Int J Clin Exp Pathol 2015;8(6):7002-7008
www.ijcep.com /ISSN:1936-2625/IJCEP0008681

*Original Article*

# Increased chemokine receptor IL-17RA expression is associated with poor survival in gastric cancer patients

Yu-Xing Jiang*, Ping-Ang Li*, Shi-Wei Yang, Ying-Xue Hao, Pei-Wu Yu

*Department of General Surgery and Center of Minimal Invasive Gastrointestinal Surgery, Southwest Hospital, Third Military Medical University, Chongqing, China. *Equal contributors.*

Received April 1, 2015; Accepted May 25, 2015; Epub June 1, 2015; Published June 15, 2015

**Abstract:** Background: Previous researchers have identified that the chemokine interleukin-17 (IL-17) was associated with survival time of patients with gastric cancer, but the roles of its receptors (IL-17R) in gastric cancer remain unknown. Our studies were designed to clarify the function of IL-17RA and to explore their potential role in gastric cancer. Materials and methods: The expression of IL-17RA was determined in primary gastric cancer tissues (n=101) using Real-time RT-PCR, immunohistochemistry, and western blotting. To investigate the functional significance of IL-17RA expression, IL-17RA expression and clinical parameters, multivariate survival was analyzed in patients with gastric cancer. Results: IL-17RA was overexpression in gastric cancer tissues compared with adjacent normal tissues ($P$<0.05). The elevated expression level of IL-17RA was observed correlated significantly with tumor progression ($P$=0.003), Lymphatic invasion ($P$=0.019), lymphoid nodal status ($P$=0.001), distant metastasis ($P$<0.001) of gastric cancer patients, TNM stage ($P$=0.0013) and was one of the independent prognostic factors for patient's overall survival. Conclusions: These results demonstrated that the expression of IL-17RA plays an important role in gastric cancer progression, migration and prognosis of gastric cancer. The IL-17-IL-17RA signaling mechanism may be a potential novel target.

**Keywords:** Interleukin-17 receptor, gastric cancer, progression, prognosis

## Introduction

Gastric cancer is the one of most common causes of cancer mortality worldwide and has an especially poor prognosis [1]. Despite new chemotherapeutic regimens and improved surgical outcomes, gastric cancer remains one of the three leading causes of cancer-related death worldwide.

The chemokine IL-17 as a proinflammatory cytokine that mainly produced by T-helper cells (Th17), macrophages and $CD8^+$ T cells [2]. Accumulating evidences have shown that IL-17-positive cells were frequently involved in multiple inflammation-associated cancers, including breast cancer, colorectal cancer, prostate cancer and ovarian cancer [3-8]. In other cancers, it has been suggested that IL-17 contributed to tumor malignancy by promoting capabilities of chemoresistance, tumor growth, angiogenesis and invasion of cancer cells [9-11]. In the previous study, we also have reported that IL-17 is associated with survival time of patients with gastric cancer [12]. Although IL-17 may promote tumor progression in gastric cancer, little is known about the specific biological mechanisms through which IL-17RA contributes to gastric cancer initiation or progression.

Here, our study report that IL-17RA was overexpressed in gastric cancer and high levels of IL-17RA expression was correlated with gastric cancer progression and a poor prognosis in patients with gastric cancer. IL-17RA may be as a novel target for clinical therapy.

## Materials and methods

### *Patients and specimens*

Tumor tissues (101 cases without foci of necrosis) were obtained from patients with GC who underwent surgical resection at the Southwest Hospital of Third Military Medical University. Meanwhile, 6 specimens of adjacent tissues

