

Prognostic significance of IL-17 mRNA expression in peritoneal lavage in gastric cancer patients who underwent curative resection

TAKESHI IIDA¹, MAKOTO IWAHASHI¹, MASAHIRO KATSUDA¹, KOICHIRO ISHIDA¹,
MIKIHITO NAKAMORI¹, MASAKI NAKAMURA¹, TEIJI NAKA¹, TOSHIYASU OJIMA¹,
KENTARO UEDA¹, KEIJI HAYATA¹, HIRONAO YASUOKA² and HIROKI YAMAUE¹

¹Second Department of Surgery, School of Medicine, and ²Division of Pathology, Department of Clinical Laboratory Medicine, School of Medicine, Wakayama Medical University, Wakayama 641-8510, Japan

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Abstract. Peritoneal dissemination is frequently detected in patients with advanced gastric cancer. The peritoneal cavity is a compartment in which an immunologic host-tumor interaction can occur. There are no reports on the relationship between IL-17 expression in peritoneal lavage and prognosis in gastric cancer patients. Therefore, we investigated the expression of IL-17 mRNA in peritoneal lavage from gastric cancer patients and assessed the association of its expression with clinicopathological parameters and prognosis. Peritoneal lavage was obtained from 114 patients with gastric cancer at initial surgery. Seventy-nine patients underwent curative resection. Among these 79 patients, IL-17 mRNA expression was associated with the depth of tumor invasion ($P<0.05$). Twelve of the 79 patients who underwent curative resection died, and 9 of those 12 developed peritoneal metastasis. Notably, among the 79 patients who underwent curative resection, those with high expression of IL-17 mRNA in peritoneal lavage had significantly prolonged survival when compared to these patients with low expression of IL-17 mRNA in peritoneal lavage ($P<0.05$) as evidence by the survival curves. In a multivariate analysis, low expression of IL-17 mRNA in peritoneal lavage and tumor size were found to be independent significant predictive factors for prognosis (HR, 7.91; 95% CI, 1.65-38.03) in the patients who underwent curative resection. IL-17 mRNA expression in peritoneal lavage is a reliable prognostic factor for patients undergoing curative resection for gastric cancer. Low IL-17 expression in the peritoneal cavity may correlate with cancer development in the peritoneal cavity in patients with gastric cancer.

Introduction

Although the prognosis of gastric cancer has improved with the development of early diagnosis and new therapeutic strategies, it remains one of the main causes of cancer-related mortality worldwide (1), with peritoneal carcinomatosis, often associated with malignant ascites, being the most frequent cause of death in patients with advanced gastric cancer. Peritoneal dissemination is considered to arise from free cancer cells in the peritoneal cavity exfoliated from the serosal surface of the stomach penetrated by the primary tumor (2). Therefore, cytologic examination of peritoneal washes has been performed at laparotomy to detect free cancer cells in patients with advanced gastric cancer, and it is recognized as one of the most important prognostic factors (3-7). Since 1999, it has been reported that the presence of free cancer cells in the peritoneal cavity should be considered as an independent prognostic factor in patients with gastric cancer by the Japanese Gastric Cancer Association. Moreover, in the International Union Against Cancer's TNM classification 7th edition for gastric cancer, positive peritoneal cytology is defined as stage IV.

Therefore, detection of free cancer cells in peritoneal washes is a standard method for the assessment of risk for peritoneal carcinomatosis. However, patients who are diagnosed as having no free cancer cells in the peritoneal cavity by conventional cytology sometimes develop peritoneal recurrence after curative resection. This may occur since cytology is considered to have low sensitivity. In fact, recently, real-time RT-PCR techniques have been developed in order to increase the sensitivity of conventional peritoneal lavage cytology (8,9). Concerning another cause for the development of recurrent peritoneal disease, the cytokine network may play an important role in the immunosuppressive and immunostimulatory properties of cancer-related ascites fluid (10,11). However, the role of cytokines produced by cells in the peritoneal cavity on tumor growth in gastric cancer patients is still unclear.

