• GASTRIC CANCER •

Significance of effector protease receptor-1 expression and its relationship with proliferation and apoptotic index in patients with primary advanced gastric adenocarcinoma

Xue-Quan Yao, Fu-Kun Liu, Jie-Shou Li, Xiao-Ping Qi, Bo Wu, Hong-Lin Yin, Heng-Hui Ma, Qun-Li Shi, Xiao-Jun Zhou

Xue-Quan Yao, Fu-Kun Liu, Jie-Shou Li, Xiao-Ping Qi, Bo Wu, Hong-Lin Yin, Heng-Hui Ma, Qun-Li Shi, Xiao-Jun Zhou, School of Medicine, Nanjing University. Department of General Surgery, Jinling Hospital, 305 Zhongshangdong Road, Nanjing, 210002, Jingsu Province, China
Correspondence to: Xue-Quan Yao, Department of General Surgery, Jinling Hospital, 305 Zhongshangdong Road, Nanjing, 210002, Jingsu Province, China. yaoxuequan@yahoo.com
Telephone: +86-25-84826808-860005
Received: 2003-04-02   **Accepted:** 2003-06-07

Abstract

AIM: To investigate the expression of effector protease receptor-1 (EPR-1), proliferative index ki-67 and apoptosis index in patients with primary advanced gastric adenocarcinoma and to clarify the significance of EPR-1 expression and its correlationship with the proliferation and apoptosis indexes.

METHODS: Using immunohistochemical staining and terminal deoxynucleotidyl transferase mediated nick end labelling (TUNEL) technique, we determined the expression of EPR-1, proliferative index (Ki-67) and apoptotic index (AI) in 120 paraffin-embedded specimens of primary advanced gastric adenocarcinoma as well as lymph node metastasis and adjacent normal tissues.

RESULTS: EPR-1 expression was distributed in the cytoplasm of normal gastric mycoderma, carcinoma cells and smooth muscle cells. The positive rate of EPR-1 expression in the primary gastric adenocarcinomas, invasion tumor node and lymph node metastasis was 65.83%, 55.29% and 68%, respectively. While the positive rate in normal gastric mycoderma and smooth muscle cells was 46.7% and 53.3%, respectively. The average positive rate of ki-67 in EPR-1-positive tumors was 7.00% which was significantly lower than that of 8.53% in EPR-1-negative tumors, but the average AI in EPR-1-positive tumors was 1.25%, which was significantly higher than that of 1.00% observed in EPR-1-negative tumors. On the other hand, the average positive labeling index for Ki-67 (ki-67) in EPR-1-positive lymph node metastasis was 7.65%, which was significantly lower than that of 9.44% observed in EPR-1-negative lymph node metastasis. However, the average AI in EPR-1-positive lymph node metastasis tumors was 0.99%, which was significantly higher than that of 0.67% observed in EPR-1-negative lymph node metastasis.

CONCLUSION: The frequency of EPR-1 expression was significantly higher in primary gastric adenocarcinoma and in its lymph node metastasis than that in normal gastric mucosa. Expression of EPR-1 was significantly correlated with tumor histological subtypes and tumor differentiation. Weighted EPR-1 Score is positively correlated with apoptosis index, but is negatively related with proliferative index. Thus, Weighted EPR-1 Score and EPR-1 expression in gastric adenocarcinoma cells maybe a potential marker in clinical setting.

INTRODUCTION

Gastric cancer is the second most common malignancy worldwide. Approximately 95% of all malignant gastric neoplasmas are adenocarcinoma. This study aimed to investigate the correlationship among expression of EPR-1, proliferative index (PI, also referred to as ki-67) and apoptosis index (AI) in patients with primary advanced gastric adenocarcinoma and to clarify the significance of EPR-1 expression in human primary gastric adenocarcinoma, its lymph node metastasis and adjacent non-tumor tissues.

MATERIALS AND METHODS

Patients and tissue samples

Primary gastric adenocarcinoma specimens from 120 patients who had undergone gastrectomy in the period of January 1991 and December 1995 were selected from the files of the Pathology Department, Nanjing Jinling Hospital. None of the patients had received chemotherapy or radiotherapy before surgery. There were 72 males and 48 females, with a median age of 64.8 years (range, 45-78 years). Samples were taken from the representative cancerous lesions as well as lymph node metastasis and adjacent non-cancerous mucosa.

Specimens of the adenocarcinoma were classified into papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma. Each subclass had 30 cases.