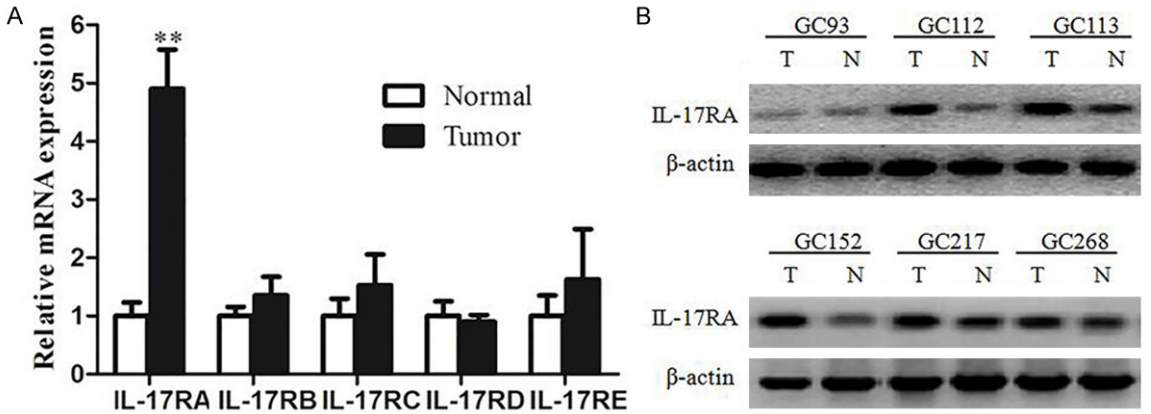


**Figure 1.** IL-17RA expression was elevated in human gastric cancer. A. The IL-17RA mRNA levels were assessed in patients with gastric cancer compared with normal tissues by qRT-PCR. The expression of IL-17RA was normalized against β-actin expression. B. Western blot analysis demonstrated the expression of IL-17RA protein in gastric cancer tissues. The IL-17RA proteins were overexpressed in most tumor tissues (T) compared with matched adjacent normal tissues (N). We collected samples from 6 gastric cancer patients with matched normal tissues (GC93, GC112, GC113, GC152, GC217 and GC268). All experiments were performed at least in triplicate and data are presented as the mean ± SD. **, $P<0.01$.

were obtained from these patients as the paired adjacent normal controls. None of these patients had received chemotherapy or radiotherapy before sampling. The clinical stages of tumors were determined according to the TNM classification system of the International Union Against Cancer (7th edition) and clinical information was collected. The study was approved by the Ethics Committee of the Southwest Hospital of Third Military Medical University. Written informed consent was obtained from all subject involved in the study.

*Real-time RT-PCR*

Total RNA from frozen tissues was extracted using Trizol-reagent (Takara). For measurement of IL-17RA, PrimeScript RT Master Mix (Takara) and SYBR Premix Ex Taq II were used. The IL-17R Specific primers sequence was: (forward) 5'-GCTGCCTAAATGAC-3'; (reverse) 5'-TGTGAGTAGCGGT-3'. The primer sequence for β-actin was: (forward) 5'-CCTTGCACATGCCGGAG-3', (reverse) 5'-GCACAGAGCCTCGCCTT-3'. All reactions were performed on a CFX96 Real-Time PCR Detection System (Bio-Rad). Each reaction was using 250 ng of total RNA and PCR reaction was subjected to 40 cycles at 95°C for 30 seconds, 60 for 60 seconds. The all samples results of IL-17RA real-time PCR were normalized using the threshold cycle (Ct) of β-actin, values of IL-17RA expression for all gastric cancer tissues were acquired and normalized, respectively. Normalized IL-17RA expression values were utilized to determine differences in IL-17RA expression among the gastric cancer samples. One hundred and one primary gastric cancer samples were included in this analysis. Finally, IL-17RA gene expression from primary tumors was analyzed to identify correlations with invasion, metastasis and survival.

*Western blotting*

Western blotting was carried out as previously described [12]. The primary antibodies used in this study were as follows: anti-IL-17RA (Cell Signaling Technology), and anti-β-actin (Cell Signaling Technology).

*Immunohistochemistry*

The immmunohistochemistry was performed as previously report [12]. The human gastric cancer tissues were cut into 5 μm sections for immunohistochemical staining. Primary antibodies included anti-IL-17RA (Cell Signaling Technology) and subsequently counterstained with hematoxylin. Slides were determined under microscope at ×200.