Th17 cells have recently been identified as having a distinct Th cell lineage and were found in an experimental animal model of cancer and in human cancers. Th17 cells have been found in several types of human cancers, such as ovarian, prostate, colorectal, and other malignancies, as well as gastric

Correspondence to: Dr Makoto Iwahashi, Second Department of Surgery, School of Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8510, Japan
E-mail: makoto@wakayama-med.ac.jp

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cancer (12-17). Research has found that IL-17 promotes tumor growth through angiogenesis and inflammation (18), and several other studies have demonstrated that IL-17 contributes to reduced tumor growth by promoting dendritic cell, cytotoxic T cell (CTL), and NK cell trafficking to, and retention within, the tumor microenvironment (19,20). Therefore, whether these cells promote tumor growth or regulate antitumor responses remains controversial. In the case of human gastric cancer, there are several reports concerning the prevalence of Th17 cells in the tumor microenvironment, tumor-draining lymph nodes and peripheral blood (12,13). In our previous study, the expression level of IL-17 mRNA in gastric tumors was associated with the depth of tumor invasion, lymphovascular invasion and lymph node involvement suggesting that IL-17 is clearly associated with tumor progression (21). However, since no previous studies have been carried out concerning the proportion of IL-17 in the peritoneal cavity in human gastric cancer patients, we hypothesized that the expression level of IL-17 mRNA in peritoneal lavage may be involved in the development of peritoneal carcinomatosis in gastric cancer.

In the present study, we quantitatively investigated expression of IL-17 messenger RNA (mRNA) in the peritoneal lavage of gastric cancer patients who underwent curative resection. The association of IL-17 expression levels with clinicopathological factors and prognosis was also assessed. Since IL-2 and IL-12 have been used for intraperitoneal immunotherapy in patients with various types of cancers, including gastric cancer (22-25), the possibility of IL-17 as a therapeutic target for patients with gastric cancer was also investigated.

Materials and methods

Patients. Included in the present study was a series of 114 patients (80 men and 34 women) with gastric cancer who underwent surgery at Wakayama Medical University Hospital (WMUH) from 2003 to 2006. At the beginning of the operation, we examined tumor metastases in the abdominal cavity. When it was thought that curative resection was possible, we performed gastrectomy with lymphadenectomy. We performed gastrectomy in 114 patients with gastric cancer. Seventy-nine patients underwent surgically curative resection and 35 underwent non-curative resection. None of the patients received anticancer therapy prior to surgery. Individuals with autoimmune disease, inflammatory bowel disease or viral infections were excluded. The clinicopathological characteristics of the 114 patients are summarized in Table IA. Clinical stages of the tumors were determined according to the International Union Against Cancer's TNM classification for gastric cancer. After surgery, all patients underwent a follow-up, with the median follow-up at analysis being 61 months (range, 1.3-98.5) for all patients. Every 3 to 6 months, physical examination, blood chemistry, including carcinoembryonic antigen (CEA) and cancer antigen (CA) 19-9, and computed tomography were performed for each patient. Written informed consent was obtained from all patients before participation in the present study. In addition, the local ethics committee of WMUH approved the study.

Preoperative peritoneal wash examination. At the beginning of each operation, 100 ml saline was introduced into the

Douglas cavity and aspirated by gentle stirring. These washes were centrifuged at 1,800 rpm for 5 min to collect intact cells. A part of each peritoneal wash was examined cytopathologically after conventional Papanicolaou staining.

RNA extraction and DNA synthesis. Total RNA was extracted using an RNeasy Mini kit (Qiagen, Hilden, Germany) followed by RNase-Free DNase Set treatment (Qiagen). Complementary DNA was synthesized from 1 μ g of total RNA using a reverse transcription system (Promega, Madison, WI, USA) according to the manufacturer's instructions. Samples were stored at -80°C until use.

Quantitative real-time RT-PCR. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed with isolated total RNA (1 μ g) on the LightCycler system (Roche Molecular Biochemicals, Mannheim, Germany). The following oligonucleotide primers and hybridization probes were used: human IL-17 (GenBank accession no. NM 002190; 53-231 bp): sense, 5'-CTGGGAAGACCTCATTTGG-3'; antisense, 5'-CCTTTTGGGATTGGTATTGG-3'; fluorescein-labeled probe, 5'-TCCTCAGAATTGGGCATCCTGGATTTC-3'; and LC Red 640-labeled probe, 5'-TGGGATTGTGATTCTCCTGCCTTCACTATGG-3'; human glyceraldehyde 3-phosphate dehydrogenase (GAPDH; GenBank accession no. NM 002046; 746-1052 bp): sense, 5'-TGAACGGGAAGCTCACTGG-3'; antisense, 5'-TCCACCACCTGTTGCTGTA-3'; fluorescein-labeled probe, 5'-TCAACAGCGACACCCACTCCT-3'; and LC Red 640-labeled probe, 5'-CACCTTTGACGCTGGGGCT-3'. Primers and probes were designed by Nihon Gene Research Laboratories, Inc. (Miyagi, Japan). After 10 min of initial denaturation at 95°C, the cycling protocol entailed 40 cycles of denaturation at 95°C (10 sec), annealing at 62°C (15 sec) and elongation at 72°C (8 sec). For GAPDH, the thermocycling protocol was the same, except that annealing was performed at 55°C (15 sec) and 50 cycles were run. On each run, all samples were quantified according to the LightCycler software program, version 3.8 (Roche Molecular Biochemicals). The levels of mRNA for IL-17 were corrected with GAPDH house-keeping control amplifications. We used the following for quantitative RT-PCR analysis: IL-17 ratio = IL-17 value/GAPDH value $\times 10^4$.