For fomalin-fixed and paraffin-embedded tissue specimens, consecutive 4-μm thick sections were cut and representative sections were used for immunohistochemistry (IHC) and haematioxylin-eosin (H&E) staining as well as the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) for apoptostic index. Using the H&E-stained sections, the tumor histology according to Lauren's grade of tumor differentiation and criteria of the World Health Organization were reassessed. Tumors were staged according to TNM classification.

Immunohistochemistry

The deparaffinized and rehydrated slides were boiled in a 10 mmol/L EDTA buffer (pH 8.0) for 5 min in an autoclave at 121℃. The sections were immunohistochemically stained by

the labeled streptavidin-biotin peroxidase method (LSAB2 Kit; Dako Japan Inc., Kyoto, Japan) with the following primary antibodies: monoclonal antibodies EPR-1(ADI, Alpha Diagnostic International, dilution 1:100), and Ki-67 (Maxim, China, dilution 1:50). The slides were immersed for 10 min in 3 mL/L hydrogen peroxide/methanol to deplete endogenous peroxidase. Then, nonspecific binding sites were blocked with 3 mL/L normal goat serum for 10 min. The primary antibody was then applied, and the sections were incubated overnight at 4 ℃. After washed with PBS (0.01 mol/L pH7.4), biotinylated goat antimouse IgG was applied onto the tissue sections and incubated at room temperature for 10 min. After washed with PBS, a streptavidin peroxicase reagent was applied and incubated at room temperature for 10 min. Finally, the reaction product was visualized using developing color by incubating the slides in a solution of 3 mL/L hydrogen peroxide and AEC chromogen. The sections were counterstained slightly with hematoxylin. Negative controls included parallel sections treated without the primary antibody, in addition to negat an adjacent section of the same block in which the primary antibody was replaced by phosphate-buffered saline (PBS).
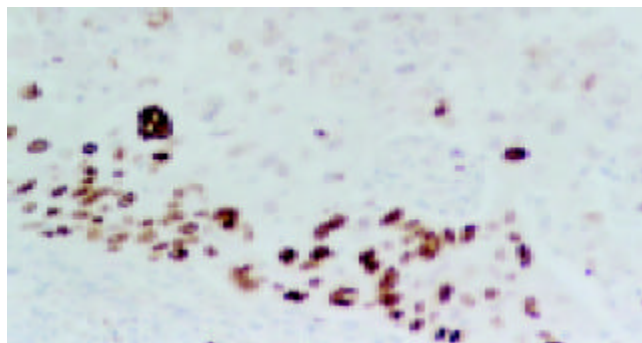
TUNEL staining

For detection of apoptotic cells, apoptotic index was examined by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate fluorescence nick end labeling (TUNEL) method. TUNEL: *In situ* cell death detection *kit* POD (ISCDD, Boehringer Mannheim, Germany) was used to detect the apoptotic cell. The procedures was according to the protocol of the kit and other references. Briefly, the sections were deparaffinized, rehydrated, and washed in distilled water (DW). The tissues were digested with 20 g/mL proteinase K (Boehringer Mannheim, Mannheim, Germany) at room temperature for 15 min. Endogenous peroxidase activity was blocked by incubating it in 3 mL/L hydrogen peroxide/methanol in PBS at 37 ℃ for 30 min. The sections then were incubated with terminal deoxynucleotidyl transferase at 37 ℃ for 60 min, and dioxigenin-conjugatd dUTP was added to the 3'-OH ends of fragmented DNA. Anti-digoxigenin antibody peroxidase was applied to the sections to detect the labeled nucleotides. The sections were stained with DAB and counterstained slightly with hematoxylin. The positive cells were identified, counted and analyzed under the light microscope.

Evaluation of EPR-1 expression

Two experienced pathologists in a blinded fashion (without knowledge of the clinico-pathological features of the tumors) examined the expression of EPR-1 and Ki-67 independently in terms of intensity and positive rate of the immunostaining in each specimen tissue section. The positive staining for EPR-1 protein was expressed as red brown granules, which were mainly located in cell cytoplasm at microscopy. At least 5 high-power (×400 field) fields were chosen randomly and 1 000 cells were counted. The ratio of EPR-1-positive cells was calculated by dividing the number of positive cells over the total number of cells, and was expressed as percentage.

