BRIEF ARTICLE

Clinicopathologic significance of expression of nuclear factor-κB RelA and its target gene products in gastric cancer patients

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Abstract

AIM: To assess the prognostic significance of nuclear factor-κB (NF-κB) and its target genes in gastric cancer.

METHODS: The tumor tissues of 115 patients with gastric cancer were immunohistochemically evaluated using monoclonal antibodies against NF-κB RelA. Preoperative serum levels of vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) were assessed *via* enzyme-linked immuno-sorbent assay. C-reactive protein (CRP) and serum amyloid A (SAA) were measured *via* immunotrubidimetry.

RESULTS: Positive rate of NF-κB RelA was 42.6%. NF-κB RelA expression in tumor tissues was also related to serum levels of IL-6 (*P* = 0.044) and CRP (*P* = 0.010). IL-6, SAA, CRP were related to depth of invasion, VEGF and SAA were correlated with lymph node metastasis. IL-6, VEGF, SAA and CRP were related to the stage. Univariate analysis demonstrated that immunostaining of NF-κB RelA, levels of IL-6, VEGF, SAA were significantly related with both disease free survival and overall survival (OS). Multivariate analysis verified that NF-κB RelA [hazard ratio (HR): 3.40, *P* = 0.024] and SAA (HR: 3.39, *P* = 0.045) were independently associated with OS.

CONCLUSION: Increased expression of NF-κB RelA and high levels of serum SAA were associated with poor OS in gastric cancer patients.

INTRODUCTION

Gastric cancer continues to pose a significant health prob-

lem, despite its declining incidence in the West. It is the 4th most common cancer worldwide, accounting for 8.6% of all new cancer diagnoses in 2002[1]. In South Korea, gastric cancer is the most common carcinoma in men, and the third-most common cancer type in women[2]. Surgery is the only potentially curative treatment for localized gastric cancer. However, the majority of patients will relapse after definitive surgery, and the 5-year overall survival (OS) rates of this condition remain lower than 30% to 40% in Western countries[1]. These high levels of gastric cancer mortality can be attributed to the high incidence of serosal invasion, direct invasion into the adjacent organs, peritoneal seeding, lymph node metastasis, and distant metastasis of gastric cancer. The assessment of biological prognostic factors is of great clinical importance in gastric cancer. The widely used tumor node metastasis (TNM) staging exhibits high prognostic power, but cannot predict outcomes perfectly for particular individuals. The outcome of cancer patients may be affected by variability in tumor biology. Thus, tumors with similar clinical or pathologic characteristics frequently evidence different clinical outcomes. Gaining insight into the underlying molecular mechanisms of initiation and progression of gastric cancer will be a necessary step in definitively identifying groups of patients with poor prognoses. The transcription factor nuclear factor-κB (NF-κB) is a heterodimeric complex of the Rel-family proteins. The most common dimer is the RelA (p65)/p50 heterodimer, i.e., NF-κB[3]. NF-κB is sequestered in the cytoplasms in an inactive form *via* interaction with the inhibitor of nuclear factor-κB proteins (IκBs). A variety of stimuli, including cytokines and infectious agents, induce the phosphorylation, ubiquitination, and degradation of IκBs, and also allow the nuclear translocation of NF-κB[4]. Once in the nucleus, NF-κB binds to DNA at its response elements and activates the expression of genes. Recent studies using anti-RelA immunostaining have shown that NF-κB is constitutively activated in gastric cancer[5-7], Additionally, there is some evidence to suggest that NF-κB levels are higher in gastric cancer cells than in normal adjacent epithelial cells, and that NF-κB activation in gastric cancer is associated with lymphatic invasion, advanced stage, and poor clinical outcomes[5,7]. However, other investigators have reported conflicting data with regard to the relationship of NF-κB protein with patient survival[6].