Determination of the cut-off value. The cut-off value of the IL-17 mRNA ratio was determined as the median value based on the quantified values of 114 samples in the present study. The cut-off value was 1.22.

Immunohistochemistry and quantitative microscopy. Sections (4 μ m) were prepared from paraffin-embedded blocks derived from gastric tumors. Sections were deparaffinized in xylene and graded alcohols, and rinsed in phosphate-buffered saline. Antigen retrieval from the tissues was carried out by autoclaving the tissues in 0.01 M citrate buffer (pH 6.0) at 100°C for 10 min. The antibody used was goat anti-IL-17 (dilution at 10 μ g/ml; R&D Systems, Minneapolis, MN, USA). The antibodies were incubated overnight at 4°C. The immunocomplex was visualized by a polymer envision method, EnVision™ + Kit (DakoCytomation, Glostrup, Denmark). For quantification

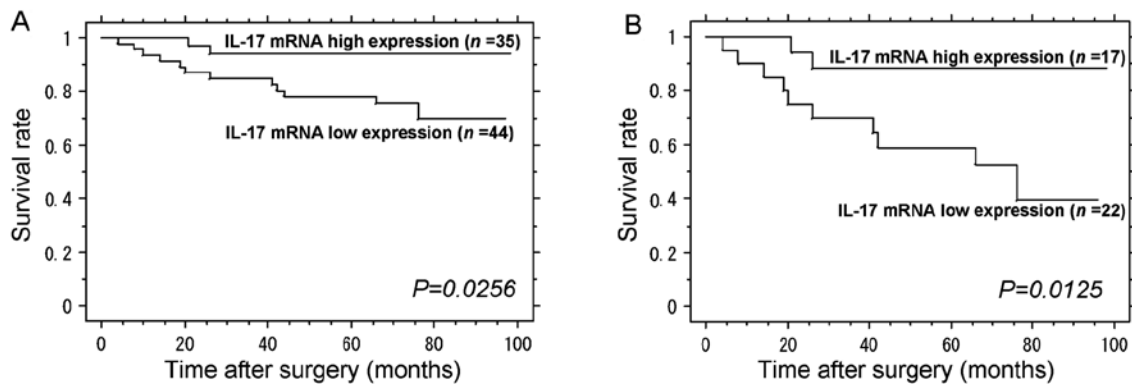


Figure 1. (A) Overall survival curves of the 79 gastric cancer patients who underwent curative R0 resection stratified according to IL-17 mRNA high or low expression in peritoneal lavage as determined by real-time RT-PCR. A significant difference in survival was noted between the IL-17 mRNA high expression and IL-17 mRNA low expression groups ($P=0.0256$; log-rank test). (B) The overall survival of the 79 patients who underwent curative resection with clinical stage II/III tumors according to IL-17 mRNA high or low expression in peritoneal lavage as determined by real-time RT-PCR. The IL-17 mRNA low expression group had a significantly poorer prognosis than the high expression group among the clinical stage II/III patients ($P=0.0125$; log-rank test).

of IL-17-positive cells, highly positive areas were initially identified by scanning tumor sections using light microscopy. Data were obtained by manually counting positively stained cells in five separate areas of intratumoral regions under x400 high power magnification. The regions were counted by a pathologist who had no knowledge of the other clinicopathological features and survival outcomes.