For quantitation of the EPR-1 expression in various samples examined, a semi-quantitative scoring system was used as previously described[1]. An average percentage of positive tumor cells was determined in at least five areas at×400 magnification and assigned to one of the five categories: (a) 0≤5%, (b) 1=5-25%, (c) 2=25-50%, (d) 3=50-75%, and (e) 4≥75%. The intensity of EPR-1 immunostaining was scored on a scale of 1-3 as following: (a) weak, 1+; (b) moderate, 2+; and (c) intense, 3+. The percentage of positive tumor cells and the staining intensity were multiplied to produce a weighted score for each case. The case with the weighted score less than 1 was defined as negative, otherwise as positive.

Ki-67-positive cells showed a distinct brown staining of the nuclei with strong intratumoral heterogeneity (Figure 1) The percentages of Ki-67-positive tumor cells were evaluated with a light microscope holding a ×100 oil immersion objective by scoring a minimum of 1 000 tumor cells in randomly selected fields.

Figure 1 Immunohistochemical staining patterns of Ki-67 in gastric adenocarcinomas. Positive nuclear staining in LSAB, ×200. Poorly-differentiated adenocarcinoma, demonstrated cell proliferative activity.

The percentage of apoptotic cells was calculated as the number of TUNEL-positive cancer cells per 1 000 cancer cells in the most frequently identified areas, and determined as the apoptotic index (AI). Under microscopy, morphological evidences of cell shrinkage, nuclear chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies were frequently observed as the characteristic changes of apoptosis cells (Figure 2).
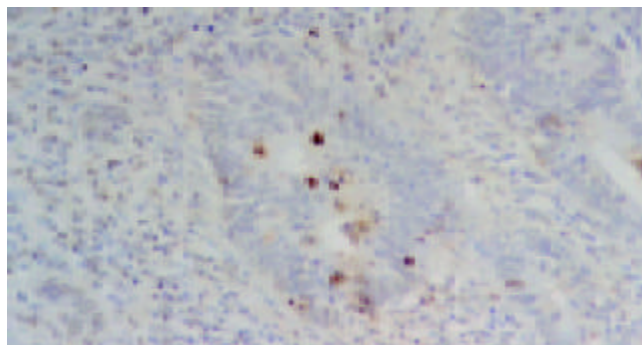
Figure 2 Apoptotic cancer cells in well-differentiated adeno-carcinoma detected by TUNEL method. LSAB, ×200.

Statistical analysis

Data were expressed as mean±SD. The two-tailed χ^2 test was used to examine the correlation between various clinical or pathologic parameters and the expression of EPR-1. Statistical significance between different groups was determined by independent Student's *t*-test (two-tailed). The Pearson test was used to examine linear correlation between Weighted EPR-1 Score and proliferative or apoptotic indexes. A *P* value less than 0.05 was considered statistically significant. All the calculations were performer by SPSS 10.0 for windows.

RESULTS

Correlation between EPR-1 protein expression and clinicopathological parameters

No significant relation was found between the expression of EPR-1 and patient age, sex, tumor location, lymph node metastasis or depth of invasion (Table 1, *P*>0.05).

Table 1 No Correlation between EPR-1 protein expression and clinicopathological parameters

