little improvement has been achieved within the last decade in terms of prognosis and quality of life for patients with gastric adenocarcinoma. Given the frequent failure of conventional treatment strategies, many cancer-related molecules have been characterized with the goal of developing novel anticancer therapies, including targeted drugs

Table 2. Univariate analysis and multivariate analysis identifies factors influencing the overall survival rate of gastric adenocarcinoma patients

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	*P* value
RSF-1	1.532	1.192-3.371	0.002	1.321	0.679-1.722	0.005
Age	1.341	0.834-2.725	0.623			
Gender	1.291	0.629-1.826	0.739			
Tumor size	1.679	1.162-2.523	0.003	1.286	0.728-2.329	0.002
Histologic grade	1.438	0.718-2.174	0.004	1.156	0.795-1.892	0.005
Lymph node involvement	1.456	0.986-2.546	0.005	1.678	1.123-2.134	0.006
Tumor stage	1.283	0.716-1.874	0.016	1.152	0.783-1.563	0.007
Ki67	1.521	1.023-2.621	0.009	1.732	0.864-2.785	0.008

or antibodies and cancer vaccines [17, 18]. RSF-1, also known as hepatitis B X-antigen associated protein (HBXAP), is a subunit of an ISWI chromatin remodeling complex, remodeling and spacing factor (RSF). The overexpression or amplification of the RSF-1 gene has been reported in various solid tumors such as breast, ovarian cancers and oral squamous cell carcinoma [13-15]. However, there has been no report on the expression profile of RSF-1 in gastric cancer. In this study, we examined the expression status of RSF-1 in gastric adenocarcinoma tissues and to evaluate whether the level of RSF-1 expression correlates with clinicopathological parameters and the prognosis of gastric adenocarcinoma patients.

In our tumor specimens, we observed that high RSF-1 expression associated with poor tumor differentiation, large tumor size, TNM stage and lymph node metastasis. This strengthens the hypothesis that RSF-1 acts as an oncogene in gastric adenocarcinoma. The Ki-67 antigen is a cell proliferation marker; Ki-67 expression strictly correlates with cell cycle progression, and can be observed in G1-, S-, and G2-phase and mitotic cells. In this study, we observed that high RSF-1 overexpression associated with a high Ki-67 index.

In the Kaplan–Meier survival analysis, the overall survival period of patients with tumors with high RSF-1 expression was significantly shorter than that of patients with low RSF-1 expression. Univariate analyses showed that increased RSF-1 expression in gastric adenocarcinoma tissues is significantly associated with the overall survival rate. Moreover, multivariate analysis demonstrated that RSF-1 expression, together with some traditional prognostic factors such as tumor size, lymph node status, and TNM stage, is an independent risk factor in the prognosis of gastric adenocarcinoma patients. These results suggest that the detection of increased RSF-1 expression might help identify gastric adenocarcinoma patients with a poor prognosis, and could therefore be a novel prognostic marker for gastric adenocarcinoma patients.

In conclusion, our results indicate that high RSF-1 expression in gastric adenocarcinoma may be important for tumor progression and thus serves as an independent biomarker for poor survival. Therefore, high RSF-1 expression identifies high-risk patients and is a otential novel therapeutic target for gastric adenocarcinoma.

Conflict of interest statement

All authors have no conflict of interest.

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