The active NF-κB transcription factor promotes the expression of more than 150 target genes[8]. The majority of proteins encoded for by NF-κB target genes participate in host immune responses, whereas others appear to be involved in the proliferation, invasion, angiogenesis, and metastasis of cancer cells. NF-κB promotes the production of cytokines that function as growth factors for transformed enterocytes[9]. One of these growth factors was subsequently identified as interleukin-6 (IL-6), which is encoded for by a NF-κB target gene. IL-6 acts on a broad range of tissues and cell lines, and induces cell growth and differentiation, the production and expression of other cytokines, and acute-phase protein synthesis. IL-6 also promotes growth arrest[10], and promotes

angiogenesis *via* the induction of vascular endothelial growth factor (VEGF) expression[11]. A previous study reported that the preoperative serum level of IL-6 was associated with lymph node metastasis, depth of invasion, advanced stage, and poor survival in gastric cancer patients[12].

C-reactive protein (CRP) and serum amyloid A (SAA) are nonspecific, acute-phase, hepatic proteins secreted in response to cytokines, including IL-1, IL-6, and tumor necrosis factor-α[13]. CRP is a representative marker for inflammatory conditions, and carries out a crucial anti-infection function in the immune system. In many cancers, chronic inflammation has reportedly been involved with malignant change, and the risks of colorectal cancer appear to be increased when pre-diagnostic CRP levels are high[14]. Cancer invasion begins with inflammation around cancer cells. Thus, serum CRP levels have generally been shown to be higher in cases of invasive cancer than in cases of non-invasive cancer[12].

An increasing body of *in vitro* evidence supports the involvement of SAA in carcinogenesis and neoplastic diseases[15]. An accumulating body of evidence has been collected to suggest that SAA might be included in a group of biomarkers for the detection of a pattern of physiological events that reflects the growth of malignancy and host response. There have also been some reports indicating that the SAA-induced upregulation of matrix metalloproteinase 9 was mediated *via* formyl peptide receptor like-1 and was achieved at the transcriptional level *via* NF-κB[16], and also that preoperative SAA was useful in predicting the survival of gastric cancer patients[17].

The expression of several angiogenic factors is also known to be regulated by NF-κB. Macrophages as well as tumor cells have been shown to generate VEGF under the control of NF-κB activation[18]. VEGF is the most important pro-angiogenic growth factor, and its stimulation under hypoxic conditions carries out critical functions in promoting the survival of malignant cells, in local tumor growth and invasion, and in the development of metastases[19]. We demonstrated that VEGF expression was correlated with the depth of invasion and NF-κB expression in stage III colorectal cancer patients[20].

The objective of the current investigation was to evaluate the prognostic significance of NF-κB RelA and its target gene products in gastric cancer patients.

MATERIALS AND METHODS

Patients

From March 2005 to July 2006, a total of 115 patients with histologically-confirmed gastric carcinomas at the Dong-A University Hospital were enrolled in this study. After operation, the disease status of patients was evaluated *via* gastroendoscopy and abdominal computed tomography (CT). During the first 2 years, we checked CT and endoscopy at 6-mo intervals, and afterwards CT and endoscopy were conducted every 12 mo. We also carried out abdomen CT when we observed abdominal distension, acute abdominal pain, severe diarrhea and

chest X-ray abnormalities suggestive of lung metastasis. Endoscopy was conducted in cases in which patients complained of unexplained abdominal discomfort and dyspepsia. On average, in the stage Ⅲ and Ⅳ patients, the first evaluation was conducted within 3 mo after operation. All patients provided informed consent, and the hospital review board approved the study.

Construction of tissue microarray

Two cores of 2-millimeters in diameter of formalin-fixed, paraffin-embedded tissue blocks were obtained from each gastric cancer patient's sample and arranged in new recipient paraffin blocks using an UNITMA apparatus (UNITMA, Seoul, South Korea) after careful histological assessment. All cases except for one endoscopic biopsy sample and the normal control tissue, were embedded in 6-tissue microarray blocks. Tissue microarray blocks were serially cut into 4 μm sections and stained for routine hematoxylin and eosin staining and immunohistochemistry.