Clinico-pathological parameters	Cases	EPR-1 positive cases (%)	P value
Age (yr)			
<60	37	21 (56.8)	
≥60	83	56 (67.5)	0.258
Sex			
Male	72	43 (59.7)	
Female	48	34 (70.8)	0.214
Tumor location			
Upper 1/3	39	22 (56.4)	
Middle 1/3	32	20 (62.5)	
Lower 1/3	19	11 (57.9)	
More than 2 locaitons	30	24 (80.0)	0.199
Differentiation			
Well	60	46 (76.7)	
Poorly	60	31 (51.7)	0.004
Subtypes			
Papillary	30	24 (80.0)	
Tubular	30	22 (73.3)	
Mucinous	30	13 (43.3)	
Signet-ring cell	30	18 (60.0)	0.017
Invasion mucosa			
No	66	40 (60.6)	
Yes	54	37 (68.5)	0.369
Lymph node metastasis			
No	70	43 (61.4)	
Yes	50	34 (68.0)	0.459
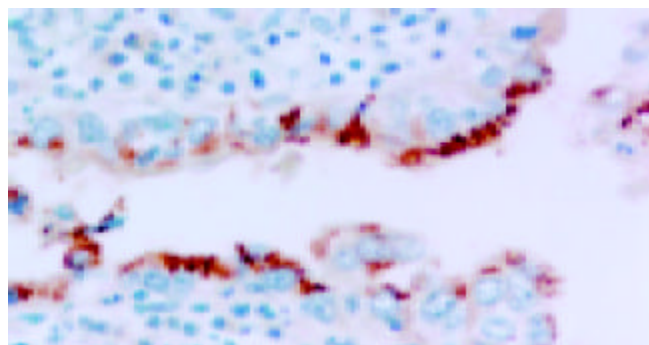
Figure 3 Strong Positive cytoplasma staining of EPR-1 in gastric adenocarcinomas (LSAB×400).
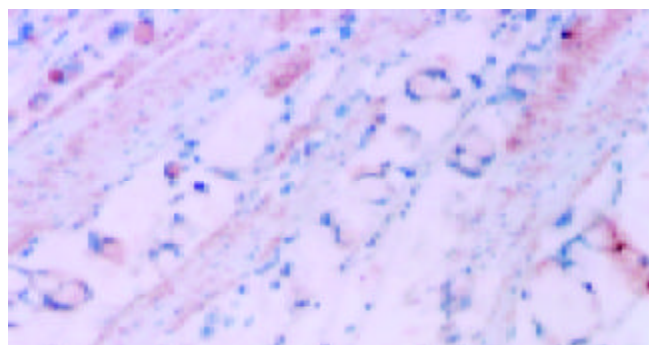
Figure 4 Positive staining of EPR-1 in signet-ring cell carcinoma invasion in smooth muscle and smooth muscle cells (LSAB×200).
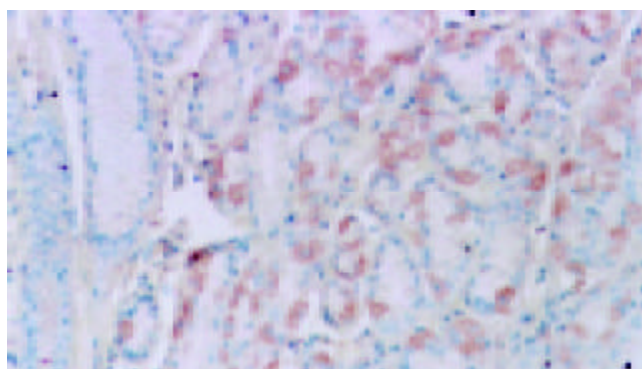
Figure 5 Moderate positive nuclear staining in benign gastric adenocytes (LSAB×200).
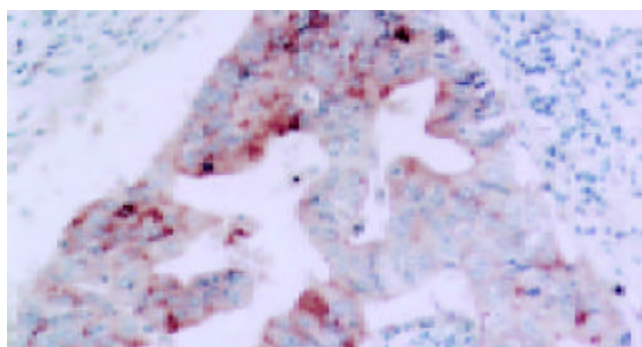
Figure 6 Positive cytoplasma staining in well-differentiated lymph node metastasis (LSAB×200).

### Expression of EPR-1 between histological subtypes

There were a variety of positive rates of EPR-1 protein in different histological subtypes (Figures 3, 4, 5, 6 and Table 2). The positive rate of EPR-1 expression nodes was significantly different in the invasive tumor (P=0.007), while it was differently expressed among the 4 subtypes and adjacent non-tumor tissues in the primary carcinoma and its lymph node metastasis (P<0.05). But there was no difference of EPR-1 expression in the adjacent normal gastric mucosa cells and smooth muscle cells among the 4 histological subtypes (P=0.849 and 0.720).

### Expression of EPR-1 in the highly-differentiated adenocarcinomas

The positive rate of EPR-1 expression in the primary gastric adenocarcinoma was significantly higher than that in normal gastric mucosa (Table 3, P=0.006), while no significant difference was found in the invasion tumor nodes, lymph node metastasis and smooth muscle (P=0.20, 0.632, 0.088), respectively. However, in the primary highly-differentiated gastric adenocarcinomas, the positive expression rate of EPR-1 was significantly different from that of normal gastric mucosa, invasive tumor node, lymph node metastasis and smooth muscle while no significant difference was found in the poorly-differentiated adenocarcinomas.