Flow cytometry. For intracellular molecule measurements, cells were stimulated with PMA (10 ng/ml) and ionomycin (500 ng/ml) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) for 5 h in the presence of GolgiPlug (BD Biosciences, San Diego, CA, USA). Then, the cells were harvested and stained with PerCP-Cy5.5-conjugated anti-CD3 mAb, FITC-conjugated anti-CD4 mAb, FITC-conjugated anti-CD8 mAb and FITC-conjugated anti- $\gamma\delta$ TCR mAb (BD Biosciences) for 30 min on ice. For intracellular staining, after fixation and permeabilization using BD Cytofix/Cytoperm (BD Biosciences), the cells were stained with PE-conjugated anti-IL-17 mAb (BD Biosciences) for 30 min at 4°C. After washing, the cells were analyzed by FACSCalibur, using CellQuest (BD Biosciences).

Statistical analysis. The Mann-Whitney test and the Kruskal-Wallis test were used to determine statistical significance between the covariates. The Cox proportional hazards model was used to compute the univariate and multivariate hazards ratios for the study parameters. Survival curves were computed using the Kaplan-Meier method and compared by means of the log-rank test. The survival curve was calculated from the date of surgery. In Fig. 3, we used the Spearman rank correlation coefficient. All statistical analyses were performed with StatView 6.0 (Abacus Concepts, Inc., Berkeley, CA, USA) statistical software program. A value of $P<0.05$ was considered to indicate a statistically significant result.

Results

Relationship between IL-17 mRNA and clinicopathological factors. To evaluate the biological significance of IL-17

expression in peritoneal lavage from patients with gastric cancer, the association between mRNA expression levels of IL-17 and clinicopathological factors was investigated. In all patients, the IL-17 mRNA expression level in the peritoneal lavage increased according to the depth of tumor invasion and peritoneal metastases, while the expression level was not associated with cytologic examination (Table IA). In the patients who underwent curative resection, IL-17 mRNA expression levels in peritoneal lavage increased according to the depth of tumor invasion (Table IB). On the other hand, no significant association was recognized between the expression level of IL-17 mRNA and histological type, lymph node metastases, lymphatic invasion, vessel invasion, clinical stage or tumor size.

Correlation between patient survival and IL-17 mRNA expression in peritoneal lavage. Kaplan-Meier survival curves indicated the overall survival of gastric carcinoma patients stratified according to the results of the IL-17 mRNA expression status in peritoneal lavage. The survival curves of all 114 patients displayed no significant difference between the IL-17 mRNA low expression group and the IL-17 mRNA high expression group (data were not shown). Importantly, however, based on the survival curves, among the 79 patients who underwent curative R0 resection, the patients in the IL-17 mRNA low expression group ($n=44$) had a significantly poorer prognosis when compared with the patients in the IL-17 mRNA high expression group ($n=35$) (Fig. 1A; $P<0.05$). During the median 61 months of postoperative surveillance, 14 (17.8%) of the 79 patients who underwent curative resection died, and 12 (85.7%) of these 14 patients developed peritoneal metastasis. Regarding peritoneal recurrence, 10 (22.7%) of the 44 cases in the IL-17 mRNA low expression group developed peritoneal metastases, while 2 (5.7%) of the 35 cases in the IL-17 mRNA high expression group developed peritoneal metastases.

Correlation between survival and IL-17 mRNA in peritoneal lavage in advanced gastric cancer. Among the patients who underwent curative resection with clinical stage II/III tumors, the prognosis of the IL-17 mRNA low group was significantly

Table I. Clinicopathological data and IL-17 mRNA expression of the 114 patients and the 79 patients who underwent curative resection.

A, Data of the 114 patients			
Factor	No. of patients	Expression of IL-17 mRNA ^a	P-value
Age (years)			
≤65	50	2.11±0.648	0.448
>65	64	1.57±0.399	
Gender			
Male	80	1.75±0.415	0.718
Female	34	1.93±0.724	
Depth of tumor invasion			
T1	30	1.00±0.523	0.023 ^c
T2	13	1.49±0.130	
T3	31	2.44±0.809	
T4	40	2.51±0.582	
Lymph node metastasis			
N0	43	2.25±0.681	0.477
N1	28	1.26±0.581	
N2	21	1.61±0.708	
N3	22	1.84±0.894	
Histological type			
Differentiated	54	2.08±0.601	0.387
Undifferentiated	60	1.55±0.423	
Lymphatic invasion			
Negative	37	1.90±0.659	0.338
Positive	77	1.76±0.432	
Vessel invasion			
Negative	57	1.83±0.544	0.292
Positive	57	1.78±0.479	
Peritoneal metastasis			
Negative	100	2.07±0.439	0.031 ^d
Positive	14	7.21±0.290	
Cytologic examination			
Negative	91	1.22±0.591	0.801
Positive	23	1.89±0.404	
Stage ^b			
I	40	1.95±0.660	0.412
II	18	2.29±0.109	
III	21	1.38±0.784	
IV	33	1.64±0.531	
Tumor size (cm)			
≤5	60	1.75±0.516	0.059
>5	54	1.87±0.506	

poorer than that of the patients in the IL-17 mRNA high group (Fig. 1B; $P<0.05$). From the point of view of the depth of the invasion, patients in the IL-17 mRNA low group had significantly poorer outcome than those in the IL-17 mRNA high