Immunohistochemistry

All immunohistochemistry processes were automatically performed using a Ventana autostainer (Benchmark; Ventana Medical Systems, Tuscon, Arizona, United States). For antigen retrieval, retrieval solution (Ventana) was automatically poured onto the sections, and heated for 60 min at 100 ℃. Endogenous peroxide activity was blocked *via* immersion in 3% hydrogen peroxide for 4 min. With diluted primary antibody for NF-κB p65 (rabbit monoclonal antibody, 1:250, Epitomics, CA, United States), the tissue sections were incubated for 32 min at 36 ℃. Immunoperoxidase staining was carried out using a DAB system (iView DAB detection kit, Ventana) and the sections were counterstained lightly with hematoxylin.

Interpretation of immunohistochemical staining

The percentage and intensity of the immunoreactive tumor cells in each core was recorded and the final values of the positive tumor cells were evaluated as the mean of the immunoreactivity in three cores. The presence of tumor tissue in at least two interpretable cores was required for the inclusion of a case in the statistical analysis. All of the slides were evaluated independently by an experienced pathologist (Kim SJ) who was blinded to all of the clinicopathologic data. Immunostaining for NF-κB RelA was regarded as positive when tumor cells evidenced granular cytoplasmic immunostaining of the lesion, with a minimal background. The percentage scoring of the immunoreactive tumor cells was as follows: 0 (0%), 1 (1%-10%), 2 (11%-50%) and 3 (> 50%). The staining intensity was visually scored and stratified as follows: 0 (negative), 1 (weak, if it was a blush), and 2 (strong, if it was obviously positive at × 20 magnification). A final score was obtained for each case by multiplying the percentage by the intensity score. Therefore, tumors with multiplied scores exceeding 4 (i.e., tumors with a strong intensity of > 10% of the tumor cells) were regarded as positively immunoreactive to NF-κB RelA; all other scores were considered negative.

Assay of serum IL-6, VEGF, SAA and CRP

Peripheral venous blood samples were obtained within 7 d prior to gastrectomy, collected in plain tubes, permitted to clot, and centrifuged for 10 min at 3000 r/min at 4 ℃ within one hour of collection to obtain the serum. The sera were aliquoted and stored at -80 ℃ until being assayed. The VEGF and IL-6 levels from the sera of patients and the standard solutions supplied were measured using a commercial system (Quantikine hVEGF, hIL-6 Immunoassay, R and D Systems, United States) in accordance with the manufacturer's instructions. All serum samples were measured by an investigator who was blinded to the clinical data. The blood samples for CRP analysis were collected in serum separation tubes, and the serum CRP levels were measured *via* immunoturbidimetry (Denka Seiken Co. Ltd., Japan). SAA protein was measured *via* latex agglutination turbidoimmunometric assays (Eiken Chemical Co., Japan).

Statistical analysis

The associations between NF-κB RelA expression and the relevant clinicopathological parameters were assessed *via* an χ^2-test or Fisher's exact test. Serum levels of VEGF, IL-6 and CRP were expressed as mean values. The Mann-Whitney *U*-test was used to evaluate the relationship between protein concentration and clinicopathological features. Disease-free survival (DFS) was defined as the length of time from surgery to initial recurrence of disease. OS was defined as the length of time from surgery to death. The Kaplan-Meier method was utilized to construct curves for DFS and OS. Data on patients who died without any evidence of disease recurrence were censored at the time of death for DFS calculations. The log-rank test was employed to compare distributions. In order to identify the independent factors that were significantly related to patient prognosis, we utilized Cox's proportional hazard analysis *via* a stepwise procedure. All tests were two-sided, and *P* < 0.05 was considered statistically significant. Analyses were conducted using SPSS version 18.0 (SPSS, Chicago, IL, United States).