Relation of EPR-1 expression with AI and PI in primary adenocarcinoma

The apoptotic index for EPR-1 positive in primary adenocarcinoma group was 1.25% and was, 1.00% for EPR-1 negative group, while the proliferative index for the two group was 7.00% and 8.53%, respectively. The difference of the two indexes between the EPR-1 positive and negative groups was statistically significant (Table 4, P<0.05). The relation of EPR-1 score with AI and PI is shown in Figure 7.
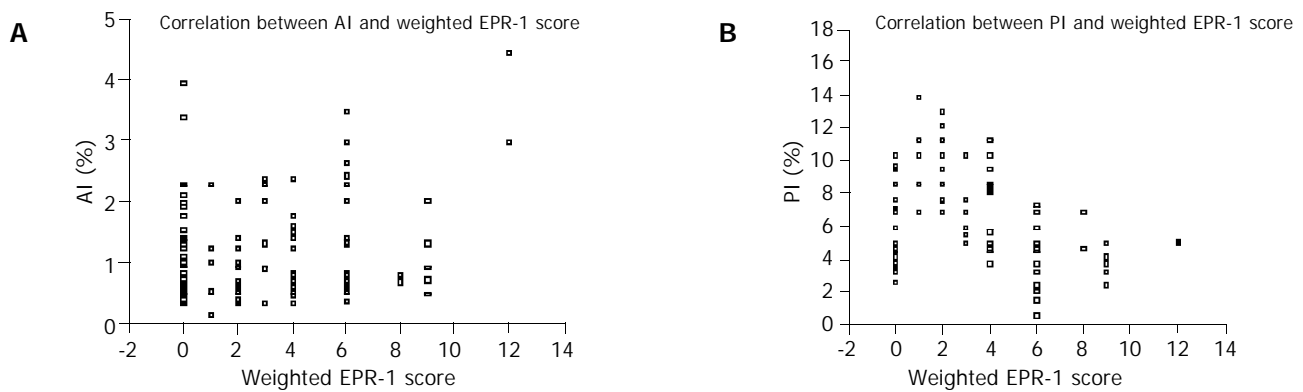
Figure 7 Significant difference of AI and PI with EPR-1 score in primary adenocarcinoma. A: The results of Pearson correlation coefficient, *r*=0.296, *P*=0.001<0.05; B: The results of pearson correlation coefficient, *r*=-0.204, *P*=0.025<0.05 Weighted EPR-1 Score was positively correlated with apoptotic index, but negatively related with proliferative index in primary adenocarcinoma.
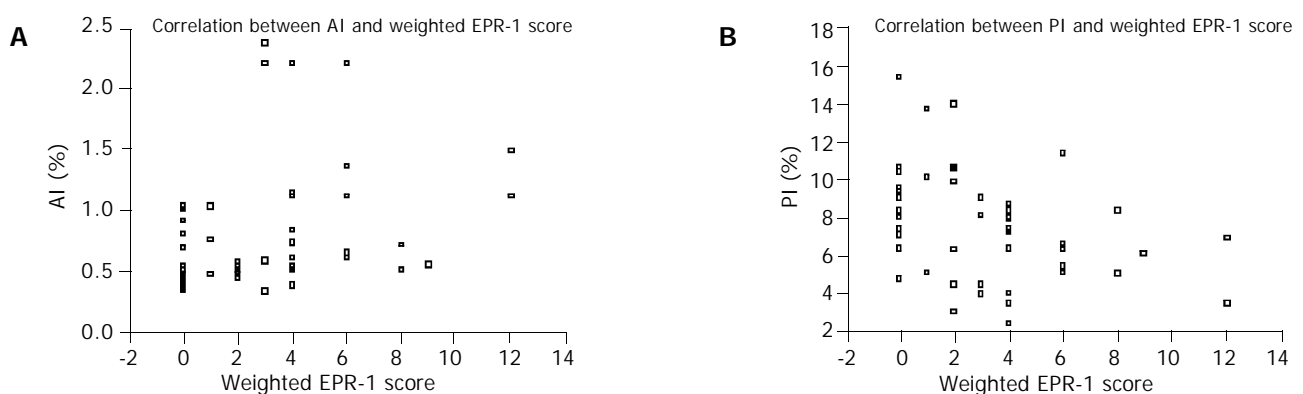
Figure 8 Significant difference of AI and PI with EPR-1 score in lymph node metastasis. A: The results of pearson correlation coefficient, *r*=0.332, *P*=0.019<0.05. B: The results of pearson correlation coefficient, *r*=-0.336, *P*=0.017<0.05. Weighted EPR-1 score is positively correlated with apoptosis index, but negatively related with proliferative index in lymph node metastasis.

