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ORIGINAL ARTICLE

Interleukin-33 Expression does not Correlate with Survival of Gastric Cancer Patients

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Abstract The aim of the study was to investigate IL-33 expression in gastric cancer (GC) and its association with the clinical characteristics and the prognosis. IL-33 protein in tumor and corresponding adjacent tissues were detected by immunohistochemistry in 179 GC patients and clinical features plus prognostic value were analyzed via Pearson's chi-square test and Kaplan–Meier test in Cox proportional hazards model, respectively. IL-33 protein levels were significantly lower in tumor tissues than adjacent tissues (29.05% vs. 78.77%, χ^2 = 89.05, $P < 0.001$). The positive rate of IL-33 in the ulcerative type group was the lowest among all groups ($P < 0.05$). IL-33 levels were correlated with age ($P = 0.025$) and invasion depth ($P = 0.030$) while not significantly associated with the overall survival of GC patients. IL-33 expression is associated with age and invasive depth of GC patients but not an independent risk factor of prognosis.

Keywords Interleukin-33 · Gastric cancer · Clinical characteristics · Survival

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Introduction

Gastric cancer (GC) is the third common malignancy in China, and the second leading cause of cancer-related death among all cases [1]. Most patients were diagnosed in late stage, which evidently shorten overall survival (OS) of GC patients. It is crucial to find novel biomarkers for diagnosing GC and predicting prognosis.

Interleukin-1 (IL-1) family members are known to alter host response to an inflammatory, infectious, or immunological challenge [2]. ST2 is a well-recognized IL-1 receptor [3] and include two forms: a full-length transmembrane form (ST2L) and a soluble, secreted form (sST2) [4]. Interleukin-33 (IL-33) is a recently identified member of IL-1 family as a potent activator of immune system. A number of studies have demonstrated that IL-33 could promote $CD8^+$ T cell function and inhibit cell growth metastasis of tumor cells [5, 6]. In a few studies, IL-33 level in serum of gastric cancer patients was higher than that of healthy controls, which could be used as a negative predictor of prognosis. [7–10]. Recently, there have been reports that a high expression of IL-33 and its receptor ST2 are negative predictive factors for survival in GC [7, 11], hepatocellular carcinoma [11] and breast cancer [12]. In gastric cancer, the present study suggests that IL-33 is mainly expressed in glandular epithelial cells, and its expression is associated to the prognosis. Though the role of IL-33

in cancers has emerged, its expression in GC tissues has never been investigated.

The purpose of this study is to investigate the expression of IL-33 in GC and corresponding adjacent tissues, analyze the association of IL-33 and GC patients' clinicopathological characteristics and evaluate its prognostic value in Chinese population.

Materials and Methods

Tissue Microarray

Human GC high density tissue microarray (HStm-Ade180Sur-02, HStm-Ade180Sur-04) was purchased from Shanghai Biochip Company and contained primary tumor specimens and the corresponding adjacent tissues with clinical follow-up data from 180 GC patients. All patients underwent resection of GC between May 2007 and August 2008. All the patients were followed up by September 2014. None of these patients received preoperative chemotherapy or radiotherapy. All cases were confirmed pathologically. Tumor-node-metastasis (TNM) stage was classified according to the American Joint Committee on Cancer Criteria (AJCC) 7th version.

Immunohistochemistry

Immunohistochemical staining was performed using the ElivisionTM method. The antibody to IL-33 (1:450 dilution, Sigma-Aldrich, USA) was the primary antibody. The secondary antibody and diaminobenzidine (DAB) color-developing agent solution were obtained from Dako Company (Glostrup, Denmark). Tissue microarray was dewaxed in dimethylbenzene and then rehydrated in graded ethanol solution. Antigen retrieval was performed at 100 degrees centigrade for 30 min in citrate solution (10 mmol/L, pH 6.0). Then the tissue microarray was incubated for 15 min in 0.3% hydrogen peroxide solution to block endogenous peroxidase activity, followed by incubation with the polyclonal rabbit antibody against human IL-33 in a humidified chamber at 4 °C overnight. Then tissue microarray was stained with DAB, counterstained with hematoxylin. Finally, tissue microarray was dehydrated and mounted. Negative controls were done with similar procedures except for phosphate buffer solution instead of primary antibody.

IL-33 immunostaining intensities were assessed by two pathologists independently. Positive IL-33 staining mainly localized in the nuclei of epithelial cells. Tissues with medium brown staining were considered positive. Every sample in the tissue microarray with more than 50% of positive staining area was considered as positive.