Table I. Continued.

B, Data of the 79 patients who underwent curative resection			
Factor	No. of patients	Expression of IL-17 mRNA ^a	P-value
Age (years)			
≤65	35	2.01±0.781	0.329
>65	44	1.72±0.527	
Gender			
Male	58	1.80±0.517	0.975
Female	21	1.93±0.724	
Depth of tumor invasion			
T1	30	1.00±0.523	0.020 ^c
T2	13	1.49±0.130	
T3	23	2.39±0.927	
T4	13	2.90±0.124	
Lymph node metastasis			
N0	43	2.25±0.681	0.307
N1	20	1.48±0.700	
N2	10	1.90±0.106	
N3	6	3.26±0.284	
Histological type			
Differentiated	41	2.31±0.719	0.613
Undifferentiated	38	1.33±0.524	
Lymphatic invasion			
Negative	34	1.72±0.690	0.118
Positive	45	1.95±0.610	
Vessel invasion			
Negative	49	1.72±0.597	0.087
Positive	30	2.05±0.708	
Stage ^b			
I	40	1.95±0.660	0.497
II	18	2.29±0.109	
III	21	1.44±0.819	
Tumor size (cm)			
≤5	54	1.62±0.509	0.097
>5	25	2.36±0.943	

^aExpression of mRNA for IL-17 was corrected with GAPDH house-keeping control amplifications. Values represent mean ± SEM. ^bStage according to the TNM classification for gastric cancer (UICC). ^cP-value of Kruskal-Wallis test as appropriate. ^dP-value of Mann-Whitney test as appropriate.

group for patients in the T2/3/4 subgroups (Fig. 2A; $P<0.05$). In the T4 subgroup, patients with IL-17 mRNA low expression in peritoneal lavage had a significantly poorer survival than those with IL-17 mRNA high expression (Fig. 2B; $P<0.05$).

Preoperative peritoneal wash assay as an independent prognostic factor. We evaluated prognostic factors in the 79 patients who underwent curative R0 resection. With the overall

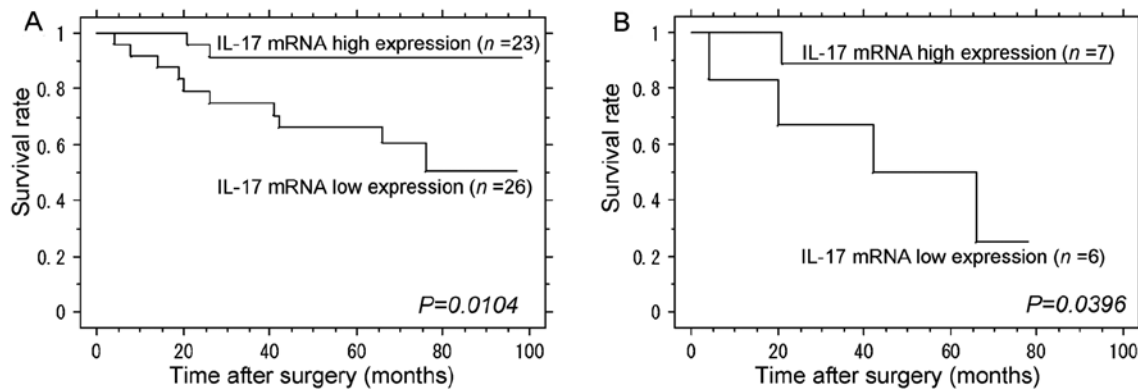


Figure 2. Overall survival curves of the 79 gastric cancer patients who underwent curative resection with pT2/3/4 stage tumors stratified according to IL-17 mRNA high or low expression in peritoneal lavage. (A) Overall survival of the 79 patients subdivided according to IL-17 mRNA high or low expression in peritoneal lavage with pT2/3/4 stage tumors. The IL-17 mRNA low expression group had a significantly poorer prognosis than the high expression group in the combined pT2/3/4 subgroup ($P=0.0104$; log-rank test). (B) Overall survival of the 79 patients with pT4 stage tumors subdivided according to IL-17 mRNA high or low expression in peritoneal lavage. The IL-17 mRNA low expression group had a significantly poorer prognosis than the high expression group among the pT4 patients ($P=0.0396$; log-rank test).