RESULTS

Patient characteristics

The mean age of the patients was 59 years (range: 24-75 years), and the study population included 47 (40.92%) females and 68 (59.1%) males. Thirty-six of the patients (31.3%) underwent radical total gastrectomy, 75 (65.2%) underwent radical subtotal gastrectomy, and four patients (3.5%) did not have operation due to distant metastasis. The majority of the tumors were located in the middle or lower stomach (72.1%), and were moderate to poorly differentiated carcinomas (75.7%). Thirty-four of the patients (30.6%) had pathological T3 (pT3) or pT4 tumors, 69 (63.2%) had lymph node metastases, and 26 patients (23.4%) had a positive lymph node ratio of ⩾ 0.2. The postoperative stages of the patients were Ⅰ, Ⅱ, Ⅲ and Ⅳ in 43 (37.4%), 23 (20.0%), 31 (27.0%) and 18 (15.7%)

Table 1 Correlation of nuclear factor-κB RelA expression and clinicopathological findings n (%)

	NF-κB RelA (-)	NF-κB RelA (+)	P
Sex			1.000
Male	39 (57.4)	29 (42.6)	
Female	27 (57.4)	20 (42.6)	
Age (yr)			0.192
< 60	38 (63.3)	22 (36.7)	
≥ 60	28 (50.9)	27 (49.1)	
Site			0.002
Upper	9 (37.5)	15 (62.5)	
Middle	9 (50.0)	9 (50.0)	
Lower	48 (72.7)	18 (27.3)	
Diffuse	0 (0)	3 (100)	
Size (cm)			0.045
< 5	47 (67.1)	23 (32.9)	
≥ 5	19 (46.3)	22 (53.7)	
Lauren			0.988
Intestinal	27 (58.7)	19 (41.3)	
Diffuse	26 (57.8)	19 (42.2)	
Mixed	9 (60.0)	6 (40.0)	
Differentiation			0.631
Well	11 (68.8)	5 (31.3)	
Moderate	14 (50.0)	14 (50.0)	
Poor	34 (60.7)	22 (39.3)	
Mucinous	7 (63.6)	4 (36.4)	
Lymphovascular invasion			0.012
-	44 (69.8)	19 (30.2)	
+	22 (45.8)	26 (54.2)	
T stage			0.012
1-2	52 (67.5)	25 (32.5)	
3-4	14 (41.2)	20 (58.8)	
Node			< 0.001
-	34 (81.0)	8 (19.0)	
+	32 (46.4)	37 (53.6)	
Lymph node ratio			< 0.001
< 0.2	60 (70.6)	25 (29.4)	
≥ 0.2	6 (23.1)	20 (76.9)	
Stage			< 0.001
1	36 (83.7)	7 (16.3)	
2	14 (60.9)	9 (39.1)	
3	16 (51.6)	15 (48.4)	
4	0 (0)	18 (100)	
CEA (ng/mL)			0.134
< 5	64 (59.3)	44 (40.7)	
≥ 5	2 (28.6)	5 (71.4)	
CA19-9 (U/mL)			< 0.001
< 37	63 (67.7)	30 (32.3)	
≥ 37	3 (13.6)	19 (86.4)	

NF-κB: Nuclear factor-κB; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19-9.

patients, respectively. With regard to the Lauren classifications, 46 (40.0%) patients were of the intestinal type, 43 (37.4%) of the diffuse type, and 15 (13.0%) of the mixed type. Sixteen patients (14.4%) had well, 28 (25.2%) had moderate, and 56 (50.5%) had poorly differentiated adenocarcinoma. Fifty-five patients (47.8%) received oral 5-fluorouracil, and 46 patients (40.0%) received cisplatin-based adjuvant chemotherapy after gastrectomy.