Statistical Analysis

SPSS 19.0 (IBM Company, Chicago, USA) was used. Pearson's chi-square test was utilized to analyze the relationship of IL-33 with clinical characteristics. OS curves were graphed using Kaplan–Meier method and compared using log-rank test. The Cox proportional hazards model was performed to estimate hazard risk (HR) with 95% confidence intervals (CI) between various clinical characteristics and IL-33 expression, and

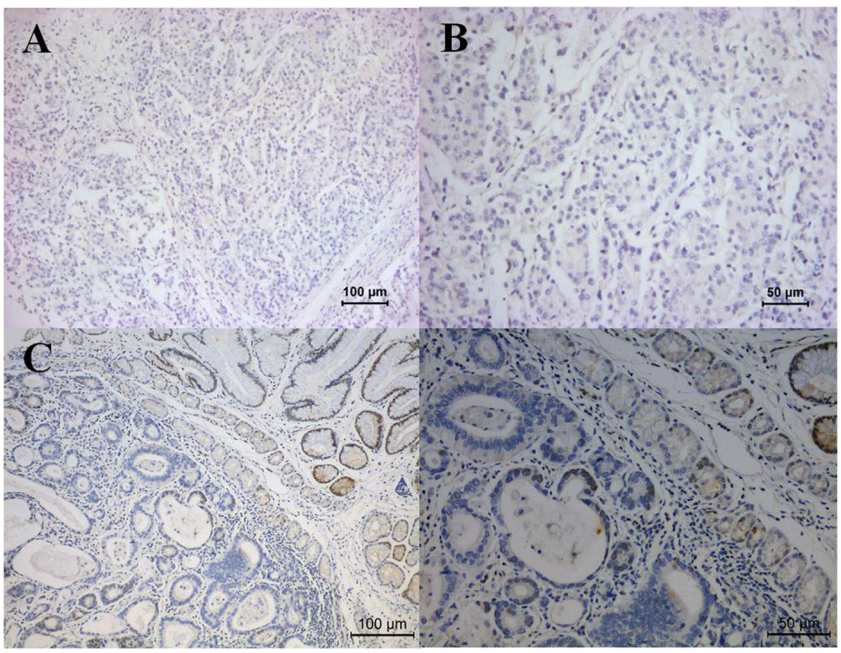

Fig. 1 Immunohistochemical staining of IL-33 in tumor tissue (**a** and **b**) and adjacent tissue (**c** and **d**)

death risks. The *P*-value of <0.05 was considered statistically significant. All statistical tests were two-sided. GraphPad Prism 6.02 (CA 92037, USA) was utilized for creating statistical graphs.

Results

IL-33 Expression in Gastric Cancer and Corresponding Adjacent Tissues

As Fig. 1 shows, no or weak IL-33 staining were observed in tumor tissues (Fig. 1a and b). IL-33 staining was predominantly observed in the nucleus of adjacent epithelial cells (Fig. 1c and d). Among the 180 cases, 1 tumor tissue was not found in 1 case thus was excluded. Among a total of 179 cases, 52 cases (29.05%) were considered IL-33 positive. A total of 141 (78.77%) in 179 cases of corresponding adjacent tissues were IL-33 positive. The difference between the positive rates was statistically significant (χ^2 = 89.05, P < 0.001).

Association of IL-33 Expression and Clinicopathological Characteristics

To investigate the relationship between IL-33 expression and clinicopathological features, we divided all cases into various subgroups according to the clinicopathological characteristics including patients' gender, age, maximum tumor diameter, pathological grading, tumor location, depth of invasion, lymphatic metastasis, distant metastasis and TNM staging.

As shown in Table 1, age, depth of invasion and pathomorphology were associated with IL-33 expression level in tumor tissues (P < 0.05). IL-33 expression was higher in subjects with age more than 60 years old, with invasive depth not reaching muscular layer and with morphology other than ulcerative type. Nevertheless, no significant associations were observed between IL-33 expression and other clinicopathological parameters (P > 0.05).

Association between IL-33 Expression and Prognosis

As shown in Table 2, the result of Cox regression univariate analysis showed that the maximum tumor diameter (P = 0.001, HR = 2.078, 95%CI 1.348–3.208), depth of invasion (P = 0.01, HR = 3.745, 95%CI 1.379–10.167), TNM staging (P < 0.001, HR = 3.221, 95%CI 2.106–4.928) were negative prognostic factors for OS,but IL-33 expression was not associated to overall survival time in patients with gastric cancer (P = 0.543, HR = 0.802, 95% CI 0.531–1.211). Multivariate analysis showed that the maximum tumor diameter (P = 0.046, HR = 1.631, 95%CI 1.009–2.634) and TNM staging (P = 0.001, HR = 3.091, 95% CI 1.902–5.024) were negatively associated with OS. And the multivariate analysis also showed that IL-33