Table II. Univariate and multivariate analysis of the overall survival for the 79 patients who underwent R0 curative resection.

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Age (years)						
≤65 vs. >65	1.62	0.542-4.840	0.388	-	-	-
Gender						
Male vs. female	1.07	0.335-3.438	0.906	-	-	-
Lymph node metastasis						
Negative vs. positive	9.98	1.296-76.95	0.027	2.94	0.175-49.39	0.454
Serosal invasion						
Negative vs. positive	9.04	2.014-40.53	0.004	1.56	0.215-11.35	0.659
Lymphatic invasion						
Negative vs. positive	9.98	1.296-76.95	0.027	1.05	0.046-23.79	0.976
Vessel invasion						
Negative vs. positive	10.38	2.320-46.42	0.0022	4.16	0.459-37.68	0.205
Histological type						
Differentiated vs. undifferentiated	2.16	0.720-6.497	0.169	-	-	-
Tumor size (cm)						
≤5 vs. >5	7.32	2.289-23.40	0.0008	4.61	1.19-17.78	0.027
IL-17 mRNA expression						
Low expression vs. high expression	4.69	1.049-20.99	0.043	7.91	1.65-38.03	0.0098

CI, confidence interval.

survival as an endpoint, lymph node metastasis, serosal invasion, lymphatic invasion, vessel invasion, tumor size and IL-17 mRNA expression were found to be significant as prognostic factors by univariate analysis. Moreover, when multivariate analysis was performed with these six covariates and the same endpoint, IL-17 mRNA low expression in peritoneal lavage and tumor size were found to be independent significant predictive

factors for prognosis (Table II; HR, 7.91; 95% CI, 1.65-38.03; $P=0.0098$).

Correlation between IL-17-positive cells in primary tumor tissues and IL-17 mRNA expression in peritoneal lavage. To examine the correlation in IL-17 production between the level in peritoneal lavage and in the primary tumor tissues,

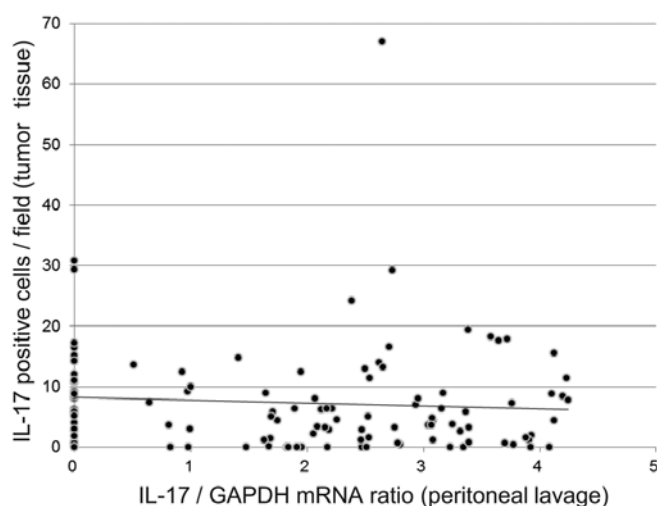


Figure 3. Correlation between IL-17-positive cells in tumor tissues and IL-17 mRNA expression in peritoneal wash. Immunohistochemical staining for IL-17 in primary tumor tissues was performed in the same patient samples whose peritoneal lavage was examined for IL-17 mRNA. The longitudinal axis shows IL-17-positive cells per field in the tumor tissues. The horizontal axis shows the IL-17/GAPDH mRNA ratio in peritoneal lavage.

we performed immunohistochemical staining for IL-17 in the primary tumors in the same patient samples. In the primary tumor tissues, IL-17 immunoreactive cells were detected in the cytoplasm of mononuclear cells; however, none of the tumor cells were stained for IL-17. IL-17-producing cells in the tumor tissues were 7.30 ± 0.82 (mean \pm SE) per field. There was no correlation between the number of IL-17-positive cells in the tumor tissues and IL-17 mRNA expression in the peritoneal wash ($r=0.092$; $P=0.329$) (Fig. 3).