Correlations between the NF-κB RelA expression, target gene expression and clinicopathological parameters
A representative tumor specimen evidencing increased NF-κB RelA expression in the cytoplasm is shown in

Table 2 Correlation of nuclear factor-κB RelA and level of target genes (mean ± SD)

	Nuclear factor-κB		P
	- (n = 66)	+ (n = 49)	
IL-6 (pg/mL)	8.1 ± 8.6	11.8 ± 10.7	0.044
VEGF (pg/mL)	117.2 ± 154.2	178.9 ± 202.7	0.066
SAA (mg/mL)	8.1 ± 20.3	14.5 ± 16.3	0.072
CRP (mg/mL)	0.1 ± 0.1	0.3 ± 0.4	0.010

IL-6: Interleukin-6; VEGF: Vascular endothelial growth factor; SAA: Serum amyloid A; CRP: C reactive protein.

Figure 1. NF-κB RelA expression was found to be significantly correlated with several findings (Table 1). NF-κB RelA expression was more common in cases of more than 5 cm ($P = 0.045$), deeper invasion ($P = 0.012$), lymph node metastases ($P < 0.001$), advanced stage ($P < 0.001$), positive lymphovascular invasion ($P = 0.012$), and increased Carbohydrate antigen 19-9 ($P < 0.001$). However, no significant correlations were noted between Nf-κB a RelA nd other parameters. We noted that VEGF, SAA levels were significantly correlated with tumor size ($P = 0.022$, $P < 0.001$, respectively). IL-6, SAA and CRP levels were related to the depth of invasion ($P = 0.007$, $P < 0.001$, $P = 0.015$, respectively). We also noted significant differences in serum VEGF and SAA levels between patients with lymph node metastasis and those without lymph node metastasis ($P < 0.001$, respectively). The mean levels of IL-6, VEGF, SAA and CRP were increased proportionally with the cancer stage ($P = 0.031$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively). Additionally, NF-κB RelA expression was correlated positively with IL-6 ($P = 0.044$), and CRP ($P = 0.010$) levels (Table 2).
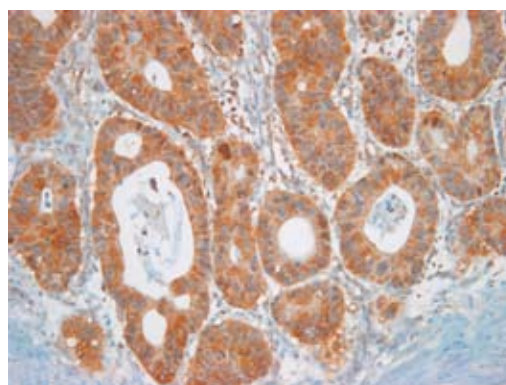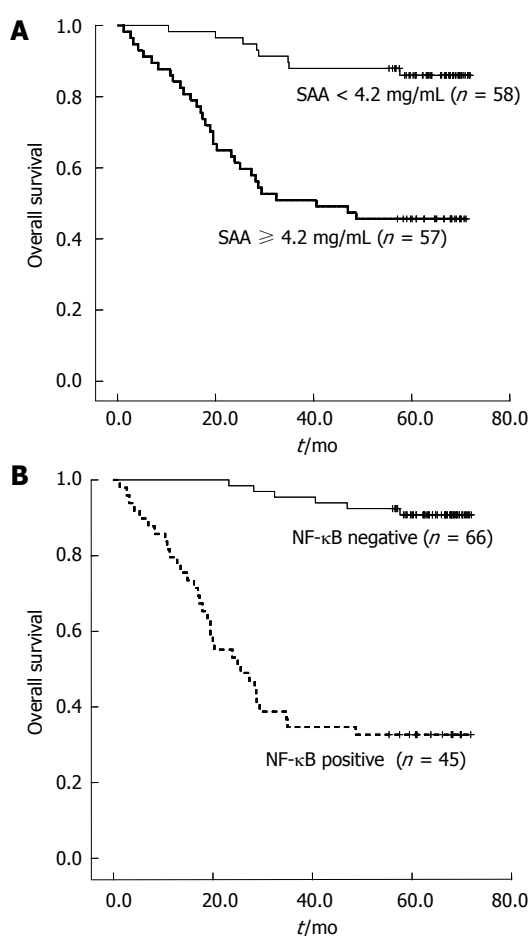
NF-κB RelA expression, target gene expressions and clinical outcomes
The median follow-up duration of the patients was 66.6 mo (range: 9.3-88.8 mo). Univariate analysis of clinicopathologic parameters and DFS and OS are provided in Table 3. Tumor size, location, lymphovascular invasion, stage, and positive lymph node ratio were significantly associated with both 5-year DFS and OS. Positive correlations were detected between immunostaining of NF-κB RelA, levels of IL-6, VEGF, SAA, and DFS and OS. CRP levels were not correlated with 5-year DFS or OS. The OS curves of SAA and NF-κB RelA are provided in Figure 2. In order to determine the independent prognostic values of these markers, we employed a multivariate Cox proportional hazard analysis to control for other prognostic factors (Table 4). Accordingly, stage was identified as an independent prognostic factor of both DFS [hazard ratio (HR): 7.83, 95% confidence interval (CI) = 2.44-25.18, $P = 0.001$], and OS (HR: 6.15, 95% CI = 1.97-19.22, $P = 0.002$). Positive lymph node ratio was also identified as a significant predictor of both DFS (HR: 1.68, 95% CI = 1.01-2.79, $P = 0.043$), and OS (HR: 2.46, 95% CI = 1.01-5.94, $P = 0.047$), after controlling for other clinicopathologic parameters. The expressions of NK-κB and