*Statistical analysis*

Results are expressed as mean ± SEM. A logarithmic transformation was performed for IL-17RA values. The statistical significance of

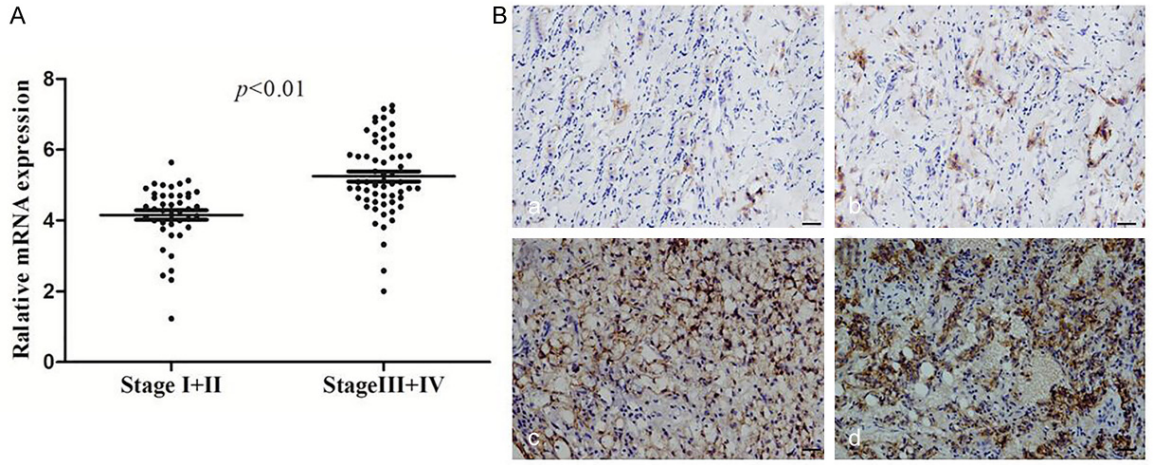


**Figure 2.** IL-17RA expression correlates with gastric cancer grade of patients. A. IL-17RA expression in gastric cancer tissues from 101 patients assessed by qRT-PCR. The relative expression of IL-17RA in specimens was determined and normalized against β-actin. The average expression of IL-17RA was performed in all cancer tissues from gastric cancer in different stages. B. Immunohistochemistry (IHC) demonstrated expression of IL-17RA proteins in stage I (a), stage II (b), stage III (c) and stage IV (d) of patients with gastric cancer. The representative microphotographs of IL-17RA expression are displayed.

differences between 2 groups was determined by the Student t test. $\chi^2$ tests or Fisher exact tests were used to assess the relationship between IL-17RA expression and clinical features. Cumulative survival time was calculated by the Kaplan-Meier method, and survival was measured in months or days. All statistical analyses were performed using SPSS statistical software (version 13.0; SPSS Inc, Chicago, IL). All data were analyzed using 2-tailed tests with a significance level $P<0.05$.

## Results

*Expression of IL-17RA was upregulated in human tumor tissues*

We have identified upregulated IL-17 is associated with survival time of patients with gastric cancer [12]. To validate the expression of IL-17 receptor family members' mRNA levels, real-time qRT-PCR was carried out in frozen tumor sample and adjacent normal tissues to detect the expression of IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE. As a result, only IL-17RA expression was significantly increased in the tumor tissues compared with normal tissues ($P < 0.001$). The IL-17RA expression in tumor tissues was about 4.8-fold higher than that found in adjacent normal control (**Figure 1A**). We then examined IL-17RA expression in six fresh gastric cancer samples and their matched normal tissues by western blot analysis. And found that all gastric cancer samples were displayed increased IL-17RA expression compared with matched adjacent normal tissues except one case (GC93) was no difference between tumor and its matched normal tissue (**Figure 1B**). These data indicate a significantly upregulation of IL-17RA in gastric cancer.

*Expression of IL-17RA demonstrated by qPCR and immunostaining in gastric cancer tissues and the association with tumor progression*

To identify potential differences of IL-17RA mRNA levels in gastric cancer progression, the mRNA levels of IL-17RA were measured by using qRT-PCR analysis for 101 primary gastric cancer tissues (from all stages of gastric cancer). Our data showed that IL-17RA expression was significantly overexpression in high-grade gastric cancer as compared with low-grade gastric cancer ($P < 0.05$; **Figure 2A**). Immunohistochemical staining showed an increased expression of IL-17RA in gastric cancer and high expression in high stages of gastric cancer, and low expression was observed in low stages cancer tissues (**Figure 2B**). The etiology of the gender difference in IL-17RA expression was still unknown and remained for future study. These results revealed that overexpression of IL-17RA in gastric cancer and association with advanced stage.