Analysis of IL-17-producing cells in peritoneal lavage. Immunohistochemical staining of peritoneal wash revealed

that mononuclear cells were stained for IL-17. However, neither tumor cells nor mesothelial cells were stained for IL-17. To identify which mononuclear cells produced IL-17 in the peritoneal lavage, we performed flow cytometric analysis using anti-IL-17, -CD3, -CD4, -CD8 and $\gamma\delta$ TCR antibodies. CD3⁺ T cells produced IL-17, while $\gamma\delta$ T cells did not produce IL-17 (Fig. 4A). CD4⁺ T cells mainly produced IL-17, and a small population of CD8⁺ T cells also produced IL-17. The mean percentage of IL-17-positive CD8⁺ T cells among the total IL-17-positive cell population was only $27.6 \pm 4.85\%$ ($n=5$), and in contrast, IL-17-positive CD4⁺ T cell population was $72.2 \pm 4.86\%$ ($n=5$). Representative flow cytometry analysis is shown in Fig. 4B.

Discussion

In the present study, we demonstrated that in patients who underwent R0 resection, the prognosis of patients in the IL-17 mRNA low expression group was significantly poorer than those in the high expression group. This is the first study evaluating the prognostic value of IL-17 detection by real-time RT-PCR in peritoneal lavage as a valuable prognostic factor in gastric cancer.

IL-17 was originally identified as a proinflammatory cytokine that induces neutrophils, and previous studies also have shown that inflammation is linked to cancer development and progression. It has recently been reported that the levels of IL-17-producing cells are significantly increased in tumor tissues, peripheral blood, malignant ascites fluid, and malignant pleural effusion from a variety of cancer patients (13,14,26-28). Despite recent advances in our understanding of the function of Th17 cells in humans, very little is known about their prevalence and tumor immunosurveillance.

In mice, overexpression of IL-17 by gene transduction into tumor cells promoted tumor growth through angiogenesis (18), but seemingly in contrast, IL-17 also suppressed tumor growth

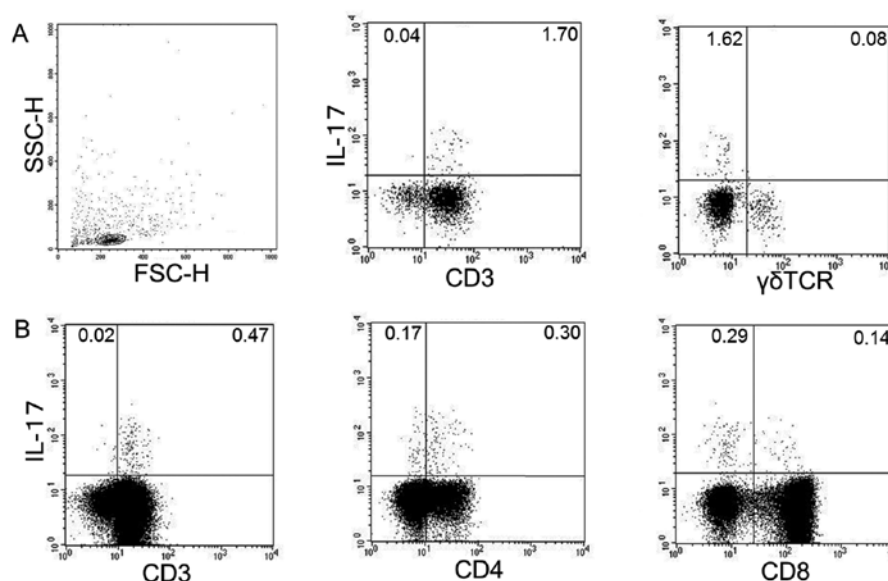


Figure 4. Flow cytometric analysis using anti-IL-17, -CD3, -CD4, -CD8 and $\gamma\delta$ TCR antibodies to identify IL-17-producing cells in peritoneal washes. (A) Cells were stained with PE-anti-IL-17 mAb, PerCP-anti-CD3 mAb and FITC-anti- $\gamma\delta$ TCR mAb after stimulation with PMA and ionomycin for 5 h. (B) Cells were stained with PE-anti-IL-17 mAb, PerCP-anti-CD3 mAb, FITC-anti-CD4 mAb and FITC-anti-CD8 mAb after stimulation with PMA and ionomycin for 5 h. Representative flow cytometry plots using peritoneal washes from subjects with cytology class II.

via a T-cell-dependent mechanism (19). Whether IL-17 promotes tumor growth or regulates antitumor responses remains controversial.