Table 3 Univariate analysis according to the clinicopathologic finding					
	n	5-yr DFS (%)	*P*	5-yr OS (%)	*P*
Sex					0.824
Male	68	64.7	0.827	65.8	
Female	47	68.1		70.2	
Age (yr)					0.367
< 60	60	70	0.355	71.7	
≥ 60	65	61.8		62.7	
Site					< 0.001
Upper	24	41.7	< 0.001	45.8	
Middle	18	72.2		77.8	
Lower	16	80.3		79.8	
Diffuse	3	0		0	
Size (cm)					0.005
< 5	70	77.1	0.006	78.1	
≥ 5	41	53.7		56.1	
Lauren					0.301
Intestinal	46	71.7	0.345	73.3	
Diffuse	43	60.5		62.8	
Mixed	15	80		80.8	
Differentiation					0.779
Well	16	68.8	0.879	75	
Moderate	28	75.2		75	
Poor	56	66.1		67	
Mucinous	11	63.6		63.6	
Lymphovascular invasion					< 0.001
-	63	82.5	< 0.001	82	
+	48	50		54.2	
T stage					< 0.001
1-2	77	79.2	< 0.001	80	
3-4	34	44.1		47.1	
Node					< 0.001
-	42	97.6	< 0.001	97.6	
+	69	50.7		52.9	
Lymph node ratio					< 0.001
< 0.2	85	81.2	< 0.001	81	
≥ 0.2	26	26.9		26.9	
Stage					<0.001
1	43	100	< 0.001	100	
2	23	60.9		63.2	
3	31	54.8		58.1	
4	18	11.1		11.1	
CEA (ng/mL)					< 0.001
< 5	108	69.4	< 0.001	69.4	
≥ 5	7	14.3		14.3	
CA19-9 (U/mL)					< 0.001
< 37	93	73.1	< 0.001	73	
≥ 37	22	36.4		36.4	
Nuclear factor-κB					< 0.001
-	66	90.9	< 0.001	90.8	
+	49	32.7		32.7	
IL-6 (pg/mL)					< 0.001
< 6.83	59	81.4	< 0.001	81.4	
≥ 6.83	56	50		49.6	
VEGF (pg/mL)					0.002
< 68.1	57	80.7	0.001	80.5	
≥ 68.1	58	52.6		52.6	
SAA (mg/mL)					< 0.001
< 4.2	58	86.2	< 0.001	86	
≥ 4.2	57	45.6		45.6	
CRP (mg/mL)					0.899
< 0.1	59	66.1	0.858	66.1	
≥ 0.1	56	66.1		65.9	

DFS: Disease free survival; OS: Overall survival; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19-9; IL-6: Interleukin-6; VEGF: Vascular endothelial growth factor; SAA: Serum amyloid A; CRP: C-reactive protein.