**Table 1.** Correlations between IL-17RA expression and clinical characteristics of patients with gastric cancer

| Clinical factor | IL-17RA expression | | *P* value |
|---|---|---|---|
| | Low (n=60) | High (n=41) | |
| Sex | | | |
| Male | 33 | 19 | 0.392 |
| Female | 27 | 22 | |
| Age (y) | | | |
| < 65 | 29 | 24 | 0.313 |
| ≥ 65 | 31 | 17 | |
| Tumor size (cm) | | | |
| < 5 | 41 | 23 | 0.214 |
| ≥ 5 | 19 | 18 | |
| Histologic type | | | |
| Differentiated | 27 | 23 | 0.273 |
| Undifferentiated | 33 | 18 | |
| Lymphatic invasion | | | |
| Absent | 30 | 11 | 0.019 |
| Present | 30 | 30 | |
| Tumor (T) invasion | | | |
| T1 + T2 | 29 | 8 | 0.003 |
| T3 + T4 | 31 | 33 | |
| Lymphoid nodal (N) status | | | |
| N0 + N1 | 35 | 11 | 0.001 |
| N2 + N3 | 25 | 30 | |
| Distant metastasis (M) status | | | |
| M0 | 50 | 15 | < 0.001 |
| M1 | 10 | 26 | |
| TNM stages | | | |
| I + II | 21 | 3 | 0.0013 |
| III + IV | 39 | 38 | |

*Correlation of IL-17RA expression and clinicpathologic changes*

Next, to better understand the pathobiological implications of the expression of IL-17RA in gastric cancer, Patients were dichotomized as high or low IL-17RA expression based on the previous description. 60 patients with tissues express low IL-17RA and 41 patients with high expression of IL-17RA. The correlation of expression with different clinicaopathological features was showed in **Table 1**. By analysis, no significantly difference in age, sex, tumor size and histologic type. However, significant difference was found between the expression of IL-17RA and lymphatic invasion (*P*=0.019), tumor invasion (*P*=0.003), lymphoid nodal status (*P*=0.001), distant metastasis status (*P*<0.001) and TNM stages (*P*=0.0013). The IL-17R expression had no correlation with other parameters.

*High expression levels of IL-17RA correlates with poor prognosis of patients with gastric cancer*

The median normalized IL-17RA expression ratio of the primary gastric cancer specimens from 101 patients stratified these tumors as high or low IL-17RA expressing groups. Accordingly, 41 tumors had high expression and 60 had low expression. Kaplan-Meier analyses showed that the patients with high IL-17RA expression tissues had significantly decreased overall survival (log-rank *P*=0.014; **Figure 3A**). To determine the prognostic significance of IL-17RA as a predictor of overall survival in patients, the prognostic of IL-17RA in gastric cancer was further verified in 293 patients with gastric cancer from TCGA database. The high expression of IL-17RA patients had shorter overall survival than low expression of IL-17RA patients (**Figure 3B**). These data indicate a consistent association of IL-17RA expression with gastric cancer malignancy, and reveal that IL-17RA may be a potential biomarker for prognosis in patients with gastric cancer.

## Discussion

Recently a newly pro-inflammatory cytokine IL-17 has been identified produced by TH17 cells, macrophages and $CD8^+$ cells. Accumulation evidences have demonstrated that IL-17 positive cells were frequently involved foster growth and progression in many cancers [3-8]. However, research defining the relationships of IL-17R and cancer has lagged. In the present study, we observed various expression patterns of IL-17RA in gastric cancer tissues using qRT-PCR and immunohistochemistry, which probably suggested their distinct biological effects on tumor growth. The expression levels of IL-17RA exhibited specificity in prognostic ability to dismal outcome of patients with gastric cancer compared to low subgroup, patients with high-density of IL-17RA have shorter over survival. Therefore, patients with high density of IL-17RA need a close monitor-