Table 2 Different expression of EPR-1 between histological subtypes

Subtypes	Primary carcino ma	P value	Normal gastric mucos a	P vlaue	Invasion node	P value	Lymph node metastasis	P value	Smooth muscle cell	P value
Papillary	24/30 (80.0)		14/30 (46.7)		14/17 (82.4)		11/13 (84.6)		17/30 (56.7)	
Tubular	22/30 (73.3)		15/30 (50.0)		12/16 (75.0)		11/15 (73.3)		15/30 (50.0)	
Mucinous	13/30 (43.3)		12/30 (40.0)		10/24 (41.7)		3/10 (30.0)		14/30 (46.7)	
Signet-ring cell	18/30 (60.0)	0.017	15/30 (50.0)	0.849	11/28 (39.3)	0.007	9/12 (75.0)	0.033	18/30 (60.0)	0.720

Table 3 Different expression of EPR-1 in the highly-differentiated adenocarcinomas

Differentation	Primary gastric adenocar cinoma	Normal gastric mucosa cells	Invasion node adenocarcinoma	Lymph node metastasis	Smooth muscle cell	P value
Well	46/60 (76.7)	29/60 (48.3)	26/33 (78.8)	22/28 (78.6)	32/60 (53.3)	0.002
Poorly	31/60 (51.7)	27/60 (45.0)	21/52 (40.4)	12/22 (54.5)	32/60 (53.3)	0.594

Table 4 Significant expression difference of the AI and PI between EPR-1 positive and negative groups in primary adenocarcinomas

	n	AI (%)	P value	PI (%)	P value
EPR-1		mean±SD		mean±SD	
+	77	1.25±0.86		7.00±3.28	
-	43	1.00±0.80	0.024	8.53±2.70	0.010

Relation of EPR-1 expression with AI or PI in cancer with lymph node metastasis

The apoptotic index for EPR-1 positive lymph node metastasis group was 0.99% and was 0.67% for EPR-1 negative lymph node metastasis group, while the proliferative index for the two groups was 7.65% and 9.44%, respectively. The difference of the two indexes between the EPR-1 positive and negative groups was statistically significant (*P*<0.05). The EPR-1 score was showed with the AI and PI in (Table 5, Figure 8).

Table 5 Significant expression difference of the AI and PI between EPR-1 positive and negative groups in lymph node metastasis

	n	AI (%)	P	PI (%)	P
EPR-1					
+	34	0.99±0.58		7.65±2.60	
-	16	0.67±0.24	0.010	9.44±3.05	0.037

DISCUSSION

Effector cell Protease Receptor-1 (EPR-1) has been recognized as a receptor of the transmembane glycoprotein coagulation factor Xa and its transcripts are present in terminally differentiated adult tissues[2-9]. Survivin gene was identified by Ambrosini *et al*. during hybridization screening of a human EPR-1 genomic library with the cDNA of EPR-1[10,11]. The gene spanand nearly 15 kb and was co-located at 17q25 with EPR-1[12]. Survivin has been found to be a new member of the inhibitors of apoptosis proteins (IAP) family[13-16], and is selectively overexpressed in common human cancers but not in normal adult tissues.

Transfecting with EPR-1 mRNA into cells could inhibit endogenous expression of survivin gene and induce apoptosis as well as inhibit cell proliferation[17]. Shinozawa *et al*.[18] used RT-PCR method to detect the expression of Survivin and EPR-1 in leukemia and lymphoma, and found that the abnormality of the expression ratio of EPR-1 and Survivin might exert anti-colon tumor proliferation functions. Yamamoto *et al*.[19] reported that EPR-1 in a human colon cancer cell line downregulated survivin expression, with a similar decrease in cell proliferation and an increase in apoptosis. This antitumour efficacy was further enhanced in combination with anticancer agents 5-Fu and CDDP. These findings suggest that regulation of survivin by induction of EPR-1 may have a significant potential as a therapy for human cancer. But to our knowledge, EPR-1 expression in gastric adenocarcarcinaoma has not been report.

In order to gain insight into the role of EPR-1 in gastric adenocarcarcinaoma, we investigated the expression of EPR-1 in a group of gastric adenocarcarcinaomas, and examined the relationship of its expression with cell proliferation and apoptosis index. By immunohistochemical analysis performed in 120 cases, our study shows that positive staining for EPR-1 protein was expressed as red brown granules, which were mainly located in cell cytoplasm. There was no significant relationship between the expression of EPR-1 and patient age, sex, tumor location, lymph node metastasis or depth of invasion. However, a significantly differences expressions of EPR-1 was found between histological subtypes.