Figure 1 Immunohistochemical staining for nuclear factor-κB RelA in gastric cancer. Gastric cancer cells evidence intense cytoplasmic immunoreactivity for nuclear factor-κB (× 200; inset, × 40).

Figure 2 Overall survival curve. A: Serum amyloid A (SAA) expression (*P* < 0.001); B: Nuclear factor-κB (NF-κB) expression (*P* < 0.001).

SAA levels were shown to significantly affect OS on multivariate analysis (HR: 3.40, 95% CI = 1.17-9.87, *P* = 0.024; HR: 3.39, 95% CI = 1.02-11.23, *P* = 0.045, respectively).

DISCUSSION

There have been a few reports regarding the expression of NF-κB in human gastric cancer assessed by immu-

Table 4 Multivariate analysisclinicopathological findings

	5-yr disease free survival			5-yr overall survival		
	HR	95% CI	P	HR	95% CI	P
Stage	7.83	2.44-25.18	0.001	6.15	1.97-19.2	0.002
Site	1.21	0.66-2.22	0.536	1.22	0.67-2.23	0.510
Size	0.48	0.188-1.27	0.142	0.577	0.22-1.41	0.218
LN ratio	1.68	1.01-2.79	0.043	2.46	1.01-5.94	0.047
LVI	0.79	0.30-2.11	0.650	1.02	0.41-2.52	0.951
IL-6	1.40	0.53-3.66	0.488	1.19	0.45-3.14	0.726
VEGF	1.22	0.77-1.95	0.390	2.11	0.86-5.17	0.102
SAA	3.21	0.96-10.79	0.058	3.39	1.02-11.23	0.045
CEA	2.17	0.66-7.07	0.198	2.38	0.76-7.48	0.135
CA19-9	0.93	0.35-2.45	0.898	1.96	0.76-5.02	0.158
NF-κB RelA	2.87	0.96-8.49	0.057	3.40	1.17-9.87	0.024

LVI: Lymphovascular invasion; IL-6: Interleukin-6; VEGF: Vascular endothelial growth factor; SAA: Serum amyloid A; LN: Lymph node; CEA: Carcinoembryonic antigen; NF-κB: Nuclear factor-κB; CA19-9: Carbohydrate antigen 19-9; HR: Hazard ratio; 95% CI: 95% confidence interval.

nohistochemistry using the same anti-RelA (p65) antibody[5,7]. The present study confirms constitutively active NF-κB RelA in gastric carcinoma, which is consistent with the findings of previous studies[5,7]. The expression rate of NF-κB RelA in gastric carcinoma is higher than that observed in normal adjacent epithelial cells, and NF-κB RelA expression was observed primarily in gastric carcinoma cytoplasm with an expression rate of 42.6%; this is somewhat higher than in other studies[5,7]. We showed that NF-κB RelA expression was correlated with tumor size, depth of invasion, lymph node metastasis, lymphovascular invasion and advanced stage. Furthermore, positive NF-κB RelA staining was associated with both DFS and OS, and NF-κB RelA was identified as an independent prognostic factor in multivariate analysis (HR: 3.40, 95% CI = 1.17-9.87, P = 0.024).

Sasaki *et al*[5] also reported that NF-κB activation was correlated with tumor size and lymphatic invasion. However, Lee *et al*[6] previously demonstrated that NF-κB activation was not correlated with poor patient prognosis, but rather was significantly associated with better prognosis of early stage gastric carcinoma patients. The absence of clear-cut criteria in the evaluation of NF-κB immunoexpression may be the major reason for the conflicts among the previously published data regarding the prognostic impact of this molecule in gastric cancer.