Table 1 Association between IL-33 expression and clinicopathological parameters

Characteristic	Negative	Positive	Total	χ^2	*P*
Gender				0.000	0.983
Female	34	14	48		
Male	93	38	131		
Age				5.029	0.025
< 60	49	11	60		
≥ 60	78	41	119		
Maximum tumor diameter				0.018	0.892
< 5	43	17	60		
≥ 5	82	34	116		
Pathological grade				1.784	0.182
I-II	25	15	40		
III-IV	102	37	139		
Tumor location				2.386	0.496
Cardia	18	10	28		
Antrum	55	25	80		
Body of stomach	28	10	38		
Angle of stomach	12	2	14		
Invasion depth				4.684	0.030
Not reached muscular layer	7	8	15		
Reached muscular layer	120	44	164		
Morphology				7.239	0.007
Other types	18	17	35		
Ulcerative type	101	34	135		
Lymph node metastasis				1.290	0.256
No	31	17	48		
Yes	96	35	131		
Distant metastasis				0.299	0.584
No	124	50	174		
Yes	3	2	5		
AJCC TNM stage-1				2.467	0.481
I	7	6	13		
II	42	17	59		
III	75	27	102		
IV	3	2	5		
AJCC TNM stage-2				0.489	0.484
I-II	49	23	72		
III-IV	78	29	107		

Data of 4 for maximum tumor diameter, 1 for pathological grading, 20 for tumor location, 1 for invasion depth, 10 for morphology, 1 for lymph node metastasis, 1 for distant metastasis and 1 for AJCC TNM staging were not obtained

Table 2 Univariate and multivariate analysis of clinicopathological parameters for overall survival

Clinicopathological characteristics	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Gender (male vs. female)	0.839 (0.560 ~ 1.256)	0.393	0.817 (0.528 ~ 1.265)	0.365
Age (≥ 60 yrs. vs. < 60 yrs)	1.241 (0.837 ~ 1.839)	0.283	1.455 (0.942 ~ 2.248)	0.091
Tumor diameter (≥ 5 cm vs. < 5 cm)	2.078 (1.348 ~ 3.208)	0.001	1.631 (1.009 ~ 2.634)	0.046
Pathological grading (grade III/IV vs. grade I/II)	1.416 (0.883 ~ 2.272)	0.149	1.020 (0.618 ~ 1.682)	0.938
Invasion to muscular layer (yes vs. no)	3.745 (1.379 ~ 10.167)	0.010	1.480 (0.515 ~ 4.249)	0.467
Morphology (Ulcerative vs. other)	1.088 (0.682 ~ 1.736)	0.723	0.827 (0.506 ~ 1.351)	0.449
AJCC TNM stage (III/IV vs. I/II)	3.221 (2.106 ~ 4.928)	<0.001	3.091 (1.902 ~ 5.024)	<0.001
IL-33 expression (positive vs. negative)	0.802 (0.531 ~ 1.211)	0.543	0.761 (0.485 ~ 1.193)	0.203

expression was not a prognostic factors and predictor for OS (P = 0.203, HR = 0.761, 95% CI 0.485–1.193).

Discussion

The evidence regarding the role of IL-33 signaling in cancers is limited. Brunner et al. reported that the tumor-infiltrating, IL-33–producing effector-memory CD8$^+$ T cells could prolong patients' survival in resected hepatocellular carcinoma [13]. Deng et al. found that tristetraprolin can inhibit GC progression through suppression of IL-33 [14]. Bergis et al. suggested that high plasma sST2 in GC was significantly associated with a more advanced tumor stage, metastatic disease and significantly associated with the duration of the disease [15]. Yu et al. reported that IL-33 could promote GC cell invasion and migration via ST2-ERK1/2 pathway [8]. Ye et al. considered that IL-33 protected against platinum-induced apoptosis and promoted cell invasion via activation of the JNK pathway in GC cells [16]. Bie et al. found that the polarization of ILC2s in peripheral blood might contribute to immunosuppressive microenvironment. The immunosuppressive effect of ILC2s may be achieved by the increase of IL-33 mRNA [9].

In opposite, a series of studies suggest that IL-33 can enhance antitumor immunity. Gao et al. confirmed that tumoral expression of IL-33 inhibits tumor growth and modifies the tumor microenvironment through CD8$^+$ T and NK cells [6]. These researches suggested that IL-33 can promote the activation and proliferation of CD8$^+$ T cells, and thus played an anti-tumor role. These results suggest that the role of IL-33 in tumors is complicated.

Here, we found that he positive expression rate of IL-33 in tumor tissues was significantly lower than that in corresponding adjacent tissues, and the expression rate of IL-33 in tumor tissues was associated to the age, the depth of tumor invasion and the morphology of the tumor in GC patients. It has been reported that patients have a well or moderately differentiated histology [17]. The result of our study suggested a high expression level of IL-33 in the elderly and it may be associated with higher degree of pathological differentiation. IL-33 expression was associated to the depth of tumor invasion and the morphology of the tumor, suggesting that IL-33 is involved in the process of inflammatory reaction in the development of GC. IL-33 plays an important role in the local inflammatory response caused by GC. But we did not find that IL-33 expression was significantly associated with prognosis. These findings suggested that the role of IL-33 in GC is complicated. Moreover, the potential mechanism of IL-33 in GC tumorigenesis and metastasis warrants progressive investigation.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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