In humans, there are several reports concerning intratumoral expression of IL-17 and its prognostic role in several cancer types such as hepatocellular carcinoma (HCC), colon, esophageal and gastric cancer. In HCC, colon, and prostate cancer patients, intratumoral IL-17-positive cells were found to be correlated with poor survival (17,29,30). Our previous study showed that Th17 cells infiltrated the tumor and secreted IL-17 in the tumor microenvironment, leading to tumor progression through angiogenesis and neutrophil infiltration in patients with gastric cancer. In the present study, we hypothesized that IL-17 promotes tumor progression in the peritoneal cavity, based on our previous study suggesting that IL-17 is related to tumor progression in the tumor microenvironment. We quantitatively analyzed the expression levels of IL-17 mRNA in peritoneal lavage from gastric cancer patients. Based on the survival curves, among the 79 patients who underwent R0 resection, the patients in the IL-17 mRNA low expression group had a significantly poorer prognosis than the patients in the IL-17 mRNA high expression group. This result was contradictory to our hypothesis. This discrepancy may be explained by the difference in the impact of IL-17 on tumor progression in the thoracoabdominal cavity and in tumor tissue. In fact, in the present study, there were no correlations noted between primary tumor tissues and peritoneal wash in terms of IL-17 expression. In lung cancer, Ye *et al* (26) reported that patients with a higher proportion of Th17 cells in malignant pleural effusion exhibited significantly longer overall survival than patients with a lower proportion of Th17 cells. Similarly, in ovarian cancer, the expression of IL-17 in ascites was analyzed, and patients with a higher IL-17 expression in ascites had a significantly lower death hazard than those with a lower IL-17 expression (28).

Most recently, it has been reported that CD8⁺ T cells that produce IL-17 (Tc17 cells) are abundant in gastric cancer tissue, and the percentage of Th17 cells is relatively lower than that of Tc17 cells in tumors. The intratumoral Tc17 cell percentage was significantly associated with tumor progression and poor prognosis (31). In the present study, flow cytometric analysis showed that CD4⁺ Th17 cells predominantly produced IL-17 in the peritoneal lavage; however, the percentage of Tc17 cells was lower than that of Th17. This suggests that IL-17-producing T cells are different between tumor tissue and the abdominal cavity, and the potential role of IL-17 could also be different in the tumor microenvironment between tumor tissue and the abdominal cavity.

There is another reason why the results of the present study were in conflict with our expectations. This may be because the role of IL-17 is different before and after the tumor is established. In the present study, the expression levels of IL-17 were significantly higher in peritoneal carcinomatosis-positive cases than those of negative cases, while they were not associated with cytologic examination (Table IA). Once tumor cells attach to the peritoneum, IL-17 may play a role as a tumor growth cytokine through angiogenesis to a greater extent than its role in regulating antitumor responses. Our results suggest that endogenous IL-17 plays different roles before tumor attachment versus in established tumor growth. Furthermore, in the

abdominal cavity, previous studies indicate that peritoneal mesothelial cells secrete various cytokines and growth factors, such as IL-6, IL-8, IL-1 α and β , granulocyte colony stimulating factor (G-CSF), as well as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)-2. These results indicate that peritoneal mesothelial cells are one of the central elements of the cytokine network controlling disease processes in the abdominal cavity. Kryczek *et al* suggested that, in the peritoneal cavity, IL-17 is positively associated with INF- γ effector T cells and Th1-type chemokines, CXCL9 and CXCL10, but not with Th2-type chemokines, CXCL12 and CCL22, in ovarian cancer ascites. Mechanistically, Th17 cell-derived IL-17 and INF- γ were found to synergistically induce the production of CXCL9 and CXCL10 and in turn promote effector T-cell migration (28). Thus, IL-17 may function as a polyfunctional cytokine profile in human tumors. There is no doubt that the role of IL-17 is highly complicated, and it remains controversial whether IL-17 promotes tumor growth or regulates the antitumor response.

In conclusion, IL-17 mRNA expression in peritoneal lavage detected by real-time RT-PCR is a reliable prognostic factor for patients with curative resection in gastric cancer. Low IL-17 gene expression in the peritoneal cavity may correlate with cancer development in the peritoneal cavity and poor prognosis in patients with gastric cancer.

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