Our study also explored the potential relationship between the Weighted EPR-1 expression score and apoptotic, proliferative indexes. Ki-67 has been regarded as a parameter of tumor proliferation[20-29] and the TUNEL method has become a popular way to detect apoptotic cells[30-32]. Using these two reliable methods, we found that the positive rate of EPR-1 expression in the primary gastric adenocarcinoma was significantly higher than that in normal gastric mucosa and in terms with degree of differentiation, the positive expression rate of EPR-1 was significantly different among the primary gastric adenocarcinoma, normal gastric mucosa, tumor invasive node, lymph node metastasis and smooth muscle in the highly-differentiated adenocarcinomas while no significant difference was found in the poorly-differentiated adenocarcinomas.

In conclusion, EPR-1 can influence the biological behaviors of tumor cells not only by inducing their apoptosis, but by inhibiting their proliferation. Whether EPR-1 can be used as a new anti-cancer therapeutics strategy is still waiting for more evidence.

REFERENCES

1   **Lu CD**, Altieri DC, Tanigawa N. Expression of a novel antiapoptosis gene, survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas. *Cancer Res* 1998; **58**: 1808-1812

2   **Altieri DC**. Molecular cloning of effector cell protease receptor-1, a novel cell surface receptor for the protease factor Xa. *J Biol Chem* 1994; **269**: 3139-3142

3   **Bouchard BA**, Silveira JR, Tracy PB. On the role of EPR-1 or an EPR-1-like molecule in regulating factor Xa incorporation into platelet prothrombinase. *Thromb Haemost* 2001; **86**: 1133-1135

4   **McLean K**, Schirm S, Johns A, Morser J, Light DR. FXa-induced responses in vascular wall cells are PAR-mediated and inhibited by ZK-807834. *Thromb Res* 2001; **103**: 281-297

5   **Blanc-Brude OP**, Chambers RC, Leoni P, Dik WA, Laurent GJ. Factor Xa is a fibroblast mitogen via binding to effector-cell protease receptor-1 and autocrine release of PDGF. *Am J Physiol Cell Physiol* 2001; **281**: C681-689

6   **Cirera S,** Fredholm M. Isolation and mapping the pig homologs survivin (BIRC5) and effector cell protease receptor 1 (EPR1) genes. *Cytogenet Cell Genet* 2001; **92**: 351-352

7   **Kawabata A**, Kuroda R, Nakaya Y, Kawai K, Nishikawa H, Kawao N. Factor Xa-evoked relaxation in rat aorta: involvement of PAR-2. *Biochem Biophys Res Commun* 2001; **282**: 432-435

8   **Monno R**, Grandaliano G, Faccio R, Ranieri E, Martino C, Gesualdo L, Schena FP. Activated coagulation factor X: a novel mitogenic stimulus for human mesangial cells. *J Am Soc Nephrol* 2001; **12**: 891-899

9   **Shinozawa I**, Inokuchi K, Wakabayashi I, Dan K. Disturbed expression of the anti-apoptosis gene, survivin, and EPR-1 in hematological malignancies. *Leuk Res* 2000; **24**: 965-970

10   **Altieri DC**. Splicing of effector cell protease receptor-1 mRNA is modulated by an unusual retained intron. *Biochemistry* 1994; **33**: 13848-13855

11   **Altieri DC**. Molecular cloning of effector cell protease receptor-1, a novel cell surface receptor for the protease factor Xa. *J Biol Chem* 1994; **269**: 3139-3142

12   **Ambrosini G**, Adida C, Sirugo G, Altieri DC. Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. *J Biol Chem* 1998; **273**: 11177-11182

13   **Zaffaroni N**, Daidone MG. Survivin expression and resistance to anticancer treatments: perspectives for new therapeutic interventions. *Drug Resist Updat* 2002; **5**: 65-72

14   **Takai N**, Miyazaki T, Nishida M, Nasu K, Miyakawa I. Survivin expression correlates with clinical stage, histological grade, invasive behavior and survival rate in endometrial carcinoma. *Cancer Lett* 2002; **184**: 105-116

15   **Fortugno P**, Wall NR, Giodini A, O'Connor DS, Plescia J, Padgett KM, Tognin S, Marchisio PC, Altieri DC. Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function. *J Cell Sci* 2002; **115** (Pt 3): 575-585