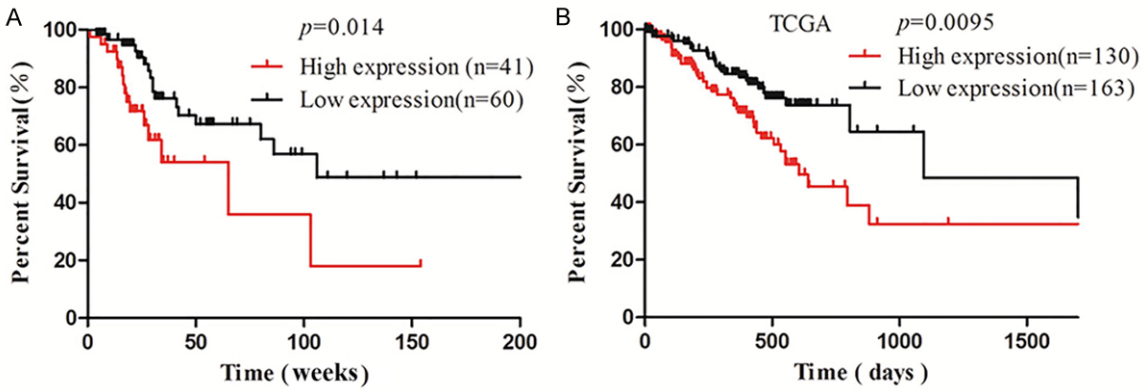


**Figure 3.** IL-17R expression correlates with prognoses of patients with gastric cancer. A. Kaplan-Meier analysis of the correlation between IL-17R and overall survival of patients with gastric cancer according to the mRNA levels of IL-17RA in tumor tissues. Patients were divided into high expression groups and low expression using mRNA expression values mentioned in Materials and Methods. B. Kaplan-Meier survival curve of gastric cancer patients from the TCGA database. Note that patients with higher IL-17R expression have a worse survival rate than patients with lower IL-17R expression ($P < 0.01$).

ing. IL-17RA may provide us a novel prognosticator for poor outcome of gastric cancer patients.

High expression of intratumoral IL-17 produced by $CD8^+$ was identified associated with the prognosis of gastric cancer patients in our previous study [12], and increasing evidence indicates that IL-17R is expressed by many types of tumor cells [13-15], which drove us to investigate its correlation with gastric cancer. Similar to previous reports, our results also shown that IL-17RA high expression in gastric cancer tissues, we found that IL-17RA was upregulated in human gastric cancer compared to non gastric cancer tissues.

We therefore assumed that high degree of IL-17RA was found to significantly correlate with the prognosis of gastric cancer. In addition, a high level of IL-17RA protein expression in gastric cancer lesions is closely associated with the size of tumor, lymph node, distant metastasis and TNM stage. In the low stages of patients, the levels of IL-17RA were significantly lower than those in patients with high stages. The different expression of IL-17RA in the etiology of gender was still needed future study. Further multivariate analysis suggested that the higher levels of IL-17RA often correlate with enhanced invasion, importantly, the levels of IL-17RA was most significantly associated with metastasis. In our previous study showed that IL-17 also significantly associated with poor outcome of gastric cancer patients. We proposed that IL-17 and IL-17RA contribution to the procession of gastric cancer and due to distant metastasis. The signaling of IL-17 transduced by IL-17RA was have been reported, including the activation of transcription factor nuclear factor NF-kappaB [16, 17], NF-kappaB signaling is accepted as a major component of pro-survival signaling and tumor initiation in breast cancer cells [18]. Also reports showed that regulates the activities of ERK1, ERK2, c-Jun N-terminal kinase, and p38MAPK [19, 20]. Interestingly, these two signaling pathways are involved in the self-renewal of tumor-initiating cells (TICs) and mesenchymal stem cells (MSCs) [21, 22]. Together, based on these evidences, we further aimed to investigate the effect of IL-17 and IL-17RA on the gastric cancer stem cell and the possible metastasis mechanisms.

In conclusion, our study suggests that the high expression of IL-17RA was associated with a worse clinical outcome. Our study identified upregulation of IL-17RA expression in gastric cancer tissues. The importance is that IL-17RA high expression in gastric cancer significant associations with patents survival and metastasis. The IL-17-IL-17RA signaling mechanism may be clinically relevant in patients with gastric cancer and represents a potential novel therapy target.

## Acknowledgments

This project was supported by Grants from National Natural Science Foundation of China (81372559).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Pei-Wu Yu, Department of General Surgery and Center of Minimal Invasive Gastrointestinal Surgery, Southwest Hospital, Third Military Medical University, 30 Gaotanyan Street, Chongqing 400038, China. Fax: 86-23-68754167; E-mail: yupeiwu01@vip.sina.com

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