The activation of NF-κB regulates a variety of genes involved in the proliferation, invasion, angiogenesis, and metastasis of cancer cells[8]. In this study, NF-κB RelA expression was correlated positively with IL-6 and CRP levels. According to some studies attempting to evaluate the clinical significance of IL-6 in gastric cancer patients, IL-6 levels were higher in malignancies than in non-malignancies, increased with increasing tumor size and depth, and were associated with poor clinical outcomes[12,21]. We found significant correlations between elevated IL-6 levels and several conditions: large tumor size, deeper invasion depth, advanced stage, and poor DFS and OS.

Findings from studies of gastrointestinal cancer patients have demonstrated that those subjects with elevated serum CRP concentrations tended toward poorer prognoses than those whose CRP levels were not increases[14,22]. CRP was related to the depth of tumor invasion, tumor differentiation, and TNM stage. However, we found no significant associations between CRP and DFS or OS.

SAA is the most sensitive plasma protein indicative of inflammatory activity[23]. SAA is superior to CRP for the detection of inflammation associated with severe burns, viral infections, kidney transplantation, Crohn's disease, and ulcerative colitis[23]. In this study, SAA was related to tumor size, depth of invasion, lymph node metastasis, clinical stage, and cancer recurrence. Similar results have been reported in other studies[17]. Those studies showed that gastric cancer patients had higher SAA levels than did patients with gastric ulcers and healthy individuals, and that SAA concentrations were correlated with prognosis[17]. SAA was related significantly to DFS and OS, and was determined to be an independent prognostic factor for OS in multivariate analysis (HR: 3.39, 95% CI = 1.02-11.23, P = 0.045).

Tumor VEGF expression was identified as a significant marker for tumor recurrence or reduced survival independent of conventional clinicopathological variables in gastric cancer[24,25]. However, the prognostic value of VEGF expression in gastric cancer remains unclear, and some studies have indicated that tumor VEGF expression may constitute an independent prognostic factor in DFS[24,25], whereas others have reported no such association[26]. In our study, VEGF expression was significantly correlated with tumor size, lymph node invasion and staging; however, our findings do not appear to confirm a significant association between VEGF expression and unfavorable prognosis in multivariate analysis.

Univariate analysis demonstrated that the immunostaining of NF-κB RelA and levels of IL-6, VEGF and SAA were all significantly related with both DFS and OS. Multivariate analysis results verified that NF-κB RelA and SAA were independently associated with OS. Although changes in the expression levels of several cytokines have been frequently reported, their proposed role in the pathogenesis and progression of gastric cancer remains controversial. The results of such studies may be confounded by several factors, including small patient sample size and variable therapeutic protocols which may potentially have affected patient outcomes. Well-designed large scale studies will be necessary to confirm our data.

COMMENTS

Background

The active nuclear factor-κB (NF-κB) transcription factor promotes the expression of target genes. The majority of proteins encoded for by NF-κB target genes participate in host immune responses, proliferation, invasion, angiogenesis, and metastasis of cancer cells.

Research frontiers

The objective of the current investigation was to clarify the prognostic significance of NF-κB RelA and its target gene products in gastric cancer patients.

Innovations and breakthroughs

In the present study, NF-κB RelA expression in tumor tissues was related to increased serum levels of interleukin (IL)-6 (*P* = 0.044) and C reactive protein (CRP) (*P* = 0.010). Levels of serum IL-6, vascular endothelial growth factor (VEGF), serum amyloid A (SAA) and CRP were related to the stage. Univariate analysis demonstrated that immunostaining of NF-κB RelA, levels of IL-6, VEGF, SAA were significantly related with both disease free survival and overall survival (OS). Multivariate analysis verified that NF-κB RelA and SAA were independently associated with OS.

Applications

Increased expression of NF-κB RelA and high levels of serum SAA were associated with poor OS in gastric cancer patients.

Peer review

The authors of this manuscript examined potential prognostic value of NF-κB RelA expression in gastric cancer patients and reported an inverse relationship between RelA expression and patient survival. In addition to RelA, the contribution of several inflammatory mediators/cytokines to the patient prognosis was also investigated. The study was well-designed and the data are convincing.

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