16   **Kanwar JR**, Shen WP, Kanwar RK, Berg RW, Krissansen GW. Effects of survivin antagonists on growth of established tumors and B7-1 immunogene therapy. *J Natl Cancer Inst* 2001; **93**: 1541-1552

17   **Adida C**, Crotty PL, McGrath J, Berrebi D, Diebold J, Altieri DC. Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. *Am J Pathol* 1998; **152**: 43-49

18   **Shinozawa I**, Inokuchi K, Wakabayashi I, Dan K. Disturbed expression of the anti-apoptosis gene, survivin, and EPR-1 in hematological malignancies. *Leuk Res* 2000; **24**: 965-970

19   **Yamamoto T**, Manome Y, Nakamura M, Tanigawa N. Downregulation of survivin expression by induction of the effector cell protease receptor-1 reduces tumor growth potential and results in an increased sensitivity to anticancer agents in human colon cancer. *Eur J Cancer* 2002; **38**: 2316-2324

20   **Scholzen T**, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol* 2000; **182**: 311-322

21   **Aoki T**, Inoue S, Imamura H, Fukushima J, Takahashi S, Urano T, Hasegawa K, Ogushi T, Ouchi Y, Makuuchi M. EBAG9/RCAS1 expression in hepatocellular carcinoma: correlation with tumour dedifferentiation and proliferation. *Eur J Cancer* 2003; **39**: 1552-1561

22   **Nieto A**, Perez-Alenza MD, Del Castillo N, Tabanera E, Castano M, Pena L. BRCA1 expression in canine mammary dysplasias and tumours: relationship with prognostic variables. *J Comp Pathol* 2003; **128**: 260-268

23   **Friedrich RE**, Hagel C, Brehme Z, Kluwe L, Mautner VF. Ki-67 proliferation-index (MIB-1) of neurofibromas in neurofibro-

matosis type 1 patients. *Anticancer Res* 2003; **23**: 953-955

24 **Sredni ST**, Alves VA, Latorre Mdo R, Zerbini MC. Adrenocortical tumours in children and adults: a study of pathological and proliferation features. *Pathology* 2003; **35**: 130-135

25 **Grabsch H**, Takeno S, Parsons WJ, Pomjanski N, Boecking A, Gabbert HE, Mueller W. Overexpression of the mitotic checkpoint genes BUB1, BUBR1, and BUB3 in gastric cancer–association with tumour cell proliferation. *J Pathol* 2003; **200**: 16-22

26 **Furuya Y,** Kawauchi Y, Fuse H. Cell proliferation, apoptosis and prognosis in patients with metastatic prostate cancer. *Anticancer Res* 2003; **23**: 577-581

27 **Watanabe J**, Sato H, Kanai T, Kamata Y, Jobo T, Hata H, Fujisawa T, Ohno E, Kameya T, Kuramoto H. Paradoxical expression of cell cycle inhibitor p27 in endometrioid adenocarcinoma of the uterine corpus - correlation with proliferation and clinicopathological parameters. *Br J Cancer* 2002; **87**: 81-85

28 **Rudolph P**, Bonichon F, Gloeckner K, Collin F, Chassevent A, Schmidt D, Coindre JM, Parwaresch R, Kloppel G. Comparative analysis of prognostic indicators for sarcomas of the soft parts and the viscerae. *Verh Dtsch Ges Pathol* 1998; **82**: 246-252

29 **Janicke F.** Value of tumor biological prognostic factors in adjuvant therapy of node-negative breast cancer. *Zentralbl Gynakol* 1994; **116**: 449-455

30 **Kase S**, Osaki M, Honjo S, Adachi H, Ito H. Tubular adenoma and intramucosal intestinal-type adenocarcinoma of the stomach: what are the pathobiological differences? *Gastric Cancer* 2003; **6**: 71-79

31 **Uysal H**, Cevik IU, Soylemezoglu F, Elibol B, Ozdemir YG, Evrenkaya T, Saygi S, Dalkara T. Is the cell death in mesial temporal sclerosis apoptotic? *Epilepsia* 2003; **44**: 778-784

32 **Bendardaf R**, Ristamaki R, Kujari H, Laine J, Lamlum H, Collan Y, Pyrhonen S. Apoptotic index and bcl-2 expression as prognostic factors in colorectal carcinoma. *Oncology* 2003; **64**: 435-442

Edited by Zhang JZ and Wang XL **Proofread by** Xu FM